



Taxonomic identification of suids, bovids, and caprines through comparative scanning electron microscopy of prismatic structure tooth enamel from prehistoric northern Italian Alpine sites

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ABSTRACT

Taxonomic identification of fragmentary faunal assemblages remains a key challenge in zooarchaeology, particularly when diagnostic macromorphological traits are absent or ambiguous. Here we test the potential of SEM-based enamel prism microstructural morphometrics as a taxonomic proxy in archaeological contexts, focusing on materials from the Adige Valley (northern Italy). A total of 64 adult permanent teeth from La Vela and Riparo Gaban (Neolithic, with additional Bronze Age levels from Riparo Gaban) were analyzed, encompassing *Bos taurus* (n = 6), *Sus* sp. (n = 28), and medium-sized herbivores (M-S herb. sp., n = 30). Sediment pH was measured to evaluate preservational conditions and potential surface alteration; both sites showed slightly acidic values (mean pH $\approx$ 5.9–6.6). Enamel surfaces were imaged with a desktop SEM (Phenom XL), and prisms were quantified and measured within standardized acquisition fields. For each prism, Dmax, Dmin, perimeter, and area were estimated, and six derived ratios were computed; to avoid pseudoreplication, all analyses were performed at the tooth level using summary descriptors (mean, SD, median, p10–p90, CV). Results show clear interspecific separation in prism morphometrics: *Bos taurus* displays markedly larger absolute values for all primary variables, *Sus* sp. occupies intermediate ranges, and M-S herb. sp. shows the lowest absolute values, with generally moderate within-taxon variability. One and Two-way ANOVA indicates an extremely strong Species effect across all variables and ratios, whereas ToothType and Species $\times$ ToothType interactions are not significant in the present dataset. Ratio variables—especially Dmax/Dmin and Perim/Area—provide particularly strong discrimination. A targeted ZooMS test (one specimen with associated bone) supported refinement of macroscopic identification. Overall, these findings support the feasibility of enamel prismatic structure morphometrics as a minimally invasive tool for taxonomic discrimination in archaeological teeth, while highlighting the need for expanded and more balanced sampling to further test within-taxon dental variation and assess performance across different soil chemistries.

1. Introduction

The study of non-human osteological material primarily aims to elucidate the relationships between humans and animals, as well as the impacts these interactions have on both societies and their surrounding environments (Sullivan et al., 2017; Oma and Goldhahn, 2020). Animal remains often reflect a complex interplay of human and animal behaviors, influenced by available environmental resources, cultural attitudes toward those resources, and the technological means employed to utilize

them (Boyd, 2017; Green, 2024). Examining how these dynamics change over time represents one of the central research objectives in this field (i.e. Brewer, 1992; Reitz and Wing, 1999; Driver, 2011).

A wide range of methods—both destructive and non-destructive—are available for taxonomic identification (hereafter TI), applicable to both osseous and dental hard tissues, as discussed in the literature cited below. This section does not aim to be exhaustive; rather, it provides an overview of the main approaches currently employed for TI.

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1.1. Methods for taxonomic identification

In terms of ease of application and instrumental requirements, macroscopic morphology of bones and teeth remains one of the cornerstone approaches for TI (Isaakidou, 2020). Its main strengths lie in its non-destructive nature, low cost—often limited to the operator's working time—and high diagnostic reliability, especially when applied to well-preserved material or diagnostically distinctive skeletal elements (Nikita, 2017). However, its limitations include a strong dependence on the observer's expertise: even under double-blind conditions, identification accuracy may vary significantly (Gobalet, 2001). Moreover, the availability and reliability of diagnostic traits decrease markedly with increasing fragmentation (Ubelaker and Wu, 2020). A comparative reference collection is also required, entailing substantial logistical constraints, and diagnoses may be influenced by intrinsic morphological variability, which is not always easily standardized for archaeological assemblages (i.e. LeFebvre and Sharpe, 2017).

Microstructural and metric analysis of bone tissue in thin section, originally developed in paleozoological research, represents a valuable tool for TI (i.e. Mao et al., 2015). However, in the case of archaeological mammalian remains, several challenges emerge (Dominguez, 2025). For example, this technique allows for the recognition of plexiform bone structure, which is useful in distinguishing human from non-human bones (Porto et al., 2022; Larentis, 2023). It should be noted that plexiform bone can also occur in growing human bone during the first years of life, making it necessary to also consider cortical thickness (Caccia et al., 2016). Metric evaluation of the Haversian system preferably requires transverse sections of long bones, such as the femur—an often difficult condition to meet in fragmented assemblages composed of multiple taxa (Crescimanno and Stout, 2011; Brits et al., 2014). Variables typically assessed include the maximum and minimum diameters of osteons and Haversian canals, osteonal perimeter length, as well as the area of lamellar bone tissue and Haversian foramen (Martiniaková et al., 2006; Cummaudo et al., 2019). Some parameters, such as osteon and canal diameters, have proven more reliable than others (Lagace et al., 2020). Nevertheless, even when selecting the most informative variables, extensive interspecific overlap in dimensional ranges often hampers unambiguous taxonomic attribution (Hillier and Bell, 2007; Martiniaková et al., 2007; Greenlee and Dunnell, 2009). Moreover, this technique is destructive and requires specialized equipment and expertise for preparing undecalcified thin sections of hard tissue.

To address some of these challenges, approaches from digital zooarchaeology have been proposed, particularly the use of micro-CT and synchrotron light, which allow for the preservation of sample integrity (Zougrou et al., 2013; Gueriau et al., 2016; Imai et al., 2023). However, these methodologies require costly and not always accessible equipment, and they remain subject to the same interpretative challenges as thin-section microscopic analyses. An additional strategy to mitigate instrumentation-related difficulties involves analyzing Haversian system morphometric parameters under reflected-light optical microscopy directly on fracture surfaces already present on diaphyses, thereby avoiding the preparation of new thin sections. This approach provides identifications with acceptable reliability, at least for the main taxa commonly recovered in post-Neolithic European archaeological contexts (O'Brien, 2019). The effectiveness of these analyses can be significantly reduced when bone structure is altered, for instance, by burning processes (which cause shrinkage and structural modifications) or by taphonomic factors such as soil chemistry interactions or fungal activity, both of which may compromise the legibility of the sample (Thompson, 2005; Huisman et al., 2017).

In recent years, TI has benefited significantly from the development of biomolecular diagnostic techniques based on mass spectrometry. Among these, the best known is Zooarchaeology by Mass Spectrometry (ZooMS), which can be applied to biological tissues containing peptides within collagen type I proteins (Brown et al., 2020; Richter et al., 2022).

ZooMS is a relatively rapid and cost-effective method, producing results that can be compared against extensive reference libraries and generally offering a high degree of reliability. However, it is typically micro-destructive, relies exclusively on collagen analysis, and in some cases does not allow for the distinction between closely related species, such as *Capra hircus*/*Capra ibex* or *Ovis aries*/*Rupicapra rupicapra* (Buckley et al., 2009a, 2009b)—a critical differentiation, for instance, when estimating the ratio between domestic and wild species.

To overcome this limitation, Species by Proteome Investigation (SPIN) can be employed. While more costly and sensitive to contamination, SPIN provides taxonomic “fingerprints” with a higher level of reliability than ZooMS (Rüther et al., 2022). Liquid chromatography–mass spectrometry (LC-MS) is another analytical technique related to ZooMS and SPIN, but capable of detecting a broader range of molecules, including in biological structures lacking collagen (Elnaggar et al., 2022). Although LC-MS is also micro-destructive and requires advanced, often inaccessible instrumentation and the support of a specialized laboratory, it is more versatile and can, among other applications, determine the biological sex of individuals (Regal et al., 2019; Koenigswald and Clemens, 1992).

