Special Issue: Integrating ZooMS and Zooarchaeology:
Methodological Challenges and Interpretive Potentials

Investigating Species Composition in the Early Aurignacian of le Piage (France) Through Collagen Fingerprinting (ZooMS) of Screen-Recovered Small Bone Fragments

PAULINE RAYMOND*
Chaire de Paléanthropologie, CIRB, Collège de France, Université PSL, CNRS, INSERM, 75005 Paris, FRANCE; pauline.raymond@college-de-france.fr

KAREN RUEBENS
Chaire de Paléanthropologie, CIRB, Collège de France, Université PSL, CNRS, INSERM, 75005 Paris, FRANCE; and, Department of Archaeology, University of Reading, Whiteknights Campus, Reading RG6 6AB, UNITED KINGDOM; k.j.ruebens@reading.ac.uk

FABRICE BRAY
UAR3290 CNRS, MSAP, University of Lille, 59655 Villeneuve d'Ascq, FRANCE; fabrice.bray@univ-lille.fr

JEAN-CHRISTOPHE CASTEL
Vertebrates, Museum of Natural History, Route de Malagnou 1, 1208 Geneva, SWITZERLAND; jean-christophe.castel@ville-ge.ch

EUGÈNE MORIN
Department of Anthropology, DNA Block C, Trent University, 2140 East Bank Drive, Peterborough ON K9J 7B8, CANADA; eugenemorin@trentu.ca

FONI LE BRUN-RICALENS
National Institute of Archaeological Research, INRA, 241 rue de Luxembourg, L-8077 Bertrange, Grand Duchy of LUXEMBOURG; foni.lebrun@inra.etat.lu

CHRISTIAN ROLANDO
UAR3290 CNRS, MSAP, University of Lille, 59655 Villeneuve d'Ascq, FRANCE; christian.rolando@univ-lille.fr

JEAN-GUILLAUME BORDES
UMR5199, CNRS, MC, PACEA, Universitiy of Bordeaux, 33318 Pessac, FRANCE; jean-guillaume.bordes@u-bordeaux.fr

JEAN-JACQUES HUBLIN
Chaire de Paléanthropologie, CIRB, Collège de France, Université PSL, CNRS, INSERM, 75005 Paris, FRANCE; jean-jacques.hublin@college-de-france.fr

*corresponding author: Pauline Raymond; *pauline.raymond@college-de-france.fr*

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Guest Editors: Geoff M. Smith (School of Anthropology and Conservation, University of Kent, and Department of Archaeology, University of Reading), Karen Ruebens (Department of Archaeology, University of Reading, and Chaire de Paléoanthropologie, CIRB, Collège de France), Virginie Sinet-Mathiot (PACEA and Bordeaux Proteome-CBMN, Université de Bordeaux), and Frido Welker (Globe Institute, University of Copenhagen)

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ABSTRACT

In the field of paleoproteomics, ZooMS (Zooarcheology by Mass Spectrometry) has been developed to identify morphologically non-diagnostic animal remains to taxon, offering insights into human subsistence practices. Here, we report new ZooMS analyses of 1,050 Early Aurignacian (ca. 37,000–34,000 cal BP) bone fragments from the site of Le Piage (Lot, France). The studied sample is heavily fragmented and was retrieved through water sieving. In our analysis, we compare the taxonomic identifications of bone remains using traditional morphological attributes with remains identified using ZooMS and discuss the implications of the taxonomic patterns that we uncovered. Our results indicate that, despite small effect sizes, the faunal spectrum identified through ZooMS differs from that obtained through morphological analyses. While reindeer remains the dominant species, bovids and other cervids are more abundantly represented in the ZooMS fraction. Two rare taxa, a hare (*Lepus* sp.) and a previously unidentified carnivore (Pantherinae/Hyaenidae/Mustelidae), were also identified using ZooMS. In addition, we note an increase of *Bos*/*Bison* remains in the sample of spongy fragments that is possibly explained by the use of grease-rich bones and bone portions as fuel. Our work adds new data on patterns of reindeer dominance during the Early Aurignacian and illustrates how ZooMS identifications of screen-recovered fragments can enhance our understanding of Paleolithic subsistence strategies and patterns of site occupation.

INTRODUCTION

Bone fragments are a dominant component of the organic material recovered from Paleolithic sites. However, both human activities and taphonomic processes can lead to high fragmentation of this material. In European Paleolithic contexts, faunal material tends to be highly fragmented, which means that the majority (typically 70–95%) of the retrieved fragments cannot be identified to taxon using macroscopic approaches (Morin et al. 2017; Romandini et al. 2015; Sinet-Mathiot et al. 2019, 2023; Smith et al. 2024). Yet, given their sheer abundance, these highly fragmented, morphologically indeterminate specimens represent a crucial, but underexplored, source of information to investigate subsistence patterns in the past.

In the last decade, a range of biomolecular methodologies have been developed that open up the possibility of assessing the phylogenetic relationships between extinct species using archaeological remains (Buckley et al. 2009; Cappellini et al. 2019; Reich et al. 2010; Rüther et al. 2022; Taniguchi et al. 2023; Welker et al. 2015a, 2017). Among the paleoproteomic approaches, Zooarcheology by Mass Spectrometry (ZooMS) has become a useful tool for identifying both morphologically identified and indeterminate faunal fragments. The method is based on the collagen preserved in faunal remains and has been successfully applied to an increasing number of Paleolithic sites (Brown et al. 2016; Buckley et al. 2009; Douka et al. 2019; García-Vázquez et al. 2023; McGrath et al. 2019; Mylopotamitaki et al. 2024; Pothier Bouchard et al. 2020; Ruebens et al. 2022, 2023; Silvestrini et al. 2022; Sinet-Mathiot et al. 2019, 2023; Welker et al. 2015b, 2016a, 2016b, 2017).

Collagen type I (COL1), a protein biomarker, is the main component (90%) of bone (Abraham et al. 2008). COL1 is formed by a triple helix composed of peptides made of amino acid chains (Richter et al. 2022). The atomic mass of these peptides tends to vary between taxa (Brown et al. 2021c; Buckley et al. 2010; García-Vázquez et al. 2023; Le Meillour et al. 2018; Welker et al. 2015b). As a mass fingerprinting method, ZooMS uses the mass of diagnostic peptides to identify the archeological material to taxon, with the level of precision ranging from family to genus for most specimens (Brown et al. 2016; Buckley et al. 2009, 2016; Eda et al. 2020; Harvey et al. 2022; Welker et al. 2015b). Taxonomic identification is possible using known differences in the molecular weight of digested diagnostic peptides as documented in published libraries that contain a wide range of species (Buckley et al. 2009; García-Vázquez et al. 2023; Richter et al. 2022; Welker et al. 2016a).

Le Piage (Lot, France) is a Paleolithic site where recent excavations have uncovered large quantities of faunal remains that are highly fragmented, including abundant material from layer GI, which is attributed to the Early Aurignacian (Bordes et al. 2006). This Early Aurignacian component is the focus of our study. ZooMS is commonly applied to plotted faunal remains, particularly those with a minimum length of 20–30mm, the same fragments that are preferentially used for AMS radiocarbon dating and DNA analyses (Brown et al. 2016; Ruebens et al. 2022; Sinet-Mathiot et al. 2019; Welker et al. 2015b). Here, we focus on the smaller fragments obtained through water screening and compare them with other ZooMS and morphological identifications from the same layer, in order to improve our knowledge of human subsistence strategies during the Early Aurignacian. Our ZooMS study aims to reveal new information on the preservation and composition of the fauna by investigating these extensively fragmented bone specimens. Our focus is on assessing variation in patterns of taxonomic representation and how they are affected by differential fragmentation in Paleolithic contexts.

SITE BACKGROUND

Le Piage (Figure 1A) is a site bordered by limestone hills and the La Relinquière stream (Bordes 2006). Located on the side of a karstic system characterized by water infiltrations, the site presents a sequence of rich and mostly well-preserved archeological assemblages (Bordes 2003; Cham-