The extraction and analysis of ancient DNA also represent valuable procedures for the TI of teeth and bones (Newman et al., 2002; Corradini et al., 2024). In particular, bulk bone metabarcoding (BBM) has demonstrated excellent results, thanks to its repeatability—achieved by quantifying both sampling and technical variability through a nested experimental design with subsamples and multi-phase replicates—its accuracy—validated by comparing morphological and molecular identifications at the family level—and its overall utility, proven by its application to two distinct Holocene fossil deposits (Grealy et al., 2015). BBM has proven to be consistent, accurate, and highly sensitive, making it applicable in many contexts. Nonetheless, it remains a destructive and costly technique, dependent on collagen preservation and requiring skilled personnel and well-equipped laboratories. As noted, the feasibility of these analyses ultimately depends on the presence and quality of aDNA preserved within biological tissues, a factor highly vulnerable to denaturation and degradation due to multiple environmental and diagenetic processes.

1.2. Teeth microstructure

Enamel, is an anisotropic material and the hardest tissue in the mammalian and some non-mammalian vertebrate bodies, forming the outermost layer of the teeth (Hillson, 2005; Ungar, 2010; Renteria et al., 2021; Berkovitz and Shellis, 2023). It is composed of approximately 95% inorganic components—primarily hydroxyapatite and its substituted forms—3.8% bound and unbound water, and 1.2% organic compounds (Borovski and Leontiev, 1991). Unlike bone tissue, enamel is acellular and therefore lacks regenerative capacity once damaged; however, ion exchange with dentin and saliva may promote localized remineralization and demineralization processes (Bergman, 1963; Zheng et al., 2019; Enax et al., 2024).

Structurally, enamel of mammals consists of prisms that mineralize according to the orientation imposed by the organic matrix during the mineralization process, forming both prismatic and interprismatic levels within the hierarchical architecture of enamel (Ippolitov et al., 2014). When examined under scanning electron microscopy, the enamel surface displays a microrelief characterized by a distinct perimeter and a central zone (Fig. 1 a). These features are most visible when observed transversely in certain areas of the tooth, but less so when viewed along longitudinal sections (Wang et al., 2023; Marsico et al., 2024). In the central portion of the tooth, prisms decussate into groups known as Hunter-Schreger Bands, where alternating prism and interprism layers create a distinctive pattern. By contrast, the incisal and cervical regions are characterized by the presence of radial enamel (Koenigswald and Clemens, 1992; Mao et al., 2015). In this type of enamel organization, the prisms run parallel to each other, thereby allowing for a clearer

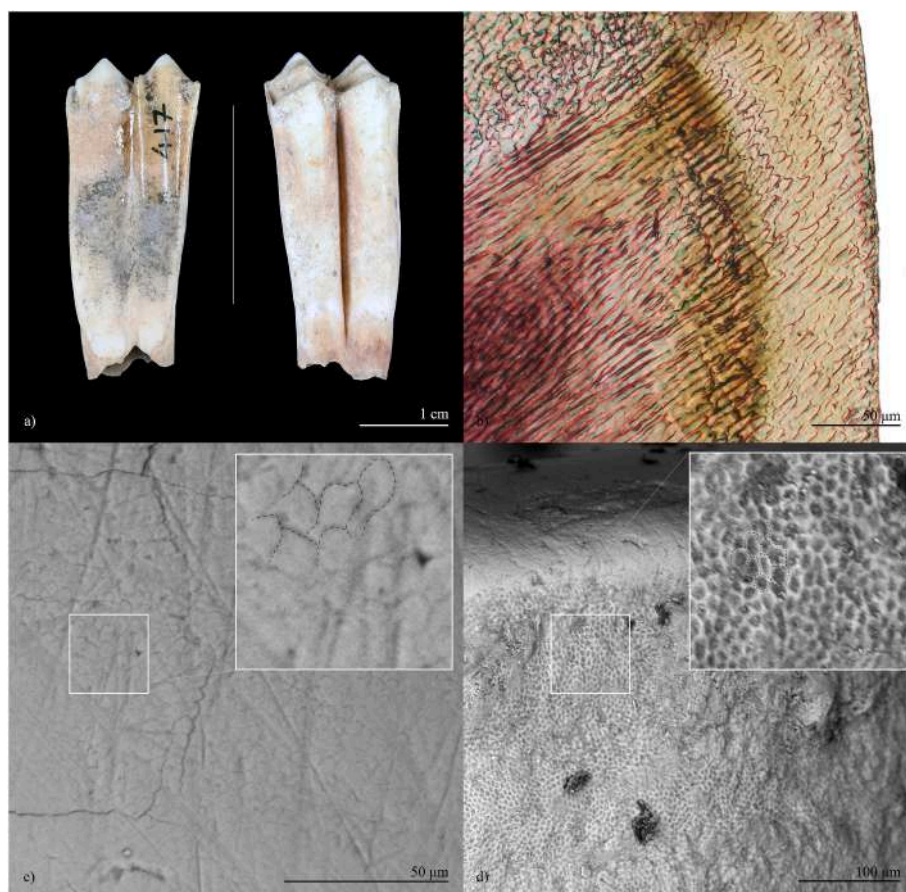


Fig. 1. (a) ID 34 lingual and buccal views; (b) linguo-buccal thin section of a mammalian molar, in which the parallel prismatic structure is visible in the cervical area; (c) SEM image of the HSB surface in one central portion of ID 6; (d) SEM image of the cervical area of the tooth, where the prisms are oriented transversely to the enamel surface near the occlusal plane in ID 7. The dashed lines within the magnifications in (c) and (d) trace the perimeters of the prisms.

visualization of their morphology (Fig. 1b–d) (Wang et al., 2023).

In particular, the shape of enamel prisms and their metric characteristics, when exposed transversely to the enamel surface, can be classified using several measurements: perimeter, corresponding to the total length of the prism sheath; internal area within the perimeter; maximum diameter; and minimum diameter. In addition, prism density per mm² and relative orientation can also be assessed (Fig. 2).

The analysis of these variables is of major importance in archaeological contexts, as the prism–interprism spatial structure has been shown to be a valuable feature for TI (Wang et al., 2021).

1.3. Archaeological contexts

The analyzed materials derive from the Neolithic levels of two major prehistoric archaeological sites located in the Adige Valley (Trentino-Alto Adige), in the central-eastern Alpine region: Riparo Gaban and La Vela di Trento (Fig. 3a and b). At Riparo Gaban, Bronze Age levels were also included in the analysis.

1.3.1. Riparo Gaban

Riparo Gaban (46°05'36"N – 11°07'21"E) is a rock shelter situated on the right bank of the Adige River, at approximately 270 m above sea level. The first scientific excavations were conducted between 1971 and 1979 under the direction of Bernardino Bagolini. Between 1982 and 1984, further investigations were carried out by Bagolini, Alberto Broglio and Stephan Kozłowski, together with Gianpaolo Dalmeri and Michele Lanzinger (Bagolini, 1972; Bagolini et al., 1973; Kozłowski and Dalmeri, 2000). More recent excavations were conducted by the University of Trento under the scientific direction of Annaluisa Pedrotti

(Pedrotti, 1998, 2009; Gibaja et al., 2025). The significance of the site lies in its stratigraphic sequence, one of the few in northern Italy documenting one of the most complete sequence of human occupation from the Mesolithic to the Bronze Age. Moreover, the distinctive characteristics of the material culture have led to the identification of a specific Neolithic facies named after the site, the so-called “Gaban Culture” (Bagolini and Biagi, 1977).