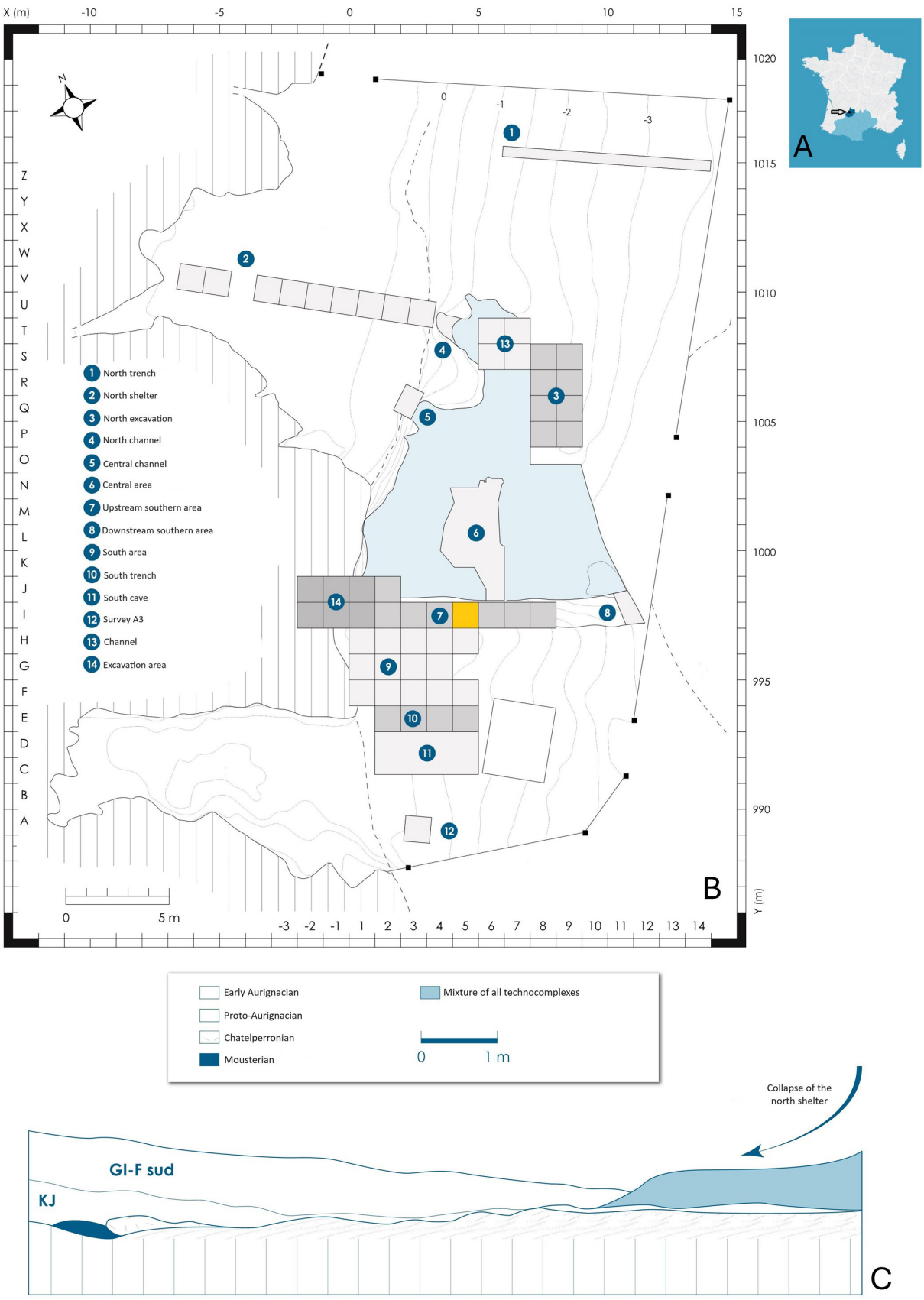

Figure 1. A) Location of Le Piage (Lot) in France. B) Ground plan of the excavated areas. The orange square indicates square I5c where the remains were selected. C) The new stratigraphy outlined for the south part of the site (after Bordes et al. 2006). In C), the letters in the layers give the correlations with the older (Champagne and Espitalié's) stratigraphy.

pagne et Espitalié 1981). During the initial excavations in 1954–1968, Fernand Champagne and René Espitalié identified eight stratigraphic units attributed to the Aurignacian, Châtelperronian, and Solutrean. The site rapidly became controversial due to the discovery of alleged interstratifications of Aurignacian and Châtelperronian layers and their interpretation as alternating occupations of *Homo sapiens* and Neanderthal groups (Champagne and Espitalié 1967, 1981). An extensive refitting program showed that this interpretation was based on flawed stratigraphic interpolations (Bordes 2002). Starting in 2004, the site has been re-excavated and re-analyzed by Jean-Guillaume Bordes and Foni Le Brun-Ricalens (Bordes 2003). Their work permitted an improved understanding of the site stratigraphy and confirmed the absence of interstratifications, now interpreted as resulting from post-depositional mixing of Châtelperronian material originating from a cave that overlooks the excavation zone (Bordes et al. 2006). These recent excavations also identified a Protoaurignacian layer (KJ), underlying the Early Aurignacian layer (GI) (Figure 1C).

Our study focuses on the southern area of the site (Figure 1B) where the stratigraphic section, which extends over 1.5m in elevation, shows moderate to good faunal preservation. This area of the site is divided into an upstream locus abutting the limestone cliff separated by a strong slope from a second locus found downstream. Square I5, which contains the material examined here (see Figure 1B), marks the transition between these two loci. The Early Aurignacian layer consists of several tapho-facies that differ in terms of sedimentation processes. The material presented in this paper was embedded in a sandy-clayey matrix within tapho-facies b, a unit that combines sub-facies b, b1 and b2 (Supplementary Information, Appendix 1: 10.5281/zenodo.12180386). With one exception, all attempts (*n*=21, three different labs) at dating the Early Aurignacian layer failed. One successful attempt provided a radiocarbon date of 32,800±700 BP (37–34 ka cal. BP, Bordes and Brun-Ricalens 2010). In marked contrast, efforts at dating the Solutrean-Badegoulian were mostly met with success (*n*=16/21 or 76.1%), the dates ranging between 22.0 and 24.5 ka cal. BP (Bordes and Brun-Ricalens 2010). Despite the difficulties encountered in dating the Early Aurignacian occupation, a recent ZooMS study provided encouraging results as it showed a success rate of 73.6% in attempts at obtaining ZooMS identifications from the associated material (Morin et al. 2023).

The recently excavated Early Aurignacian occupation (see Figure 1C; Bordes et al. 2011) from Le Piage is large with approximately 4,000 faunal specimens and 1,200 lithic objects, all piece-plotted (Bordes 2005; Bordes et al. 2011). The faunal remains have been analyzed by Jean-Christophe Castel and Eugène Morin (Bordes et al. 2006; Morin et al. 2023). Their work shows that reindeer dominates throughout the various Paleolithic occupations, and therefore, was likely the main species exploited (83.3–89.8% in the Aurignacian horizons; 86.1% in the Solutrean-Badégoulian layer, NISP counts). Horse, bison, chamois and ibex are also present (Bordes and Brun-Ricalens 2010), a picture that is consistent with what has been documented in the region during MIS 3 (Marine Isotopic Stage 3) (Grayson and Delpech 2008; Mellars 2005; Morin 2006; Soulier and Mallye 2012). However, regarding the Solutrean-Badegoulian, we note that coeval paleontological samples accumulated in sinkholes in the same region differ by showing higher proportions of bison and horse (Castel et al. 2014; and forthcoming), which raises questions on the causal factors underlying the reindeer-dominated assemblages at Le Piage. Abundant cutmarks indicate that the Early Aurignacian assemblage is anthropic, as confirmed by the virtual absence of carnivores (1.1% of the morphological identifications, Morin et al. 2023) and a low incidence of marks associated with their activities.

MATERIAL AND METHODS

We analyzed 1,050 Early Aurignacian faunal specimens from sub-square I5c (a 50x50cm unit, see Figure 1B) recovered through water screening sediments from the recently excavated tapho-facies b. Sub-square I5c was selected because it had previously yielded a human specimen, a sample that we were interested in expanding. Our study focused on bone fragments—the sample did not contain tooth or antler fragments—larger than 15mm with no visible traces of burning (based on surface color) that were considered morphologically unidentifiable by the site's zooarcheologists. These bone fragments were further classified according to the type of skeletal tissue (spongy vs cortical) and assigned to size classes (10–20mm, 20–30mm, 30–40mm, Figure 2) depending on the corresponding mesh sizes used for the water sieving. Overall, our sample size considerably exceeds that of a previous ZooMS study conducted at the same site (*n*=360, Morin et al. 2023) and is also large compared to other Paleolithic ZooMS studies (Brown et al. 2021c; Harvey et al. 2022; Mylopotamitaki et al. 2024; Oldfield et al. 2023; Ruebens et al. 2023; Sinet-Mathiot et al. 2023; Wang et al. 2023). Having a sample as large as possible is important because it reduces the possibility of sampling bias and diminishes the influence of outliers (Oldfield et al. 2023).

SAMPLING AND EXTRACTION PROTOCOLS

Sampling

All 1,050 bone fragments were sampled at the Paleoproteomics lab of the Chaire de Paléoanthropologie, Collège de France in Paris. Specimens were sampled by removing a small fragment (~5mg) using pliers as described in Welker et al. (2015b). These fragments were put in 96-well plates leaving one blank in each plate for contamination control (Richter et al. 2011). Of these, 84 specimens were sampled a second time by scraping off 5mg of bone powder using a scalpel for analysis with another method (MALDI-FT-ICR) (in prep.).

Extraction protocols

We applied three different extraction protocols (AmBic, HCl, TFA), tailored to two different methods of mass spec-

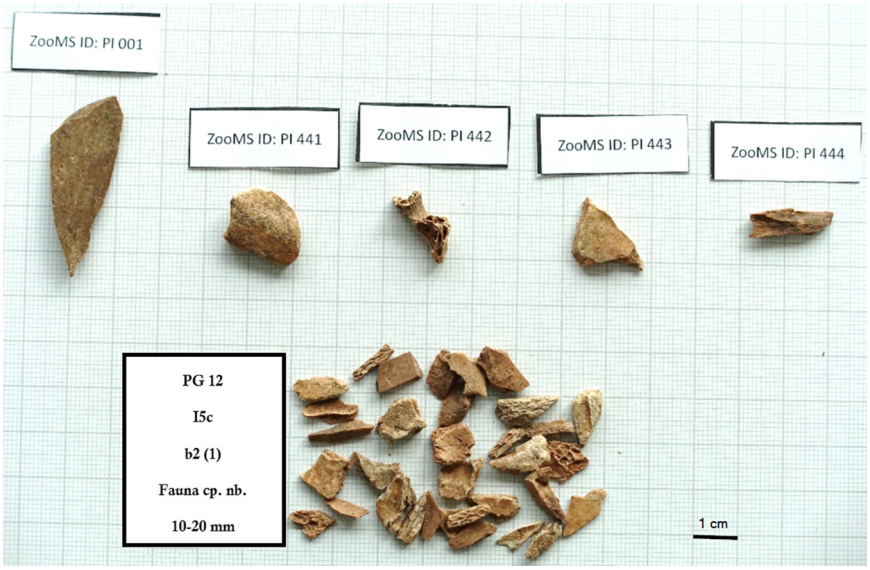

Figure 2. Examples of morphologically unidentifiable material from Le Piage recovered through water screening of the tapho-facies b sediments, Early Aurignacian layer (as defined by Bordes et al. 2011), sub-square I5c. Bones larger than 15mm were given a ZooMS ID and included in the ZooMS analysis, whereas bones smaller than 15mm were excluded (abbreviations: cp=compact ; nb=non burnt).

trometry analysis:

MALDI-TOF (Matrix-assisted laser desorption/ionization-time of flight). All samples (11 96-well plates) were processed using the AmBic extraction protocol (van Doorn et al. 2011). 100µl 50mM AmBic (ammonium bicarbonate buffer, pH 8.0) was added to each sample overnight and discarded. Then the samples were incubated in 100µl of AmBic at 65°C for 1h. The plates were subsequently centrifuged and 50µl of supernatant was collected with 1µl of trypsin (Promega) kept at 37°C for 16–18 h. To halt digestion, 1µl of 10% trifluoroacetic acid (TFA) was added. The samples were cleaned using C18 ZipTip (Hypersep) plates and a vacuum manifold. As a final step, the filtered peptides were eluted in 100µl of conditioning solution (0.1% TFA in 50:50 ACN/UHQ water). To increase the probability of identification, the acid protocol described in Buckley et al. (2009) was applied to five of the plates (#1, 2, 7, 9, and 11). To this end, the samples were demineralised in 120µl of 0.6M HCl acid overnight and rinsed three times with 100µl 50mM AmBic (pH 8.0). The subsequent steps repeat those associated with the AmBic extraction protocol (see above).