1.3.2. La Vela

The second site from which the analyzed materials derive is La Vela near the present-day city of Trento (46°04'50"N 11°06'08"E), sector VII, investigated during the 1987–1988 excavation campaigns by Bernardino Bagolini and Enrico Cavada. This archaeological area, comprising both a settlement and a necropolis, was first identified in the 1960s during urban development works (Bagolini, 1990; Barfield, 1970; Pedrotti, 1990). Subsequent systematic investigations provided a more detailed understanding of the site, leading to its subdivision into nine excavation sectors (Pedrotti, 2001; Degasperis et al., 2011; Mottes, 2007; Santaniello et al., 2016; Mottes, 2022).

2. Materials and methods

A total of 18 permanent teeth from adult specimens taxonomically identified through macroscopic analysis as belonging to bovines, suids, and medium-sized herbivores, such as ovicaprids were analyzed from the Neolithic levels of the Vela site (1987–1988 excavation campaigns), along with 24 teeth from the Neolithic levels of Riparo Gaban (1982–1984 campaigns) and 22 teeth from the Bronze Age levels of the same site (2023 campaign) (Fig. 4; Table 1).

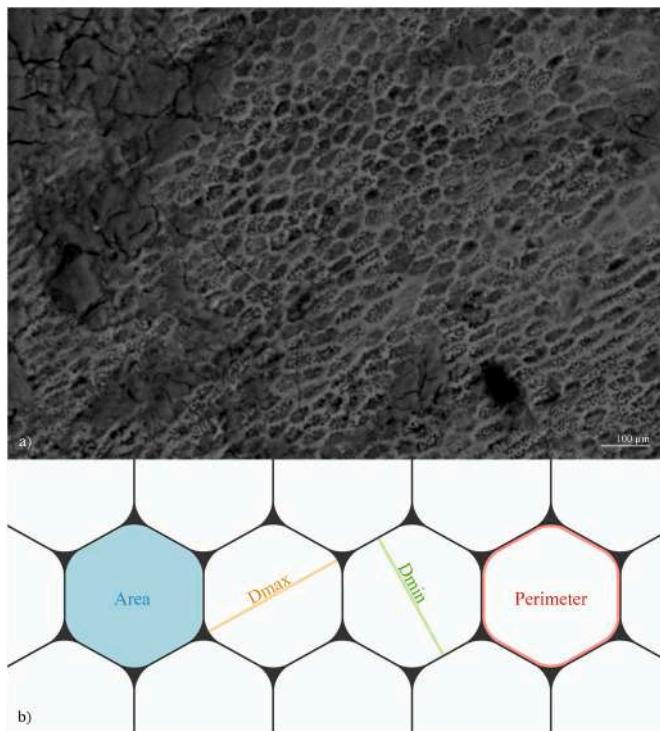


Fig. 2. (a) Representative detail of the prism–interprism spatial structure observed in the analyzed *Bos taurus* enamel samples of ID14. (b) Schematic representation of the morphometric parameters measured in this study, including maximum diameter (Dmax), minimum diameter (Dmin), perimeter, and area.

The selection of samples originating from the same geographic area but dating to different periods was intended to reduce biological

variability related to environmental factors, thereby allowing potential differences to be attributed primarily to temporal variation. Different archaeological contexts from different periods could potentially introduce preservation-related bias. To assess this issue and the possible impact of soil chemistry on dental enamel, the remains were examined macroscopically, and pH analyses were performed on sediment samples collected from the same contexts as the materials. Measurements were carried out using a portable soil pH meter (MW101-SOIL, Milwaukee, precision ± 0.1 pH). The teeth were cleaned of adhering sediment using demineralized water and a soft-bristled brush, then catalogued in Excel spreadsheets and photographically documented.

Prior to analysis, the samples were cleaned in an ultrasonic bath filled with demineralized water. The use of tools that could exert mechanical force was strictly avoided to prevent any potential alteration to the enamel surface morphology. The teeth were then placed to dry in a laboratory oven at 20°C for two days to ensure complete drying under stable conditions.

Macroscopic TI of permanent teeth was carried out through comparison with a reference collection of non-human bones of known species, both modern and archaeological, housed at the Archaeozoology Laboratory of the MUSE – Museo delle Scienze di Trento, as well as through consultation of non-human osteological atlases (e.g., Schmid, 1972; Barone, 2003). Given the difficulty in distinguishing *Ovis aries*, *Rupicapra rupicapra*, *Capra hircus* and *Capra ibex* on macroscopic grounds the teeth attributed to these taxa were grouped, and for the purposes of this study, this category was treated as representing medium-sized herbivores (medium-sized herbivores, M-S herb. sp.). To avoid issues related to the differentiation between domestic and wild forms within the same genus, suids were classified as *Sus* sp.

To strengthen the taxonomic attribution of specimens with ambiguous macroscopic traits, a proteomic approach was integrated into the study protocol. Among the analyzed sample, one specimen belonging to the M-S herb. sp. group (ID 40-41; see Fig. 4 and Table 1) retained sufficient associated mandibular bone tissue to allow for ZooMS analysis. This additional step was performed to test the consistency between



Fig. 3. (a) Location of Trento in northern Italy. (b) Geographic setting of the La Vela di Trento and Riparo Gaban archaeological sites within the Adige Valley.



Fig. 4. Table of the teeth analyzed in this study. The sample includes specimens from the Neolithic levels of the Vela site (IDD 1–18) and Riparo Gaban (IDD 19–42), as well as from the Bronze Age levels of the latter site (IDD 43–64).

macroscopic identification and molecular data, following established protocols for fragmentary remains (Buckley et al., 2009a, 2009b; Richter et al., 2022). The ZooMS results were utilized to refine the taxonomic profile and to exclude alternative taxa, thereby providing a more robust framework for the subsequent morphometric evaluation. Collagen extraction followed an acid-insoluble protocol (Buckley et al., 2009a, 2009b; Welker et al., 2015). Approximately 20 mg of bone was demineralized in 0.6 M HCl for three days, rinsed with ammonium bicarbonate, gelatinized at 65°C for 1 h, and digested with trypsin at 37°C for ~18 h. Peptides were purified using C18 ZipTips and analyzed in triplicate by MALDI-TOF-MS (Autoflex, Bruker Daltonics) with α -cyano-4-hydroxycinnamic acid matrix. TI was obtained by comparison with published reference spectra (Buckley et al., 2009a, 2010, 2017; Buckley and Collins, 2011; Kirby et al., 2013; Welker et al., 2016; Jensen et al., 2020).

Dental enamel was analyzed using a desktop scanning electron

microscope equipped with an EDS detector (Phenom XL). Our analysis aimed to investigate the morphometric characteristics and spatial distribution of enamel prisms in their cross-section, as exposed on the outer surface of the tooth. Consequently, our protocol does not require resin embedding or the subsequent steps typically involved in preparing thin sections or sectioned surfaces for microscopic evaluation like sectioning and etching. Analysis was conducted under low vacuum conditions to bypass the need for conductive coating, thereby ensuring minimal interference with the specimens. The untreated samples were mounted directly onto SEM pin stubs using conductive adhesive tabs for immediate evaluation. Two SEM images of the enamel surface were acquired for each tooth, selecting areas with optimal readability located on the buccal portion of the crown and in the cervical region (Fig. 1 d). From each acquisition, an area of 2500 μm^2 was selected in which the prism–interprism spatial structure was particularly well preserved and clearly visible (Fig. 2 a). The shape of the acquisition field does not

Table 1

List of analyzed teeth, including stratigraphic information and archaeological chronology, based on a series of calibrated radiocarbon dates (Croze et al., 2025; Mottes, 2022).