MALDI-FT-ICR (Matrix-assisted laser desorption/ionization Fourier transform ion cyclotron resonance mass spectrometry). To further increase taxonomic identifications, 79 bone powder samples from sample plate #2 were also treated following the protocol described by Bray et al. (2023)—along with 5 burnt bones—in sample plate #27 (see Supplementary Information, Appendix 1). The samples were demineralised in 200µl of 5% TFA overnight, rinsed with 100µl 50mM AmBic (pH 8.8), and incubated for 1 h at 65°C on a stirrer prior to trypsin digestion and C18 filtering (same protocol as described above). The peptides were eluted in a conditioning solution (100µL of 80% ACN, 0.1% acetic acid and 100µL of ACN), evaporated and resuspended in 10µL of H2O, 0.1% formic acid.

Peptide Mass Fingerprinting

We spotted 0.5µl of the eluted peptidic solutions on 384-well AB Sciex MALDI plates in triplicate. The analyses were run in automatic mode on the MALDI-TOF 5200 AB Sciex instrument at the Ecole Supérieure de Physique et Chimie industrielle (ESPCI, Paris), as well as the MALDI-FT-ICR instrument in the lab of Miniaturisation pour la Synthèse, l'Analyse et la Protéomique (MSAP) at the University of Lille.

The triplicate spectra obtained from the MALDI-TOF were merged in R Studio using the MALDIquant and MALDIquantForeign packages (Gibb and Strimmer 2012). Once the background noise was smoothed and the peaks aligned, the files were analyzed using mMass software (V.5.5.0, http://www.mmass.org/, Niedermeyer and Strohalm 2012). The signal to noise (S/N) ratio was set at 3.0 and we used the peak picking function to detect the peaks (Brown 2021).

These spectra transcribe the molecular weight (*m*/*z*) of the peptides extracted. We used peptide mass fingerprinting (PMF) to identify specific peptides for each taxon (Buckley 2018; Buckley et al. 2016; Cleland et Schroeter 2018). We recorded the masses of nine mammalian peptide markers using the most recent nomenclature (Brown et al. 2021a). The identifications were made manually based on published libraries (Buckley et al. 2009, 2010; Welker et al. 2015b; García-Vázquez et al. 2023; Harvey et al. 2022). In the present analysis, we only report the most precise identifications obtained for the samples that went through different protocols (for details, see Supplementary Information, Appendix 1).

Reindeer can be identified using ZooMS as it has a spe-

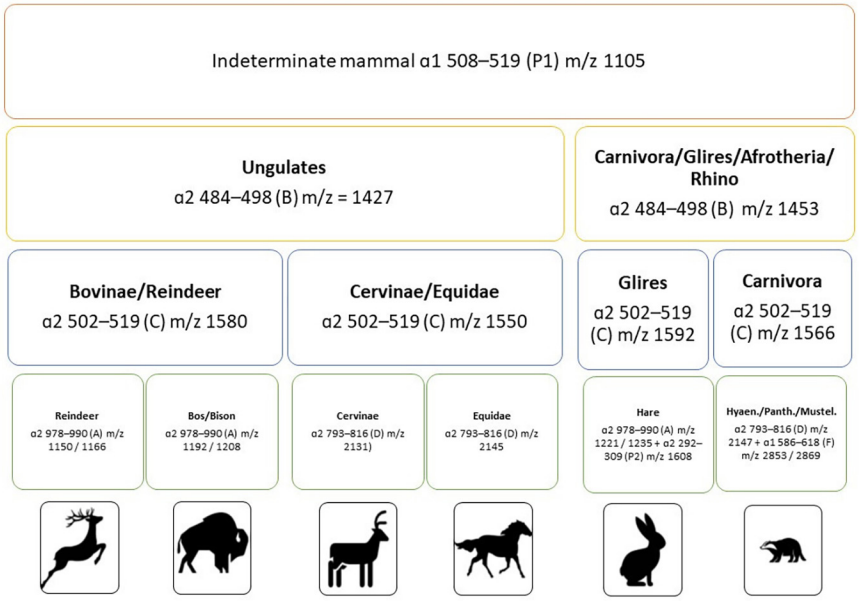

Figure 3. Flowchart showing the peptide markers used to identify the taxa at Le Piage.

cific peptide marker (COL1α2 978–990, *m/z* 1150 / 1166). This species also has a peptide marker (COL1α2 502–519, *m/z* 1580) that can be used to distinguish it from other cervids (Figure 3). However, cervids other than reindeer that might have been present in the region (e.g., red deer *Cervus elaphus*, fallow deer *Dama dama*, giant deer *Megaloceros giganteus*) cannot be discriminated from each other, nor can they be separated from the Saiga antelope (*Saiga tatarica*). Roe deer (*Capreolus* sp.) can possibly be identified when the peptide marker COL1α2 757–789 (*m/z* 3017 / 3033) is present, but this is rarely the case with poor to moderate quality spectra. Therefore, all these cervids can only be assigned to Cervid/*Saiga* (see Figure 3). However, because the evidence indicates that *Saiga tatarica* was absent in the region during the Early Aurignacian (Nadachowski et al. 2016), this taxon can safely be excluded for our assemblage.

Species of *Equus* can only be differentiated from other ungulates using peptide markers COL1α2 793–816 (*m/z* 2145) combined with COL1α2 484–498 (*m/z* 1427). Although *Bos* and *Bison* genera cannot be differentiated using ZooMS, peptide markers COL1α2 978–990 (*m/z* 1192/1208), COL1α1 586–618 (*m/z* 2853/2869) and COL1α2 757–789 (*m/z* 3017/3033) can be used to distinguish them from reindeer (see Figure 3). When these peptide markers are poorly preserved, the fragments can only be attributed to Bovinae/Reindeer; additionally, when these samples also lack the peptide markers COL1α2 978–990 and COL1α2 502–519 (*m/z* 1580), they were attributed to Bovidae/Cervidae. Carnivores and hares (*Lepus* sp.) were distinguished from ungulates using the peptide marker COL1α2 484–498 (*m/z* 1453) (see Figure 3). We also differentiated specimens for which no collagen peptide could be identified (labeled as 'Fail') from those that only showed the presence of peptide COL1α1 508–519 (*m/z* 1105) and a maximum of one other peptide marker (labeled as 'Indeterminate').

Biomolecular Preservation

We calculated deamidation values as an additional indicator of collagen preservation using the BetaCalc package (Sinet-Mathiot et al. 2019; Van Doorn et al. 2012; Welker et al. 2016b; Wilson et al. 2012). Studies have suggested that glutamine (Q) deamidation is a reliable tool for assessing collagen preservation and detecting outliers in faunal assemblages (van Doorn et al. 2011; Welker et al. 2016b), even though the precise biochemical processes that are involved remain unclear (Brown et al. 2021b; Cleland and Schroeter, 2018). We chose to include the deamidation rate from peptide COL1α1 508–519 (*m/z* 1105)—the best preserved biomarker present in all the specimens that we identified—to evaluate collagen preservation between the taxa present in our sample (Sinet-Mathiot et al. 2019). Values were only calculated for AmBic spectra because the acid extraction protocol potentially affects the deamidation values (Ruebens et al. 2022, 2023).

RESULTS

FAUNAL SPECTRUM OF THE SCREEN-RECOVERED FRACTION

Of the 1,050 remains studied, 70% (*n*=744) were identifiable at least to family level, and frequently to genus or even species level (Table 1). Reindeer (*Rangifer tarandus*) is the most common taxon in our sample, representing 85.2% of the identified specimens. Next, in decreasing order, are *Bos/Bison* (10.6%), equids (2.1%) and cervids (1.7%, Bovinae/reindeer and Bovidae/Cervidae excluded from the counts). One specimen was assigned to Pantherinae/Hyaenidae/Mustelidae (*Panthera* sp./*Crocuta* sp./*Meles* sp.) as a result of the presence of peptide marker COL1α2 793–816 (*m/z* 2147), whereas a second remain was attributed to hare (*Lepus sp.*) using the following peptide markers: COL1α2 978–990 (*m/z*

TABLE 1. ZooMS IDENTIFICATIONS IN THE EARLY AURIGNACIAN LAYER OF LE PIAGE (Lot, France).

ZooMS ID	Taxonomic identification	N	%[1]	%[2]
Reindeer	*Rangifer tarandus*	634	60.4	85.2
Bos/*Bison*	*Bison* sp./*Bos* sp.	79	7.5	10.6
Equidae	*Equus* sp.	16	1.5	2.1
Cervid	*Cervus* sp./*Dama* sp./*Capreolus* sp.	13	1.2	1.7
Hare	*Lepus* sp.	1	0.1	0.1
Hyaen./Panth./Mustel.	*Panthera* sp./*Crocuta* sp./*Meles* sp.	1	0.1	0.1
Bovinae/Reindeer	*Bison* sp./*Bos* sp./*Rangifer tarandus*	41	3.9	-
Bovidae/Cervid	*Bison* sp./*Bos* sp./Cervid	34	3.2	-
Indeterminate		50	4.8	-
Fail		181	17.2	-
Total analyzed		1050	100	100

[1]Percentages calculated using all taxonomic identifications (n=1,050), regardless of taxonomic level.
[2]Percentages calculated using taxonomic identifications made at least to family level (n=744).

1221/1235), COL1α2 502–519 (*m*/*z* 1592), COL1α2 292–309 (*m*/*z* 1608).