ID	Site	Excavation year	SU	Sector	Square	RR	ID DB	Chronology
1	La Vela	1987–1988	4	VII	7g III	-	-	VBQ 2 inhabited
2	La Vela	1987–1988	24	VII	23g II	-	-	VBQ 2 inhabited
3	La Vela	1987–1988	24	VII	-	-	-	VBQ 2 inhabited
4	La Vela	1987–1988	27	VII	13	-	-	Final Early Neolithic (Gaban Group)
5	La Vela	1987–1988	27	VII	16g I	-	-	Final Early Neolithic (Gaban Group)
6	La Vela	1987–1988	27	VII	16g III	-	-	Final Early Neolithic (Gaban Group)
7	La Vela	1987–1988	28	VII	8m III	-	-	Final Early Neolithic (Gaban Group)
8	La Vela	1987–1988	28	VII	8t I	-	-	Final Early Neolithic (Gaban Group)
9	La Vela	1987–1988	28	VII	-	-	-	Final Early Neolithic (Gaban Group)
10	La Vela	1987–1988	28	VII	-	-	-	Final Early Neolithic (Gaban Group)
11	La Vela	1987–1988	28	VII	-	-	-	Final Early Neolithic (Gaban Group)
12	La Vela	1987–1988	28	VII	-	-	-	Final Early Neolithic (Gaban Group)
13	La Vela	1987–1988	28	VII	-	-	-	Final Early Neolithic (Gaban Group)
14	La Vela	1987–1988	71	VII	17r IV	-	-	Final Early Neolithic (Gaban Group)
15	La Vela	1987–1988	166	VII	-	-	-	Final Early Neolithic (Gaban Group)
16	La Vela	1987–1988	166	VII	-	-	-	Final Early Neolithic (Gaban Group)
17	La Vela	1987–1988	166	VII	-	-	-	Final Early Neolithic (Gaban Group)
18	La Vela	1987–1988	196	VII	16w III	-	-	VBQ 1
19	Riparo Gaban	1982–1984	D1	IV	α1	-	548	Final Early Neolithic (Gaban Group)
20	Riparo Gaban	1982–1984	D3	IV	α1-2-3	-	563	Final Early Neolithic (Gaban Group)
21	Riparo Gaban	1982–1984	D7	IV	β2	-	538	Final Early Neolithic (Gaban Group)
22	Riparo Gaban	1982–1984	D7	IV	β2	-	541	Final Early Neolithic (Gaban Group)
23	Riparo Gaban	1982–1984	D8	IV	α2-3-4	-	498	Final Early Neolithic (Gaban Group)
24	Riparo Gaban	1982–1984	D9	IV	β1	-	4	Final Early Neolithic (Gaban Group)
25	Riparo Gaban	1982–1984	23	V	δ	-	377	Final Early Neolithic (Gaban Group)
26	Riparo Gaban	1982–1984	23	V	δ	-	382	Final Early Neolithic (Gaban Group)
27	Riparo Gaban	1982–1984	23	V	δ	-	391	Final Early Neolithic (Gaban Group)
28	Riparo Gaban	1982–1984	23	V	δ1	-	398	Final Early Neolithic (Gaban Group)
29	Riparo Gaban	1982–1984	23	V	δ1	-	403	Final Early Neolithic (Gaban Group)
30	Riparo Gaban	1982–1984	24	V	δ	-	17	Final Early Neolithic (Gaban Group)
31	Riparo Gaban	1982–1984	24	V	δ	-	19 bis	Final Early Neolithic (Gaban Group)
32	Riparo Gaban	1982–1984	24	V	δ	-	20	Final Early Neolithic (Gaban Group)
33	Riparo Gaban	1982–1984	24	V	δ	-	22	Final Early Neolithic (Gaban Group)
34	Riparo Gaban	1982–1984	24	V	δ1	-	417	Final Early Neolithic (Gaban Group)
35	Riparo Gaban	1982–1984	24	V	δ1	-	420	Final Early Neolithic (Gaban Group)
36	Riparo Gaban	1982–1984	24	V	δ1	-	459	Final Early Neolithic (Gaban Group)
37	Riparo Gaban	1982–1984	26	V	β1	-	502	Final Early Neolithic (Gaban Group)
38	Riparo Gaban	1982–1984	26	V	γ1	-	204	Final Early Neolithic (Gaban Group)
39	Riparo Gaban	1982–1984	26	V	δ1	-	350	Final Early Neolithic (Gaban Group)
40	Riparo Gaban	1982–1984	27	V	δ	-	460 a	Final Early Neolithic (Gaban Group)
41	Riparo Gaban	1982–1984	27	V	δ	-	460 b	Final Early Neolithic (Gaban Group)
42	Riparo Gaban	1982–1984	27	V	δ	-	465	Final Early Neolithic (Gaban Group)
43	Riparo Gaban	2023	109	VI	48E 304N	-	-	Bronze Age
44	Riparo Gaban	2023	109	VI	48E 304N	-	-	Bronze Age
45	Riparo Gaban	2023	109	VI	48E 304N	-	-	Bronze Age
46	Riparo Gaban	2023	109	VI	48E 304N	-	-	Bronze Age
47	Riparo Gaban	2023	109	VI	51E 302N	-	-	Bronze Age
48	Riparo Gaban	2023	109	VI	51E 302N	-	-	Bronze Age
49	Riparo Gaban	2023	130	VI	52E 301N	1393	663	Bronze Age
50	Riparo Gaban	2023	130	VI	52E 301N	1393	663	Bronze Age
51	Riparo Gaban	2023	130	VI	52E 301N	1393	663	Bronze Age
52	Riparo Gaban	2023	130	VI	52E 301N	1393	663	Bronze Age
53	Riparo Gaban	2023	130	VI	52E 301N	1393	663	Bronze Age
54	Riparo Gaban	2023	130	VI	52E 301N	1407	668	Bronze Age
55	Riparo Gaban	2023	130	VI	52E 301N	-	160	Bronze Age
56	Riparo Gaban	2023	130	VI	52E 303N	-	235	Bronze Age
57	Riparo Gaban	2023	130 base	VI	52E 300N	-	58	Bronze Age
58	Riparo Gaban	2023	130 base	VI	52E 300N	-	60	Bronze Age
59	Riparo Gaban	2023	130 base	VI	52E 302N	-	45	Bronze Age
60	Riparo Gaban	2023	135	VI	51E 301N	1421	669	Bronze Age
61	Riparo Gaban	2023	135	VI	51E 302N	-	605	Bronze Age
62	Riparo Gaban	2023	135	VI	51E 302N	-	611	Bronze Age
63	Riparo Gaban	2023	135	VI	51E 302N	-	620	Bronze Age
64	Riparo Gaban	2023	135	VI	52E 302N	-	396	Bronze Age

necessarily have to be square; it may be polygonal, if it encompasses the areas with the best preservation and the clearest visibility of the prismatic structure. For each image, data were collected on the spatial distribution and relative orientation of prism geometries, polygon density per unit area, and four morphometric datasets were analyzed using the ImageJ software: maximum enamel prism diameter (Dmax), minimum diameter (Dmin), prism perimeter (Perim) and prism area (Area)

(Fig. 2b), each provided as a separate spreadsheet tab. Each row corresponds to a tooth sample (unique ID, column A). For each tooth, archaeological data (column B, C), macroscopic TI (MTI column D), tooth identification (column E) and numbers of prisms evaluated are provided (column F). Prism-level measurements are stored in columns labelled numerically (e.g., 1 ... n, column from G), representing individual enamel prism measured within the tooth (Table 2).