QUANTITATIVE INTEGRATION

The ZooMS analysis of a single sub-square unit (50x50cm)—representing less than 1% of the excavated volume—yielded a sample of taxonomic identifications that corresponds to one third of the sample of morphological identifications assembled for the entire excavation zone (6m^2). This means that, unlike traditional approaches of identification, taxonomic profiles can be generated with ZooMS using a small number of fragments. One of the aims of this study was to test if taxonomic proportions varied depending on the method of identification, as previous studies have observed significant differences between morphological and ZooMS identification, particularly with respect to the representation of large ungulates (Brown et al. 2021c; Le Meillour et al. 2020; Morin et al. 2023; Oldfield et al. 2023; Sinet-Mathiot et al. 2019, 2023; Welker et al. 2015). We ran chi-square tests to compare taxonomic proportions between our ZooMS (NISP=744) and published morphological identifications (NISP=2,549, Morin et al. 2023). The results for all the identified taxa (excluding Bovinae/Reindeer and Bovidae/Cervidae) show a statistically significant difference (χ^2=85.8, df= 0, p<0.0001, Supplementary Information Table S1). However, the effect size is small (Cramér's V=0.16). A second chi-square test that focuses only on the main taxonomic classes (i.e., reindeer, *Bos*/*Bison*, equids, and cervids other than reindeer) shows a similar pattern (χ^2=64.5, df=3, p<0.0001, data from Supplementary Information Table S1). Again, the effect size is small (Cramér's V=0.14). An analysis of the residuals of both tests indicates a significant over-representation of *Bos*/*Bison* (+5.90 and +5.78) and cervids (+5.33 and +5.28) in our screen-recovered ZooMS assemblage, combined with a significant over-representation of reindeer (+3.52 and +4.88) and fox (+2.60) in the assemblage identified morphologically (Figure 4).

Our ZooMS results are significantly different from the ZooMS results obtained by Morin et al. (2023, *n*=95, χ^2=35.0, p<0.0001, Early Aurignacian indeterminate remains >2cm, data from Table 2). The comparisons of the residuals between the two ZooMS studies point to an over-representation of reindeer (+5.36) and an under-representation of *Bos*/*Bison* (-4.93) and equids (-2.94) in our sample. Like the previous tests, the effect size is small (Cramér's V=0.20). Despite differences in terms of species representation, we note that the two ZooMS studies show the same enrichment in large ungulates compared to the morphological sample (see Table 2).

BONE FRAGMENTATION

With respect to fragmentation, it is interesting to note that all of the largest specimens in the ZooMS samples were assigned to the medium-sized reindeer (Table 3)—the most common species at Le Piage—rather than *Bos*/*Bison*, a group that includes much larger animals. Across all fragment sizes, there is a clear dominance of reindeer. This is supported statistically, as the data show no significant difference between the distributions of fragment size and taxa (χ^2=6.2, df=10, p>0.05, data from Table 3) or between fragment size and rate of identification (χ^2=28.6, df=18, p>0.05, data from Table 3).

As a result of their lamellar structure (Osterhoff et al. 2016), spongy bone does not preserve as well as cortical bone, which could lead to possible biases in patterns of skeletal and/or taxonomic composition. Our results show that the proportion of fragments that could be identified—slightly over 75% of the specimens—is similar for both cortical (*n*=593) and spongy (*n*=226) specimens, indicating a comparable level of collagen preservation regardless of the

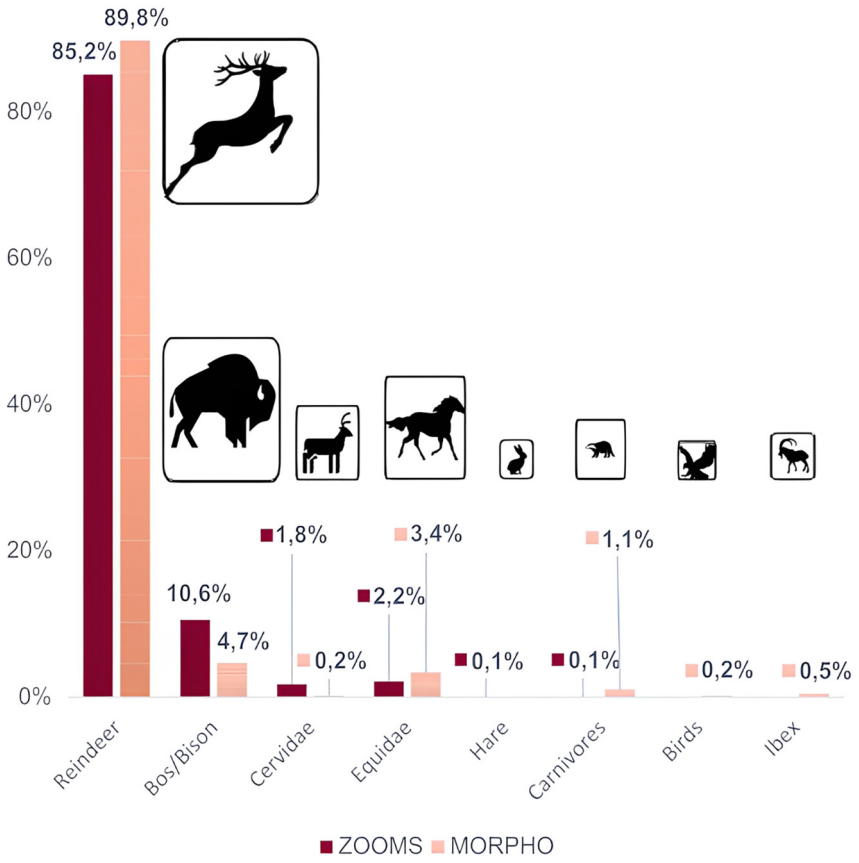

Figure 4. Histogram comparing taxonomic proportions as a function of the identification methods in the Early Aurignacian bone assemblage. NISP ZooMS=744, NISP morphology=2,549.

type of bone tissue (Table 4). However, there is a significant difference between cortical and spongy bone in terms of taxonomic composition (all identifications: χ^2= 25.7, p<0.05, Cramér's V=0.18; upper four rows only: χ^2=23.1, df=3, p<0.0001, Cramér's V=0.18, data from Table 4). Although the effect size is small in both tests, the analysis of the residuals for all taxa indicates a clear under-representation of reindeer (-3.73) and an over-representation of *Bos/Bison* (+4.55) in the spongy bone sample.

BIOMOLECULAR PRESERVATION

At Le Piage, collagen preservation is variable but nonetheless favorable given that we were able to derive identifications at least to the family level for 70% of the specimens. This value is very similar to that (73.6%) derived by Morin et al. (2023) for the same site. Out of the 480 ZooMS identifications obtained using the AmBic protocol, we could derive deamidation values for 287 bones (for details see Supplementary Information, Appendix 2: 10.5281/zenodo.12180386).

The rates of deamidation values obtained are similar for reindeer (60%, *n*=249) and *Bos/Bison* (60%, *n*=28). However, it is a bit lower for equids (56%, *n*=5) and Cervids (42%, *n*=5), although the very small sample sizes for these last taxa preclude statistical testing. Overall, the values suggest that deamidation, and by extension diagenesis, is homogeneous across taxa (Figure 5).

Deamidation values were also compared between types of tissue, with the majority of the data coming from cortical (82.9%) rather than spongy bones (17.1%, for details see Supplementary Information, Appendix 2). Comparisons of deamidation values between tissue types show no significant difference (W=6129, p>0.05, see Supplementary Information, Figure S1). The values obtained for both tissues range between 0.3 and 0.6, which is relatively low compared to published values for European Paleolithic assemblages (Brown et al. 2021b, Ruebens et al. 2022; Sinet-Mathiot et al. 2019, 2023; Welker et al. 2015b, 2016a). Among other causes, site formation processes—including solifluction, cyclic water inclusions, and debris flows—likely negatively influenced preservation of collagen at Le Piage.

DISCUSSION

TAXONOMIC DIVERSITY

In the Early Aurignacian of Le Piage, reindeer is the dominant species in both the ZooMS and morphologically identified fractions. *Bos/Bison* is also moderately represented in the ZooMS and morphological analyses. Given the environmental context of deposition, the ZooMS "Cervid" specimens probably correspond to red deer (*Cervus elaphus*), a taxon that has been identified morphologically by the

TABLE 2. PATTERNS OF TAXONOMIC REPRESENTATION IN THE EARLY AURIGNACIAN LAYER AT LE PIAGE PRESENTED AS A FUNCTION OF THE SCIENTIFIC TEAM THAT PERFORMED THE ANALYSIS AND THE METHOD OF IDENTIFICATION.

	Specimens identified morphologically (Morin et al. 2023) (n=2,549)	**ZooMS identifications of water-sieved specimens from subsquare I5C** (n=1,050)	**ZooMS identifications of piece-plotted remains (Morin et al. 2023)** (n=95)
Reindeer	**89.8 %**	**85.2 %**	**63.2 %**
Bos/*Bison*	**4.7 %**	**10.6 %**	**28.4 %**
Equidae	3.4 %	2.1 %	7.4 %
Cervid	0.2 %	1.7 %	1.1 %
Mammoth	0.04 %	-	-
Ibex	0.5 %	-	-
Fox	0.9 %	-	-
Canis	0.2 %	-	-
Hyaen./Pant./Mustel.	-	0.1 %	-
Leporidae	0.1 %	0.1 %	-
Bird	0.1 %	-	-

zooarcheologists working at the site. ZooMS also allowed more precise taxonomic identification of the Leporidae remains (assigned by ZooMS to *Lepus*) recently identified morphologically. Some differences were also noted, while canids (*Canis lupus* and *Vulpes vulpes*), *Capra ibex*, and *Gypaetus barbatus* have been identified morphologically, these taxa are absent from the ZooMS samples. Conversely, Hyaenidae, Pantherinae or Mustelidae—taxa rarely recorded in the Early Aurignacian of southwest France—were identified using ZooMS.

Despite the use of two distinct methods of identification and a large sample of remains (total of 3,293 taxonomic identifications for the two methods), the number of species is relatively low (NTAXA=10) at Le Piage. This suggests an environment with low species diversity, a trend commonly observed in cool environments (Morin 2008). At Le Piage, this low species diversity coincided with strong dominance of reindeer.

DIFFERENTIAL PROCESSING OF LARGE GAME TAXA?

Several studies have observed an over-representation of large ungulates in ZooMS assemblages (see Discamps et al. 2024, this special issue), which has been interpreted as indicating differential processing of animals (Sinet-Mathiot et al. 2019, 2023). The Early Aurignacian sample from Le Piage not only shows an over-representation of *Bos*/*Bison* in the small bone fraction, but also an increased representation of this taxon in the spongy bone sample. These data may indicate that *Bos*/*Bison* skeletal elements were more intensively fragmented than those from reindeer, perhaps in relation with the use of spongy portions of long bones as fuel. However, because the proportion of spongy parts in long bones tends to be smaller in reindeer than in large taxa such as *Bos*/*Bison* and equids, the separate examination of spongy and cortical bones in the ZooMS analysis possibly inflated the proportion of large ungulates in the spongy sample. It should also be noted that spongy fragments are more difficult to identify to taxon than cortical fragments, which potentially affected the representation of these tissues in the morphological sample.

Our analysis needs also to address the problem of specimen interdependence as all of our specimens derive from a single excavation unit. This problem is critical when a high fraction of specimens derive from a small number of individuals. In this situation, the interdependence of the

TABLE 3. DISTRIBUTION OF ZooMS IDENTIFICATIONS AS A FUNCTION OF FRAGMENT SIZE CLASSES.

Taxa	15–20mm	%	20–30mm	%	30–40mm	%
Reindeer	397	*57.2*	221	*65.4*	16	*94.1*
Bos/Bison	56	*8.0*	23	*6.8*	0	*0*
Cervid	10	*1.4*	3	*0.9*	0	*0*
Equidae	11	*1.6*	5	*1.5*	0	*0*
Carnivora	1	*0.1*	0	*0.0*	0	*0*
Hare	1	*0.1*	0	*0.0*	0	*0*
Bovinae/reindeer	20	*2.9*	21	*6.2*	0	*0*
Bovidae/Cervidae	27	*3.9*	7	*2.1*	0	*0*
Indeterminate	40	*5.7*	10	*3.0*	0	*0*
Fail	132	*19.0*	48	*14.2*	1	*5.9*
Total	695	*100*	338	*100*	17	*100*

In Supplemental Information Appendixes 1 and 2, some bone fragments are identified as >20mm, >30mm, and >40mm. The specimens were classified as follows: bones marked as >20mm were included in the 20–30mm group, while bones marked as >30mm and >40mm were included in the 30–40mm group.

specimens comparably reduces the true statistical size of the sample (Grayson 1984). Likewise, the increased representation of *Bos/Bison* in the indeterminate sample could reflect greater post-depositional fragmentation in large taxa than in reindeer. These problems aside, our results are consistent with a previous ZooMS analysis conducted on randomly selected specimens (Morin et al. 2023) in showing an increased representation of large ungulates in the ZooMS sample. For this reason, it seems reasonable to conclude that our sampling protocol did not negatively impact our data, although it might have increased the problem of specimen interdependence due to limited coverage of the excavated area.

INTEGRATION OF THE NEW ZOOMS DATA IN THE CONTEXT OF MONOSPECIFIC SITES

Our ZooMS identifications confirm the taxonomic dominance of reindeer at Le Piage, a pattern observed in other Early Aurignacian assemblages from the same region (e.g., Roc de Combe: Grayson and Delpech 2008). In France and Germany, some Early Aurignacian assemblages contain up to 99% of reindeer (Mellars 2005), although lower propor-

TABLE 4. PROPORTION OF ZooMS IDENTIFICATIONS AS A FUNCTION OF BONE TISSUE TYPE (cortical or spongy) IN THE EARLY AURIGNACIAN OF LE PIAGE.

Taxa	Cortical	%	Spongy	%	Total	%
Reindeer	479	*75.6*	155	*24.4*	634	*100*
Bos/Bison	40	*50.6*	39	*49.4*	79	*100*
Cervid	11	*84.6*	2	*15.4*	13	*100*
Equidae	11	*68.8*	5	*31.3*	16	*100*
Carnivora	1	*100*	0	*0*	1	*100*
Hare	1	*100*	0	*0*	1	*100*
Bovinae/Reindeer	29	*70.7*	12	*29.3*	41	*100*
Bovidae/Cervidae	21	*61.8*	13	*38.2*	34	*100*
Total identified	**593**	***78.6***	**226**	***76.3***	**819**	*100*

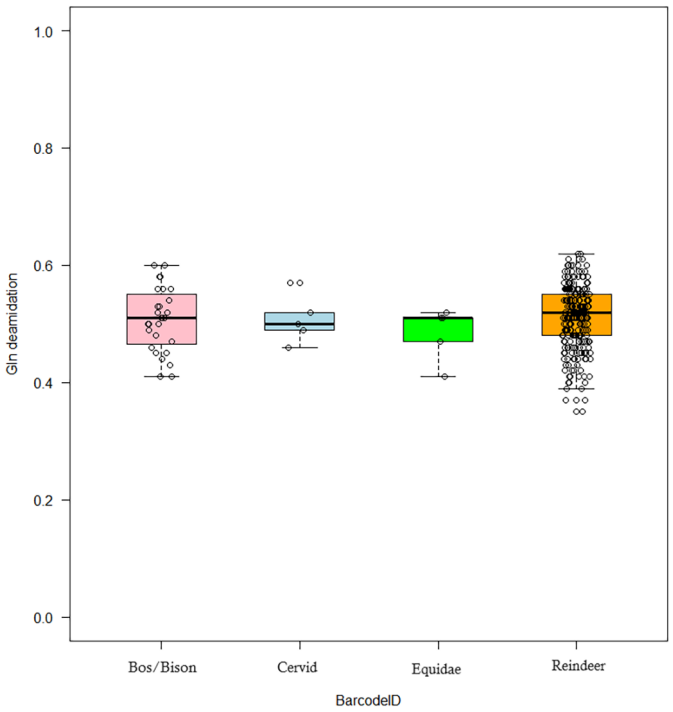

Figure 5. Boxplot of glutamine (Q) deamidation values (COL1a1 508–519, m/z 1105) in the four main taxa identified by ZooMS (AmBic extraction) in the Early Aurignacian of Le Piage. Specimens that have not been deamidated have a value of "1" whereas specimens that are deamidated have a value of "0." Bos/Bison *(n=28): pink; Cervid (n=5): light blue; Equidae (n=5): green: Reindeer (n=249): orange.*

tions (70–90%), as is the case at Le Piage, are more common. The implications of this pattern of reindeer dominance have been controversial. For instance, while earlier models have emphasized evidence for an economic "specialization" on reindeer (Mellars 2005), more recent propositions have stressed the primacy of climatic factors (Discamps et al. 2011; Grayson and Delpech, 2002; Morin 2008; Rendu et al. 2012). The high representation of reindeer at Le Piage should not obscure the fact that other taxa were likely of significance in the diet, especially *Bos/Bison*, as suggested by our ZooMS identifications and those of Morin et al. (2023). Our work is thus consistent with a pattern of reindeer dominance during the Early Aurignacian, although this does not necessarily imply a monospecific subsistence strategy (Ruebens et al. 2023; Sinet-Mathiot et al. 2023).

COLLAGEN PRESERVATION

The taxonomic identification of 744 bone fragments in the Early Aurignacian layer at Le Piage brings new insights into the problem of biomolecular preservation. Our deamidation results demonstrate that ancient, endogenous collagen was effectively extracted from the Early Aurignacian faunal material, despite the variable presence and quality of collagen in the assemblage.

Our results contrast with the multiple failed attempts at radiocarbon dating the Early Aurignacian layer at Le Piage. Among other positive outcomes, the ZooMS analyzes that we conducted should enable us to better delineate contexts that are favorable for radiocarbon dating when the assemblages are variably preserved, for instance by identifying samples with acceptable collagen yield. Approaches such as ZooMS may prove particularly useful in sites where biomolecular preservation varies both within and across layers, as documented in some more recent contexts (Harvey et al. 2016).

CONCLUSION

ZooMS provides taxonomic information on indeterminate fragments and may reveal new information on faunal preservation and composition, particularly at sites like Le Piage, where faunal remains are highly fragmented. Our study illustrates how ZooMS analyses contribute to our understanding of diet breadth and processing behavior. Our results add to the growing body of studies finding discrepancies in the representation of medium- versus large-sized ungulates in zooarcheological and ZooMS datasets. Qualitatively, our ZooMS identifications are generally consistent with the morphological identifications. However, as observed in several recent ZooMS analyses, our study shows non-marginal changes in proportions of taxa between methods. The increased representation of *Bos/Bison* and cervids (excluding reindeer) in the ZooMS record provides additional insights into faunal patterns in Early Aurignacian contexts although it remains unclear whether these changes are due to taphonomic and/or cultural factors. At Le Piage, the high rate of taxonomic identifications achieved with ZooMS contrasts with the lack of success in radiocarbon dating the assemblage. Our ZooMS study and the deamidation results support an endogenous origin for the collagen at the site, which means that further paleoproteomic studies could be conducted to better evaluate the behavioral implications of the patterns of bone fragmentation that were recorded in the faunal assemblage. Combined with other screening methods, such as near-infrared spectroscopy (Fewlass et al. 2019; Malegori et al. 2023; Ruebens et al. 2023; Sponheimer et al. 2019; Talamo et al. 2021), ZooMS represents a valuable tool for selecting faunal fragments with high potential for direct radiocarbon dating.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest to disclose.

DATA AVAILABILITY STATEMENT

ZooMS spectra as well as appendices 1 and 2, including a simplified database recording the final identifications are available on Zenodo: 10.5281/zenodo.12180386. All triplicates from the mass spectrometry data are uploaded in the format mzXML.

REFERENCES

Abraham, L.C., Zuena, E., Perez-Ramirez, B., Kaplan, D.L., 2008. Guide to collagen characterization for biomaterial studies. J. Biomed. Mater. Res. B 87B, 264–285.

Beckouche, S., 1981. Les grands mammifères du Paléolithique supérieur du Piage (Lot). In: Champagne, F., Espitalié, R. (Eds.), Le Piage: site préhistorique du Lot. Mémoires de La Société Préhistorique Française 15, Paris, pp. 165–192.