Six ratios were initially computed at the prism level: Dmax/Dmin, Dmax/Perim, Dmax/Area, Dmin/Perim, Dmin/Area, Perim/Area. These ratios were used to capture size-independent and shape-related variation. Because multiple prisms are measured within the same tooth, prisms are not independent biological replicates. Therefore, the tooth (ID) was treated as the independent analytical unit. To ensure that all morphometric variables contributed equally to the multivariate analysis, regardless of their original units of measurement or absolute magnitude (e.g., Area vs. Dmax), a Z-score normalization was performed prior to the PCA. Each variable was rescaled to have a mean of 0 and a standard deviation of 1 using the following formula:

$$z = (x - \mu) / \sigma$$

where x is the raw value, μ is the mean of the variable, and σ is its standard deviation. This procedure prevents variables with larger absolute values from disproportionately influencing the calculation of the principal components.

For each tooth and for each variable/ratio, robust summary descriptors were computed across prisms: mean, standard deviation, median; 10th percentile (p10), 90th percentile (p90), coefficient of variation (CV). Ratios were computed at the tooth level. For each tooth, Dmax/Dmin and Perimeter/Area were calculated; for medium-sized herbivores, Dmax/Dmin values were inverted (1/ratio) prior to species-level summarization. Species means and standard deviations were then computed from tooth-level ratios. To test whether prism morphometrics differ among *taxa* and tooth types, one and two-way ANOVA models were fitted at the tooth level:

$$Y \sim \text{Species} \times \text{ToothType}$$

where Y is the tooth-level mean for each variable/ratio, Species is the harmonised *taxon*, and ToothType is the categorical tooth class (molar/premolar/canine/incisor). P-values were extracted for Species, ToothType and their interaction.

To better characterize the discriminative power of the morphometric variables, LDA was performed. Original variables were projected as vectors onto the canonical axes to identify which parameters contributed most to group separation. Furthermore, to investigate potential internal heterogeneity within the 'M-S herb. sp.' group, a dedicated PCA was conducted exclusively on this subset. This secondary analysis aimed to identify cryptic clusters or trends related to site, chronology, or tooth type (molars vs. premolars) that might be obscured in the general model.

To account for the potential dependence of variance on the mean, the Coefficient of Variation (C.V.%) was calculated for all metric indices. Homogeneity of variances was assessed using Levene's test. Since the assumption of homoscedasticity was violated ($p < 0.05$), we applied a log-transformation to the data before performing ANOVA.

Osteological investigations were conducted in accordance with the guidelines of the Italian Central Institute for Archaeology (Istituto Centrale per l'Archeologia, ICA) and the Italian Central Institute for Catalogues and Documentation (Istituto Centrale per il Catalogo e la Documentazione, ICCD) (MiC, 2022). All analyses were formally authorized by the Italian Ministry of Culture (Ministero della Cultura, MiC).

Table 2

Example of the data matrix used for morphometric data collection in this study. A separate table was created for each morphometric dataset (Dmax, Dmin, Perimeter, and Area). Each row corresponds to an individual tooth (unique ID), while columns report archaeological metadata, taxonomic attribution, tooth type, number of measured prisms, and prism-level measurements.

A	B	C	D	E	F	G	H	... n
ID	Site	Sector	MTI	Tooth	Prism n	1	2	3
-	-	-	-	-	-	-	-	-

3. Results

This section presents the data acquired during the study. The presentation of the findings follows the same sequence outlined in the Methods section, which in turn mirrors the established analytical protocol.

3.1. Soil pH

Soil measurements allowed the estimation of average pH values, which were slightly acidic (Table 3).

3.2. Macroscopic TI

Macroscopic TI of the teeth allowed for the estimation of the number of remains based on the category originating from each site and the chronological periods investigated. The dataset comprises 64 teeth distributed as: M-S herb. sp. (30), *Sus* sp. (28), *Bos taurus* (6) (Table 4 and in detail Supplementary material 1).

Supplementary Material 1. Macroscopic TI of the analyzed dental remains, showing the distribution of taxa by archaeological site, chronological period, and tooth class.

3.3. ZooMS

ZooMS analysis was performed on sample IDD 40-41 (Laboratory Sample ID 47, MALDI plate spots L1, N1, O1). This analysis assigned the sample to either *Ovis aries* or *Rupicapra rupicapra* in two instances (L1, O1), and to *Ovis aries*, *Rupicapra rupicapra*, or *Cervus elaphus* in the remaining instance (Supplementary Material 2). Subsequent macroscopic re-examination, however, allowed for the conclusive TI of the remains as *Ovis aries*.

Supplementary Material 2. ZooMS results for sample IDD 40-41. MALDI-TOF-MS spectra obtained from three independent spots (L1, N1, O1) are reported, with diagnostic collagen peptides and corresponding taxonomic attributions based on reference libraries.

3.4. Enamel microstructure morphometrics

The spatial distribution and relative orientation of prism geometries were analyzed for each recognized taxonomic category (Fig. 5). As detailed in the Methods section, morphometric evaluations were standardized by analyzing a consistent surface area of 2500 μm^2 for each designated tooth region (buccal crown and cervical area). To account for localized preservation differences across specimens, these standardized areas were extracted using customized square or polygonal sampling fields targeted exclusively at zones with optimal prism-interprism visibility. In this way, unreadable surface areas caused by small matrix debris or adhering soil are systematically excluded from the analysis.

In *Bos taurus*, enamel prisms exhibit a predominantly sub-hexagonal cross-sectional shape, whereas in *Sus* sp. prisms are generally more rectangular. Prism orientation, assessed using the direction of the maximum diameter as the principal axis, reveals bundles of prisms that are sub-parallel to one another, but heterogeneously oriented with respect to the tooth. In M-S herb. sp., prisms display a sub-polygonal morphology approaching a circular outline. Although a weak tendency toward parallel arrangement is observed, their spatial distribution and

Table 3

Results of the measurements performed on the soil originating from the analyzed archaeological sites.

Site	pH (Mean $\pm$ SD)
La Vela (1987–1988)	6,6 $\pm$ 0,3
Riparo Gaban (1982–1984)	5,9 $\pm$ 0,3
Riparo Gaban (2023–2025)	6,0 $\pm$ 0,3

Table 4

Summary of the analyzed dental remains. The assemblage is categorized by site, archaeological chronology, and taxonomic identification. Counts (*n*) refer to the individual teeth selected for SEM analysis from the prehistoric sites of La Vela and Riparo Gaban.

Site	Chronology	<i>Bos taurus</i>	<i>Sus</i> sp.	M-S herb. sp.	Total
La Vela	Early Neolithic/VBQ	2	16	9	27
Riparo Gaban	Early Neolithic	1	2	21	24
	Bronze Age	3	10	0	13
Total		6	28	30	64

orientation are less clearly organized and more difficult to classify.

The count of enamel prisms per acquisition allowed the calculation of mean values according to taxonomic group and tooth type (Table 5).

The morphometric analysis of enamel prisms allowed the measurement of maximum diameter (Dmax), minimum diameter (Dmin), perimeter, and area for each identified prism (Supplementary Materials 3–6).

Supplementary Material 3. Tooth-level summary statistics of maximum enamel prism diameter (Dmax), including mean, dispersion, and percentile-based descriptors for each analyzed specimen.

Supplementary Material 4. Tooth-level summary statistics enamel prism diameter (Dmin), reporting central tendency and variability measures for each sample.