Bordes, J.-G., 2003. Lithic taphonomy of the Châtelperronian/Aurignacian interstratifications in Roc de Combe and Le Piage (Lot, France). In: Zilhão, J., d'Errico, F. (Eds.), The Chronology of the Aurignacian and of the Transitional Technocomplexes: Dating, Stratigraphies, Cultural Implications. Proceedings of Symposium 6.1 of the XIVth Congress of the UISPP (University of Liège, Belgium, September 2–8, 2001). IPA, Lisboa, pp. 223–244.

Bordes, J.-G., 2005. La séquence Aurignacienne du Nord de l'Aquitaine : variabilité des productions lamellaires à Caminade-est, Roc-de-Combe, Le Piage et Corbiac-Vignoble II. In: Le Brun-Ricalens, F. (Ed.), Productions lamellaires attribuées à l'Aurignacien: chaînes opératoires et perspectives technoculturelles. MNHA, Luxembourg, pp. 123–154.

Bordes, J.-G., 2006. News from the West: a reevaluation of the classical Aurignacian sequence of the Périgord. In: Bar-Yosef, O., Zilhão, J. (Eds.), Towards a Definition of the Aurignacian. Instituto Português de Arqueologia, Lisbon, pp. 147–171.

Bordes, J.-G., Lebrun-Ricalens, F., Castel, J.-C., Ducasse, S., Faivre, J.-P., Feruglio, V., Henry-Gambier, D., Lacrampe-Cuyaubère, F., Laroulandie, V., Lenoble, A., Martin, H., Maureille, B., Morala, A., Renard, C., Rendu, W., Rigaud, S., Rougier, H., Tartar, E., Texier, P.-J., Teyssandier, N., 2006. Les débuts du Paléolithique supérieur dans le Sud-Ouest de la France : fouilles 2004-2006 au Piage (Fajoles, Lot). Problématique et premiers résultats. Mémoire 47, Société préhistorique française, Paris, pp. 261–288.

Bordes, J.-G., Brun-Ricalens, F., 2010. Rapport d'opération de fouille triennale. Ministère de la culture et de la communication, Service régional de l'archéologie, Fajoles.

Bordes, J.-G., Bachellerie, F., Brun-Ricalens, F., Michel, A., 2011. Towards a new "transition": new data concerning the lithic industries from the beginning of the Upper Palaeolithic in Southwestern France. In: Derevianko, A.P., Shunkov, M.V. (Eds.), Characteristic Features of the Middle to Upper Paleolithic Transition in Eurasia. Proceedings of the international symposium 'Characteristic features of the Middle to Upper Paleolithic transition in Eurasia: development of culture and evolution of *Homo* Genus' (July 4 – 10, 2011, Denisova Cave, Altai). Novosibirsk, 2011, pp. 11–23.

Bray, F., Fabrizi, I., Flament, S., Locht, J.-L., Antoine, P., Auguste, P., Rolando, C., 2023. Robust high-throughput proteomics identification and deamidation quantitation of extinct species up to Pleistocene with Ultrahigh-Resolution MALDI-FTICR mass spectrometry. Anal. Chem. 95, 7422–7432.

Brown, S., 2021. Identifying ZooMS Spectra (mammals) using mMass. Protocols.io, Tübingen.

Brown, S., Higham, T., Slon, V., Pääbo, S., Meyer, M., Douka, K., Brock, F., Comeskey, D., Procopio, N., Shunkov, M., Derevianko, A., Buckley, M., 2016. Identification of a new hominin bone from Denisova Cave, Siberia using collagen fingerprinting and mitochondrial DNA analysis. Sci. Rep. 6, 23559.

Brown, S., Douka, K., Collins, M.J., Richter, K.K., 2021a. On the standardization of ZooMS nomenclature. J. Proteomics. 235, 104041.

Brown, S., Kozlikin, M., Shunkov, M., Derevianko, A., Higham, T., Douka, K., Richter, K.K., 2021b. Examining collagen preservation through glutamine deamidation at Denisova Cave. J. Archaeol. Sci. 133, 105454.

Brown, S., Wang, N., Oertle, A., Kozlikin, M.B., Shunkov, M.V., Derevianko, A.P., Comeskey, D., Jope-Street, B., Harvey, V.L., Chowdhury, M.P., Buckley, M., Higham, T., Douka, K., 2021c. Zooarchaeology through the lens of collagen fingerprinting at Denisova Cave. Sci. Rep. 11, 15457.

Buckley, M., 2018. Zooarchaeology by mass spectrometry (ZooMS). Collagen fingerprinting for the species identification of archaeological bone fragments. In: Giovas, C.M., LeFebvre, M.J. (Eds.), Zooarchaeology in Practice: Case Studies in Methodology and Interpretation in Archaeofaunal Analysis. Springer International Publishing, Cham, pp. 227–247.

Buckley, M., Collins, M., Thomas-Oates, J., Wilson, J.C., 2009. Species identification by analysis of bone collagen using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry. Rapid Commun. Mass Spectrom. 23, 3843–3854.

Buckley, M., Whitcher Kansa, S., Howard, S., Campbell, S., Thomas-Oates, J., Collins, M., 2010. Distinguishing between archaeological sheep and goat bones using a

single collagen peptide. J. Archaeol. Sci. 37, 13–20.
Buckley, M., Gu, M., Shameer, S., Patel, S., Chamberlain, A.T., 2016. High-throughput collagen fingerprinting of intact microfaunal remains; a low-cost method for distinguishing between murine rodent bones. Rapid Commun. Mass Spectrom. 30, 805–812.
Buckley, M., Pinsonneault, M., Brassey, C., Rolett, B., 2021. High-throughput microCT and ZooMS collagen fingerprinting of Scombrid bone from the Marquesas Islands. J. Archaeol. Sci. 136, 105475.
Cappellini, E., Welker, F., Pandolfi, L., Ramos-Madrigal, J., Samodova, D., Rüther, P.L., Fotakis, A.K., Lyon, D., Moreno-Mayar, J.V., Bukhsianidze, M., Rakownikow Jersie-Christensen, R., Mackie, M., Ginolhac, A., Ferring, R., Tappen, M., Palkopoulou, E., Dickinson, M.R., Stafford, T.W., Chan, Y.L., Götherström, A., Nathan, S.K.S.S., Heintzman, P.D., Kapp, J.D., Kirillova, I., Moodley, Y., Agusti, J., Kahlke, R.-D., Kiladze, G., Martínez-Navarro, B., Liu, S., Sandoval Velasco, M., Sinding, M.-H.S., Kelstrup, C.D., Allentoft, M.E., Orlando, L., Penkman, K., Shapiro, B., Rook, L., Dalén, L., Gilbert, M.T.P., Olsen, J.V., Lordkipanidze, D., Willerslev, E., 2019. Early Pleistocene enamel proteome from Dmanisi resolves *Stephanorhinus* phylogeny. Nature 574, 103–107.
Castel, J.-C., Boudadi-Maligne, M., Ducasse, S., C., R., Kuntz, D., Mallye, J.-B., 2014. Animal exploitation strategies in eastern Aquitaine (France) during the Last Glacial Maximum. In: Foulds, F.W.F., Drinkall, H.C., Perri, A.R., Clinnick, D.T.G., Walker, J.W.P. (Eds.), Wild Things. Recent Advances in Palaeolithic and Mesolithic Research. Oxbow Books, Oxford, pp. 160–174.
Champagne, F., Espitalie, R., 1967. La stratigraphie du Piage: note préliminaire. Bull. Soc. Préhist. française 64, 29–34.
Champagne, F., Espitalié, R., 1981. Le Piage, site préhistorique du Lot. Société Préhistorique Française (Mémoire de la Société Préhistorique Française; 15), Paris.
Cleland, T.P., Schroeter, E.R., 2018. A comparison of common mass spectrometry approaches for paleoproteomics. J. Proteome Res. 17, 936–945.
Discamps, E., Jaubert, J., Bachellerie, F., 2011. Human choices and environmental constraints: deciphering the variability of large game procurement from Mousterian to Aurignacian times (MIS 5-3) in southwestern France. Quatern. Sci. Rev. 30, 2755–2775.
Discamps, E., Smith, G.M., Hublin, J-J., Ruebens, K., 2024. Can ZooMS help assess species abundance in highly fragmented bone assemblages? Integrating morphological and proteomic identifications for the calculation of an adjusted ZooMS-eNISP. PaleoAnthropology 2024:2 (this special issue).
Douka, K., Brown, S., Higham, T., Pääbo, S., Derevianko, A., Shunkov, M., 2019. FINDER project: collagen fingerprinting (ZooMS) for the identification of new human fossils. Antiquity 93, e1.
Eda, M., Morimoto, M., Mizuta, T., Inoué, T., 2020. ZooMS for birds: discrimination of Japanese archaeological chickens and indigenous pheasants using collagen peptide fingerprinting. J. Archaeol. Sci. Rep. 34, 102635.
Fewlass, H., Tuna, T., Fagault, Y., Hublin, J.-J., Kromer, B., Bard, E., Talamo, S., 2019. Pretreatment and gaseous radiocarbon dating of 40–100 mg archaeological bone. Sci. Rep. 9, 5342.
García-Vázquez, A., Pinto-Llona, A.C., Maroto, J., Torres, T., Grandal-D'Anglade, A., 2023. Characterising the cave bear *Ursus spelaeus* Rosenmüller by ZooMS: a review of peptide mass fingerprinting markers. Earth Environ. Sci. Trans. R. Soc. Edinburgh 114, 1–11.
Grayson, D.K., 1984. Quantitative Zooarchaeology. Academic Press, San Diego.
Grayson, D.K., Delpech, F., 2002. Specialized early Upper Palaeolithic hunters in southwestern France? J. Archaeol. Sci.. 29, 1439–1449.
Grayson, D.K., Delpech, F., 2008. The large mammals of Roc de Combe (Lot, France): the Châtelperronian and Aurignacian assemblages. J. Anthropol. Archaeol. 27, 338–362.
Harvey, V.L., Egerton, V.M., Chamberlain, A.T., Manning, P.L., Buckley, M., 2016. Collagen fingerprinting: a new screening technique for radiocarbon dating ancient bone. PloS One 11, e0150650.
Harvey, V.L., LeFebvre, M.J., Sharpe, A.E., Toftgaard, C., deFrance, S.D., Giovas, C.M., Fitzpatrick, S.M., Buckley, M., 2022. Collagen fingerprinting of Caribbean archaeological fish bones: methodological implications for historical fisheries baselines and anthropogenic change. J. Archaeol. Sci. 145, 105642.
Le Meillour, L., Zazzo, A., Lesur, J., Cersoy, S., Marie, A., Lebon, M., Pleurdeau, D., Zirah, S., 2018. Identification of degraded bone and tooth splinters from arid environments using palaeoproteomics. Palaeogeogr. Palaeoclimatol. Palaeoecol. 511, 472–482.
Le Meillour, L., Zirah, S., Zazzo, A., Cersoy, S., Détroit, F., Imalwa, E., Lebon, M., Nankela, A., Tombret, O., Pleurdeau, D., Lesur, J., 2020. Palaeoproteomics gives new insight into early southern African pastoralism. Sci. Rep. 10, 14427.
Malegori, C., Sciutto, G., Oliveri, P., Prati, S., Gatti, L., Catelli, E., Benazzi, S., Cercatillo, S., Paleček, D., Mazzeo, R., Talamo, S., 2023. Near-infrared hyperspectral imaging to map collagen content in prehistoric bones for radiocarbon dating. Commun. Chem. 6, 1–10.
McGrath, K., Rowsell, K., Gates St-Pierre, C., Tedder, A., Foody, G., Roberts, C., Speller, C., Collins, M., 2019. Identifying archaeological bone via non-destructive ZooMS and the materiality of symbolic expression: examples from Iroquoian bone points. Sci. Rep. 9, 11027.
Mellars, P.A., 2005. Reindeer specialization in the early Upper Palaeolithic: the evidence from south west France. J. Archaeol. Sci. 31, 613–617.
Morin E. 2008. Evidence for declines in human population densities during the early Upper Paleolithic in Western Europe. Proc. Nat. Acad. Sci. U.S.A. 105, 48–53.
Morin, E., 2020. Revisiting bone grease rendering in highly fragmented assemblages. Am. Antiq. 85, 535–553.