Supplementary Material 5. Descriptive statistics of enamel prism

perimeter measured at the tooth level for all analyzed specimens.

Supplementary Material 6. Tooth-level descriptive statistics of enamel prism cross-sectional area, including measures of central tendency and variability.

Tooth-level summary statistics indicate systematic differences among *taxa* across all primary morphometric variables. *Bos taurus* generally exhibits larger prism dimensions, *Sus* sp. shows intermediate values, and medium-sized herbivores display smaller dimensions overall. Within-taxon variability is moderate and comparable across groups (Supplementary Material 7).

Supplementary Material 7. Tooth-level descriptive statistics of enamel prism morphometrics ordered by specimen ID. For each tooth, summary statistics are reported for Dmax, Dmin, perimeter (Perim), area (Area), and derived ratios. Values include mean, standard deviation (SD), median, 10th (p10) and 90th (p90) percentiles, and coefficient of variation (CV). Taxonomic attribution, tooth type, site, and archaeological phase are provided.

To statistically evaluate the significance of these morphological differences, we performed both one-way and two-way ANOVA models. The results demonstrate an extremely strong and highly significant species-level effect ($p < 0.001$) across all primary morphometric variables (Dmax, Dmin, perimeter, and area) and all derived ratios. Conversely, the effect of tooth type (e.g., molars vs. premolars) and the interaction between species and tooth type were found to be non-significant. This statistical output confirms that enamel prism morphometrics are strictly driven by taxonomic affiliation rather than dental positioning, validating the robustness of these parameters as diagnostic taxonomic proxies. Full p-values and model R^2 are provided in Tables 6

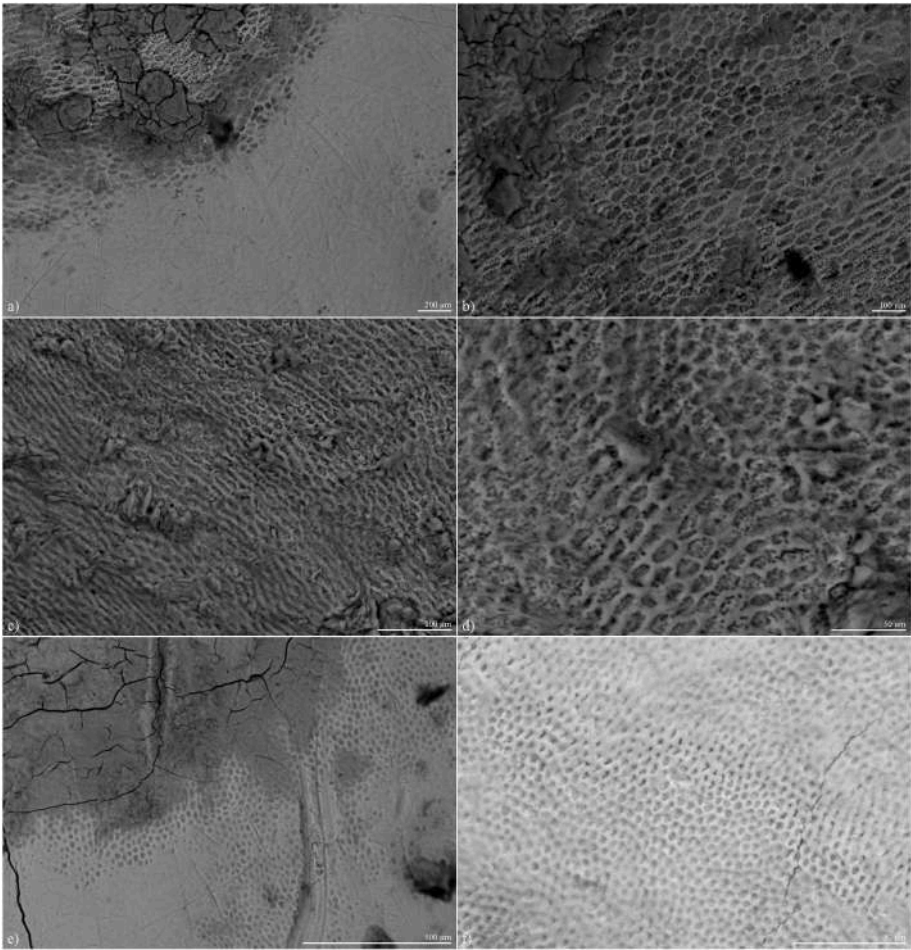


Fig. 5. SEM acquisitions of dental surface microtopography. The selection of these images was based on the particularly good preservation and high readability of the surfaces analyzed. (a) *Bos taurus* ID 13 (b) *Bos taurus* ID 14. (c) *Sus* sp. ID 17. (d) *Sus* sp. ID 44. (e) M-S herb. sp. ID 55. (f) M-S herb. sp. ID 64.

Table 5

Descriptive statistics of the number of enamel prisms measured per tooth, summarized by taxonomic group and tooth type. n indicates number of teeth; values are averages across acquisitions; mean, standard deviation (SD), minimum (Min), and maximum (Max) values refer to the number of prisms quantified per tooth.

Species	Tooth	n (acquisitions)	Mean	SD	Min	Max
<i>Bos taurus</i>	Molar	8	63,9	5,5	55	71
<i>Bos taurus</i>	Premolar	4	60,2	6,8	52	68
M-S herb.	Molar	38	122,6	2,2	116	125
M-S herb.	Premolar	22	121,9	2,5	117	125
<i>Sus</i> sp.	Canine	10	84,4	7,9	72	98
<i>Sus</i> sp.	Incisor	14	88,6	8,6	76	103
<i>Sus</i> sp.	Molar	20	86,2	8,4	74	101
<i>Sus</i> sp.	Premolar	12	85,4	11	71	102

and 7.

Ratio-based variables reduce the influence of absolute size and highlight shape-related differences among *taxa*, supporting their utility alongside raw dimensional measures. Tooth-level analyses of enamel prism morphometrics show a strong and consistent taxonomic signal across all primary variables (Dmax, Dmin, Perimeter, Area) and derived ratios. One and Two-way ANOVA results indicate a highly significant effect of Species for all morphometric descriptors ($p < 0.001$), whereas ToothType and Species $\times$ ToothType interactions are not significant (Tables 7 and 8). This pattern confirms that interspecific differences dominate the observed variance structure, while within-taxon variation across different dental elements is comparatively limited in the present dataset.

All six derived ratios showed highly significant differences among species, confirming that shape-related descriptors provide a strong taxonomic signal at the tooth level. In particular, the Dmax/Dmin and Perim/Area ratios consistently exhibited the clearest separation among *taxa*, highlighting their potential utility for taxonomic identification independent of absolute size effects. Although some variability may be associated with tooth type, interspecific differences are more pronounced, suggesting that enamel prism morphometrics retain taxonomic relevance across different dental elements (Supplementary Material 7).

For M-S herb. sp., the low Dmax/Dmin values indicate more isodiametric prism outlines compared to *Bos taurus* and *Sus* sp. (Table 8).

The use of C.V. reveals that the M-S herb. sp. group exhibits the highest morphological variability (20.3% for Dmax/Dmin), consistent with its nature as a multi-species taxonomic category. ToothType was not significant for the tooth-level means in this dataset ($p \approx 1$ across models), and Species $\times$ ToothType interactions were not significant. This pattern is consistent with a dominant taxonomic signal and limited tooth-type contribution in the available sampling (and/or incomplete combinations of species $\times$ tooth types, which can reduce estimability of interaction terms). Using tooth-level inference (avoiding pseudoreplication), enamel prism morphometrics display a very strong taxonomic signature among *Bos taurus*, *Sus* sp. and M-S herb. sp. The near-perfect cross-validated classification obtained independently by linear

Table 7

Formatted results of the one-way ANOVA testing the effect of Species on tooth-level enamel prism morphometric variables and derived ratios. For each variable, F-statistics, degrees of freedom, p -values, model R^2 , and sample size (n) are reported.