Morin, E., Ready, E., Boileau, A., Beauval, C., Coumont, M.-P., 2017. Problems of Identification and quantification in archaeozoological analysis, Part II: presentation of an alternative counting method. J. Archaeol. Method Theory 24, 938–973.

Morin, E., Oldfield, E.-M., Bakovic, M., Bordes, J.-G., Castel, J.-C., Crevecoeur, I., Monnier, G., Tostevin, G., Buckley, M., 2023. A double-blind comparison of morphological and collagen fingerprinting (ZooMS) methods of skeletal identifications from Paleolithic contexts. Sci. Rep. 13, 18825.

Mylopotamitaki, D., Weiss, M., Fewlass, H., Zavala, E.I., Rougier, H., Sümer, A.P., Hajdinjak, M., Smith, G.M., Ruebens, K., Sinet-Mathiot, V. and Pederzani, S., 2024. *Homo sapiens* reached the higher latitudes of Europe by 45,000 years ago. Nature 626, 1–6.

Nadachowski, A., Lipecki, G., Ratajczak, U., Stefaniak, K., Wojtal, P., 2016. Dispersal events of the saiga antelope (*Saiga tatarica*) in Central Europe in response to the climatic fluctuations in MIS 2 and the early part of MIS 1. Quatern. Int. 420, 357–362.

Niedermeyer, T.H.J., Strohalm, M., 2012. mMass as a Software Tool for the annotation of cyclic peptide tandem mass spectra. PloS One 7, e44913.

Oldfield, E.-M., Dunstan, M., Chowdhury, M., Slimak, L., Buckley, M., 2023. AutoZooMS: integrating robotics into high-throughput ZooMS for the species identification of archaeofaunal remains at Grotte Mandrin, France. Research Square preprint. https://doi.org/10.21203/rs.3.rs-2762261/v1

Osterhoff, G., Morgan, E.F., Shefelbine, S.J., Karim, L., McNamara, L.M., Augat, P., 2016. Bone mechanical properties and changes with osteoporosis. Injury, Osteoporotic fracture fixation — a biomechanical perspective. Injury 47, S11–S20.

Pothier-Bouchard, G., Mentzer, S.M., Riel-Salvatore, J., Hodgkins, J., Miller, C.E., Negrino, F., Wogelius, R., Buckley, M., 2019. Portable FTIR for on-site screening of archaeological bone intended for ZooMS collagen fingerprint analysis. J. Archaeol. Sci. Rep. 26, 101862.

Pothier-Bouchard, G., Riel-Salvatore, J., Negrino, F., Buckley, M., 2020. Archaeozoological, taphonomic and ZooMS insights into the Protoaurignacian faunal record from Riparo Bombrini. Quatern. Int. 551, 243–263.

Reich, D., Green, R.E., Kircher, M., Krause, J., Patterson, N., Durand, E.Y., Viola, B., Briggs, A.W., Stenzel, U., Johnson, P.L.F., Maricic, T., Good, J.M., Marques-Bonet, T., Alkan, C., Fu, Q., Mallick, S., Li, H., Meyer, M., Eichler, E.E., Stoneking, M., Richards, M., Talamo, S., Shunkov, M.V., Derevianko, A.P., Hublin, J.-J., Kelso, J., Slatkin, M., Pääbo, S., 2010. Genetic history of an archaic hominin group from Denisova Cave in Siberia. Nature 468, 1053–1060.

Rendu, W., Costamagno, S., Meignen, L., Soulier, M.-C., 2012. Monospecific faunal spectra in Mousterian contexts: implications for social behavior. Quatern. Int. 247, 50–58.

Rendu, W., Renou, S., Soulier, M.-C., Rigaud, S., Roussel, M., Soressi, M., 2019. Subsistence strategy changes during the Middle to Upper Paleolithic transition reveals specific adaptations of human populations to their environment. Sci. Rep. 9, 15817.

Richter, J., 1986. Experimental study of heat induced morphological changes in fish bone collagen. J. Archaeol. Sci. 13, 477–481.

Richter, K.K., Codlin, M.C., Seabrook, M., Warinner, C., 2022. A primer for ZooMS applications in archaeology. Proc. Nat. Acad. Sci. U.S.A. 119, e2109323119.

Richter, K.K., Wilson, J., Jones, A.K.G., Buckley, M., van Doorn, N., Collins, M.J., 2011. Fish 'n chips: ZooMS peptide mass fingerprinting in a 96 well plate format to identify fish bone fragments. J. Archaeol. Sci. 38, 1502–1510.

Romandini, M., Cristiani, E., Peresani, M., 2015. A retouched bone shaft from the Late Mousterian at Fumane cave (Italy). Technological, experimental and micro-wear analysis. C.R. Palevol 14, 63–72.

Ruebens, K., Sinet-Mathiot, V., Talamo, S., Smith, G.M., Welker, F., Hublin, J.-J., McPherron, S.P., 2022. The Late Middle Palaeolithic occupation of Abri du Maras (Layer 1, Neronian, Southeast France): integrating lithic analyses, ZooMS and radiocarbon dating to reconstruct Neanderthal hunting behaviour. J. Paleolit. Archaeol. 5, 4.

Ruebens, K., Smith, G.M., Fewlass, H., Sinet-Mathiot, V., Hublin, J.-J., Welker, F., 2023. Neanderthal subsistence, taphonomy and chronology at Salzgitter-Lebenstedt (Germany): a multifaceted analysis of morphologically unidentifiable bone. J. Quatern. Sci. 38, 471–487.

Rüther, P., Husic, I., Bangsgaard, P., Gregersen, K., Pantmann, P., Carvalho, M., Godinho, R.M., Friedl, L., Cascalheira, J., Taurozzi, A., Jørkov, M., Benedetti, M., Haws, J., Bicho, N., Welker, F., Cappellini, E., Olsen, J., 2022. SPIN enables high throughput species identification of archaeological bone by proteomics. Nature Commun. 13, 2458.

Silvestrini, S., Lugli, F., Romandini, M., Real, C., Sommella, E., Salviati, E., Arrighi, S., Bortolini, E., Figus, C., Higgins, O.A., Marciani, G., Oxilia, G., Delpiano, D., Vazzana, A., Piperno, M., Crescenzi, C., Campiglia, P., Collina, C., Peresani, M., Spinapolice, E.E., Benazzi, S., 2022. Integrating ZooMS and zooarchaeology: new data from the Uluzzian levels of Uluzzo C Rock Shelter, Roccia San Sebastiano cave and Riparo del Broion. PLoS One 17, e0275614.

Sinet-Mathiot, V., Rendu, W., Steele, T.E., Spasov, R., Madelaine, S., Renou, S., Soulier, M.-C., Martisius, N.L., Aldeias, V., Endarova, E., Goldberg, P., McPherron, S.J.P., Rezek, Z., Sandgathe, D., Sirakov, N., Sirakova, S., Soressi, M., Tsanova, T., Turq, A., Hublin, J.-J., Welker, F., Smith, G.M., 2023. Identifying the unidentified fauna enhances insights into hominin subsistence strategies during the Middle to Upper Palaeolithic transition. Archaeol. Anthropol. Sci. 15, 139.

Sinet-Mathiot, V., Smith, G.M., Romandini, M., Wilcke, A., Peresani, M., Hublin, J.-J., Welker, F., 2019. Combining

ZooMS and zooarchaeology to study Late Pleistocene hominin behaviour at Fumane (Italy). Sci. Rep. 9, 12350.

Smith, G.M., Ruebens, K., Zavala, E.I., Sinet-Mathiot, V., Fewlass, H., Pederzani, S., Jaouen, K., Mylopotamitaki, D., Britton, K., Rougier, H., Stahlschmidt, M., Meyer, M., Meller, H., Orschiedt, J., Dietl., Krause, J., Schüler, T., McPherron, S., Weiss, M., Hublin, J-J., Welker, F., 2024, The ecology, subsistence and diet of ~45,000-year-old *Homo sapiens* at Ilsenhohle in Ranis, Germany. Nature Ecol. Evol. 88, 564–577.

Soulier, M.-C., Mallye, J.-B., 2012. Hominid subsistence strategies in the south-west of France: a new look at the early Upper Palaeolithic faunal material from Roc-de-Combe (Lot, France). Quatern. Int. 252, 99–108.