Variable	p _Species	R^2	n _teeth
Dmax	1,56E-78	0,99718857	64
Dmin	1,69E-78	0,9971808	64
Perim	5,31E-69	0,99422595	64
Area	1,14E-87	0,99858932	64
Dmax_Dmin	3,91E-33	0,91341171	64
Dmax_Perim	7,47E-68	0,99370301	64
Dmax_Area	2,36E-60	0,98890849	64
Dmin_Perim	1,40E-65	0,99252374	64
Dmin_Area	1,70E-46	0,96842107	64
Perim_Area	5,95E-46	0,96709999	64

Table 8

Enamel prism shape ratios by species (tooth-level data). Values < 1 were inverted to maintain consistency with Dmax/Dmin definition.

Species	Dmax/Dmin (mean)	Dmax/Dmin (C.V.%)	Perim/Area (mean)	Perim/Area (C.V.%)	n
<i>Bos taurus</i>	1,83	9,07	1,93	3,56	6
M-S herb. sp.	0,79	20,30	1,21	5,55	30
<i>Sus</i> sp.	3,61	4,41	1,31	3,15	28

(LDA) and non-linear models (SVM-RBF, RF) indicates that the inter-specific signal is robust and consistent across different modelling frameworks and decision boundaries, although future validation on larger and more balanced datasets will be necessary to confirm its external generalizability.

The first two principal components explained 88.2% of the total variance, with PC1 accounting for 63.6% and PC2 for 24.6% (Fig. 6). Specifically, PC1 represents a size-related component, driven primarily by absolute dimensional variables such as Area, Perimeter, Dmax, and Dmin; it effectively separates *Bos taurus* (which exhibits significantly larger prisms) from the other taxa. On the other hand, PC2 behaves as a shape-related component, influenced by derived geometric ratios (such as Dmax/Dmin and Perimeter/Area). This component captures the elongation and boundary complexity of the prisms, contributing to the further isolation of *Sus* sp. from the medium-sized herbivore group. This multivariate structuring confirms that taxonomic discrimination relies on a combination of both absolute scale and specific geometric outlines.

The LDA biplot (Fig. 7a) shows a clear separation of the three taxonomic groups.

The first canonical axis (LD1) is primarily driven by Dmax and Dmin, effectively isolating *Bos taurus* due to its significantly larger prism dimensions. The second axis (LD2) aids in discriminating *Sus* sp. from the M-S group, with Area and Perimeter being the most influential vectors. The sub-set PCA focused on 'M-S herb. sp.' (Fig. 7b) revealed a structured

Table 6

Raw output of the two-way ANOVA performed on tooth-level enamel prism morphometric variables and derived ratios. Reported values include p -values for the main effects of Species and ToothType, their interaction (Species $\times$ ToothType), as well as model R^2 and sample size (n).

Variable	p _Species	p _ToothType	p _Interaction	R^2	n _teeth
Dmax	2,91E-74	0,75093889	0,09778242	0,99763662	64
Dmin	2,46E-74	0,94537084	0,08767789	0,99765105	64
Perim	2,26E-64	0,43819587	0,18797289	0,99466995	64
Area	1,36E-83	0,2621445	0,02098027	0,99890262	64
Dmax_Dmin	1,22E-30	0,96500023	0,78133855	0,91468022	64
Dmax_Perim	4,07E-64	0,56077457	0,1995394	0,99455889	64
Dmax_Area	1,12E-58	0,70646783	0,00979475	0,99150471	64
Dmin_Perim	1,42E-61	0,88319611	0,28771233	0,99329264	64
Dmin_Area	6,58E-43	0,94032075	0,94632827	0,96885969	64
Perim_Area	2,90E-43	0,44010425	0,71999897	0,96982823	64

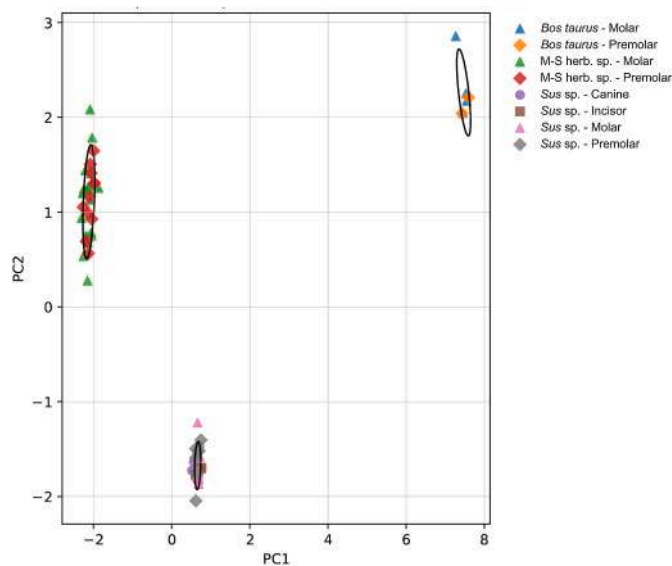


Fig. 6. Principal Component Analysis (PCA) of enamel prism morphometrics based on tooth-level mean values. The first two principal components explain 88.2% of the total variance (PC1 = 63.6%, PC2 = 24.6%). Points represent individual teeth, colored by species and symbol-coded by tooth type. Ellipses indicate 68% confidence regions for each species. The plot reveals a strong multivariate structuring of enamel prism morphology, with clear interspecific separation primarily driven by size-related variables along PC1 and additional shape-related differentiation along PC2.

internal distribution. PC1 (explaining 44.6% of the variance) is strongly correlated with Dmin and derived ratios, suggesting a morphological gradient within the group.

4. Discussion

The primary aim of this study was to test the potential of SEM-based enamel prism microstructural morphometrics as a rapid, cost-effective, and minimally invasive taxonomic proxy for fragmented zooarchaeological assemblages where macromorphological traits are ambiguous. By analyzing 64 adult teeth from two prehistoric northern Italian sites, our findings demonstrated a clear interspecific separation among the

main taxonomic groups (*Bos taurus*, *Sus* sp., and medium-sized herbivores). Absolute microstructural measurements (Dmax, Dmin, area, and perimeter) combined with size-normalized geometric shape ratios provided robust multivariate segregation, indicating that the enamel prismatic signal is strictly driven by taxonomic affiliation rather than dental position or localization. These main results validate the proposed operational protocol as a reliable tool capable of complementing biomolecular and classic morphological methods in prehistoric faunal studies.

Prior to cleaning and SEM analysis, sediment pH was measured for each archaeological context. This step was undertaken because soil chemistry is a well-known factor influencing the preservation of skeletal hard tissues, and interactions between the burial matrix and dental remains may alter the external tooth surface at the microscopic scale (Schmidt et al., 2017). It is also important to note that part of the material analyzed derives from excavations conducted in the 1980s, during which the bones had already been handled and curated, in contrast to more recently excavated materials that were sampled promptly and in an unmanipulated state. Time elapsed since excavation and post-excavation handling are known to potentially influence measured pH values (Matthiesen, 2004; Gordon and Buikstra, 1981). Nevertheless, these measurements represent the only available data related to the sediment matrices of the materials excavated in the 1980s and therefore constitute the sole attainable reference values.