Sponheimer, M., Ryder, C.M., Fewlass, H., Smith, E.K., Pestle, W.J., Talamo, S., 2019. Saving old bones: a non-destructive method for bone collagen prescreening. Sci. Rep. 9, 13928.

Talamo, S., Nowaczewska, W., Picin, A., Vazzana, A., Binkowski, M., Bosch, M.D., Cercatillo, S., Diakowski, M., Fewlass, H., Marciszak, A., Paleček, D., Richards, M.P., Ryder, C.M., Sinet-Mathiot, V., Smith, G.M., Socha, P., Sponheimer, M., Stefaniak, K., Welker, F., Winter, H., Wiśniewski, A., Żarski, M., Benazzi, S., Nadachowski, A., Hublin, J.-J., 2021. A 41,500 year-old decorated ivory pendant from Stajnia Cave (Poland). Sci. Rep. 11, 22078.

Taniguchi, K., Miyaguchi, H., 2023. COL1A2 Barcoding: bone species identification via shotgun proteomics. J. Proteome Res. 23(1), 377–385.

van Doorn, N.L., Hollund, H., Collins, M.J., 2011. A novel and non-destructive approach for ZooMS analysis: ammonium bicarbonate buffer extraction. Archaeol. Anthropol. Sci. 3, 281–289.

van Doorn, N.L., Wilson, J., Hollund, H., Soressi, M., Collins, M.J., 2012. Site-specific deamidation of glutamine: a new marker of bone collagen deterioration. Rapid Commun. Mass Spectrom. 26, 2319–2327.

Wang, N., Xu, Y., Tang, Z., He, C., Hu, X., Cui, Y., Douka, K., 2023. Large-scale application of palaeoproteomics (Zooarchaeology by Mass Spectrometry; ZooMS) in two Palaeolithic faunal assemblages from China. Proc. R. Soc. B 290, 20231129.

Welker, F., Collins, M.J., Thomas, J.A., Wadsley, M., Brace, S., Cappellini, E., Turvey, S.T., Reguero, M., Gelfo, J.N., Kramarz, A., Burger, J., Thomas-Oates, J., Ashford, D.A., Ashton, P.D., Rowsell, K., Porter, D.M., Kessler, B., Fischer, R., Baessmann, C., Kaspar, S., Olsen, J.V., Kiley, P., Elliott, J.A., Kelstrup, C.D., Mullin, V., Hofreiter, M., Willerslev, E., Hublin, J.-J., Orlando, L., Barnes, I., MacPhee, R.D.E., 2015a. Ancient proteins resolve the evolutionary history of Darwin's South American ungulates. Nature 522, 81–84.

Welker, F., Hajdinjak, M., Talamo, S., Jaouen, K., Dannemann, M., David, F., Julien, M., Meyer, M., Kelso, J., Barnes, I., Brace, S., Kamminga, P., Fischer, R., Kessler, B.M., Stewart, J.R., Pääbo, S., Collins, M.J., Hublin, J.-J., 2016a. Palaeoproteomic evidence identifies archaic hominins associated with the Châtelperronian at the Grotte du Renne. Proc. Nat. Acad. Sci. 113, 11162–11167.

Welker, F., Soressi, M., Rendu, W., Hublin, J.-J., Collins, M., 2015b. Using ZooMS to identify fragmentary bone from the Late Middle/Early Upper Palaeolithic sequence of Les Cottés, France. J. Archaeol. Sci. 54, 279–286.

Welker, F., Smith, G.M., Hutson, J.M., Kindler, L., Garcia-Moreno, A., Villaluenga, A., Turner, E., Gaudzinski-Windheuser, S., 2017. Middle Pleistocene protein sequences from the rhinoceros genus *Stephanorhinus* and the phylogeny of extant and extinct Middle/Late Pleistocene Rhinocerotidae. PeerJ 5, e3033.

Welker, F., Soressi, M.A., Roussel, M., Riemsdijk, I. van, Hublin, J.-J., Collins, M.J., 2016b. Variations in glutamine deamidation for a Châtelperronian bone assemblage as measured by peptide mass fingerprinting of collagen. STAR: Sci. Technol. Archaeol. Res. 3, 15–27.

Wilson, J., van Doorn, N.L., Collins, M.J., 2012. Assessing the extent of bone degradation using glutamine deamidation in collagen. Anal. Chem. 84, 9041–9048.

Special Issue: Integrating ZooMS and Zooarchaeology: Methodological Challenges and Interpretive Potentials

Supplement 1: Investigating Species Composition in the Early Aurignacian of le Piage (France) Through Collagen Fingerprinting (ZooMS) of Screen-Recovered Small Bone Fragments

PAULINE RAYMOND
Chaire de Paléanthropologie, CIRB, Collège de France, Université PSL, CNRS, INSERM, 75005 Paris, FRANCE; pauline.raymond@college-de-france.fr

KAREN RUEBENS
Chaire de Paléanthropologie, CIRB, Collège de France, Université PSL, CNRS, INSERM, 75005 Paris, FRANCE; and, Department of Archaeology, University of Reading, Whiteknights Campus, Reading RG6 6AB, UNITED KINGDOM; k.j.ruebens@reading.ac.uk

FABRICE BRAY
UAR3290 CNRS, MSAP, University of Lille, 59655 Villeneuve d'Ascq, FRANCE; fabrice.bray@univ-lille.fr

JEAN-CHRISTOPHE CASTEL
Vertebrates, Museum of Natural History, Route de Malagnou 1, 1208 Geneva, SWITZERLAND; jean-christophe.castel@ville-ge.ch

EUGÈNE MORIN
Department of Anthropology, DNA Block C, Trent University, 2140 East Bank Drive, Peterborough ON K9J 7B8, CANADA; eugenemorin@trentu.ca

FONI LE BRUN-RICALENS
National Institute of Archaeological Research, INRA, 241 rue de Luxembourg, L-8077 Bertrange, Grand Duchy of LUXEMBOURG; foni.lebrun@inra.etat.lu

CHRISTIAN ROLANDO
UAR3290 CNRS, MSAP, University of Lille, 59655 Villeneuve d'Ascq, FRANCE; christian.rolando@univ-lille.fr

JEAN-GUILLAUME BORDES
UMR5199, CNRS, MC, PACEA, Universitiy of Bordeaux, 33318 Pessac, FRANCE; jean-guillaume.bordes@u-bordeaux.fr

JEAN-JACQUES HUBLIN
Chaire de Paléanthropologie, CIRB, Collège de France, Université PSL, CNRS, INSERM, 75005 Paris, FRANCE; jean-jacques.hublin@college-de-france.fr

SUPPLEMENT 1

This supplement includes: Supplementary Information Figure S1 and Table S1. A simplified database of all identifications presenting the most precise identification obtained for the samples analyzed with different protocols and an appendix of the deamidation values, as well as the mzXML files of the ZooMS spectra, can be accessed on Zenodo: 10.5281/zenodo.12180386.

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Figure S1. Boxplot of glutamine (Q) deamidation values (COL1α1 508–519, m/z 1105) in the cortical and spongious bones studied by ZooMS (Ambic extraction) in the Early Aurignacian of Le Piage. Specimens that have not been deamidated have a value of "1" whereas specimens that are deamidated have a value of "0." Cortical (n=238): light blue; Spongious (n=49): green.

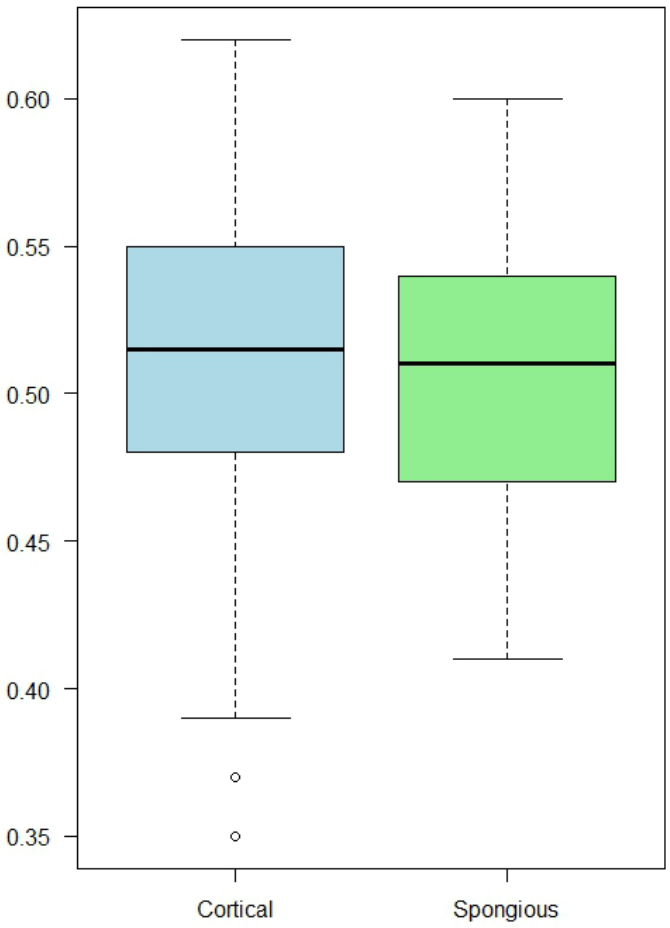

TABLE S1. RATES OF IDENTIFICATION (%) PER TAXA IDENTIFIED IN THE EARLY AURIGNACIAN LAYER AT LE PIAGE USING MORPHOLOGY AND ZooMS.

	Morin et al. 2023 Morpho identifications	**%**	**Raymond et al. ZooMS**	**%**
Reindeer	2290	*89.8*	634	*85.2*
Bos/Bison	121	*4.7*	79	*10.6*
Equidae	87	*3.4*	16	*2.2*
Cervidae	4	*0.2*	13	*1.7*
Mammoth	1	*0.0*		
Ibex	12	*0.5*		
Fox	23	*0.9*		
Canis	5	*0.2*		
Hyaen./Panth./Mustel.			1	*0.1*
Hare			1	*0.1*
Bird	6	*0.2*		
Total	2549	*100*	744	*100*