Measured pH values for both sites—La Vela, located on an alluvial fan on the left bank of the Adige River, and Riparo Gaban, a rockshelter situated mid-slope on the right bank—indicate slightly acidic conditions in both contexts (mean values reported in Table 3). Mild soil acidity may contribute to superficial demineralization of dental hard tissues, potentially improving the exposure and readability of microscopic features under SEM (Pashley et al., 1993; Kuntze et al., 2020). This aspect is particularly relevant from a methodological perspective. The approach presented here is particularly well suited to archaeological contexts because, under favorable soil pH conditions, enamel prisms can become naturally exposed over time, allowing SEM observation without any laboratory preparation of the tooth surface. It is important to stress that the microstructural features analyzed here are enamel prisms, not dentinal tubules, and that the observed structures derive from natural exposure of the enamel surface rather than from dentin demineralization. In contrast, studies on modern teeth typically require controlled chemical or mechanical treatments to expose enamel microstructures prior to SEM analysis (Pashley et al., 1993; Perdigão et al., 1999). The

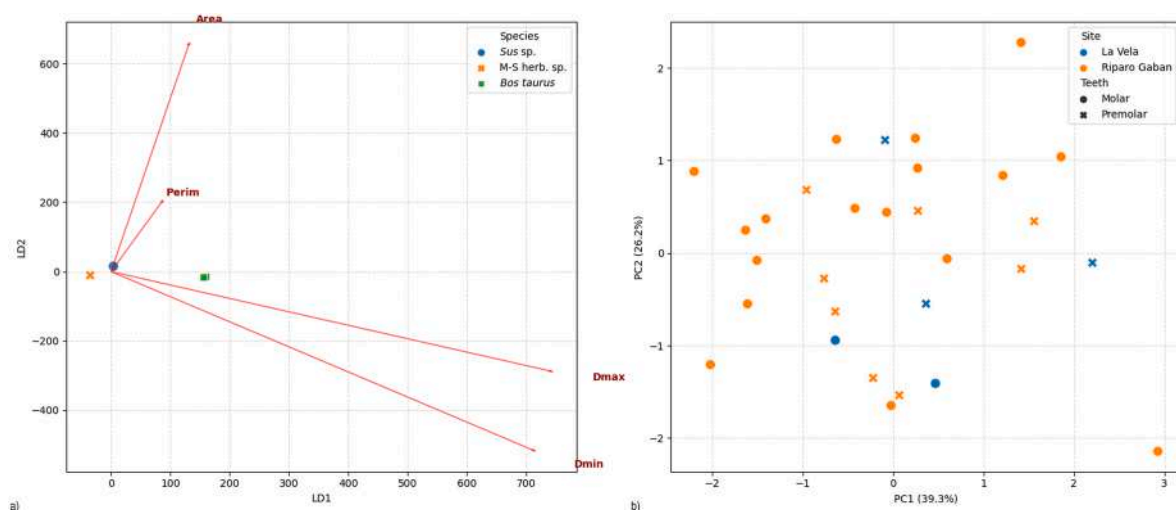


Fig. 7. Multivariate analysis of enamel prism morphometrics. (a) LDA biplot showing taxonomic group separation; red vectors indicate the discriminative influence of original variables (Dmax, Dmin, Area, and Perimeter). (b) Subset PCA of the M-S herb. sp. group, illustrating internal variance and clustering related to site provenance and tooth position (molars vs. premolars). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

absence of invasive preparation procedures represents a significant advantage when working with archaeological specimens, as it minimizes intervention on often fragile and unique materials.

At the same time, this reliance on natural soil-mediated exposure represents an intrinsic limitation of the method. Its applicability is likely reduced in neutral to alkaline soils, where demineralization processes are less effective and enamel prisms may remain concealed. The influence of soil pH on the performance and reliability of this analytical approach warrants more detailed investigation and will be addressed in future studies.

The integration of ZooMS analysis, although performed on a single specimen due to the selective preservation of bone tissue, provided a significant methodological benchmark. The proteomic data allowed for the exclusion of several alternative taxa (Table 5), enabling a more confident macroscopic and morphometric reassessment of the specimen, ultimately attributed to *Ovis aries*. From a broader perspective, while this refined identification does not substantially alter the overall statistical outcomes of the study—given the numerical dominance of other taxonomic groups—it highlights the value of an interdisciplinary approach. Combining proteomic and morphometric data proves to be a powerful strategy for resolving macroscopic uncertainties in fragmentary assemblages (Buckley and Collins, 2011; Welker et al., 2016), facilitating the standardization of taxonomic identification in prehistoric contexts.

The differences in sample size among taxonomic groups represent a common condition in archaeological and zooarchaeological datasets and do not, in themselves, preclude robust statistical analysis. Modern statistical frameworks explicitly allow for unbalanced designs, provided that the analytical methods are appropriately chosen and assumptions are carefully evaluated (Quinn and Keough, 2002; Zar, 2010).

In the present study, analyses were conducted at the tooth level rather than at the individual prism level, thereby reducing pseudo-replication and ensuring that each observation represents an independent biological unit. Summary statistics derived from multiple prisms per tooth further enhance robustness by stabilizing estimates of central tendency and dispersion (Hurlbert, 1984; Lazic, 2010). Moreover, parametric approaches such as ANOVA are known to be relatively robust to unequal group sizes, particularly when group variances are comparable and sample sizes are sufficient to characterize within-group variability (Glass et al., 1972; Underwood, 1997). When these conditions are met, ANOVA remains a valid tool for testing differences in group means even under strongly unbalanced designs.

The interpretation of results in this study focuses on effect sizes, variance structure, and multivariate patterns rather than on sample-size-driven significance alone. Multivariate techniques such as Principal Component Analysis and supervised classification methods are routinely applied in archaeometric and bioarchaeological research with uneven group representation, as their primary aim is to explore structure, separation, and discriminative potential rather than population-level inference (Lachenbruch and Mickey, 1968; Baxter, 2003). Taken together, these considerations support the validity of the statistical analyses presented here, while also acknowledging that sample imbalance represents an inherent limitation of the archaeological record rather than a methodological shortcoming. Future work based on larger and more balanced datasets will be essential to further refine and validate the observed patterns.

The qualitative differences observed in prism geometry and orientation among taxa suggest that enamel microstructural organization may vary in a taxonomically patterned manner. In *Bos taurus*, the predominance of sub-hexagonal prism cross-sections and the presence of sub-parallel prism bundles indicate a relatively ordered enamel architecture. In *Sus* sp., prisms tend to display more rectangular outlines and a less uniform orientation, with sub-parallel arrangements at the bundle level but heterogeneous orientation relative to the tooth axis. In M-S herb. sp., prisms show a sub-polygonal morphology approaching a circular outline, accompanied by only a weak tendency toward parallel

alignment and a less clearly structured spatial organization. While these observations are qualitative, they are consistent with the morphometric patterns documented in this study and support the interpretation that enamel prism organization may contribute to taxonomic differentiation.

The observed differences in enamel prism counts among taxa primarily reflect structural and dimensional differences in enamel micro-architecture, rather than biologically meaningful variation in prism density. Larger prism cross-sections, such as those observed in *Bos taurus*, result in fewer prisms per acquisition area, whereas smaller and more densely packed prisms in M-S herb. sp. lead to higher prism counts (Table 5). The observed values appear to be largely driven by species-level differences, while *Bos taurus* teeth show the lowest counts, with mean values around 60 prisms per tooth, *Sus* sp. exhibits intermediate values, with mean counts close to 85–90 prisms per tooth. In contrast, M-S herb. sp. display the highest prism densities, with molars showing mean values exceeding 120 prisms per tooth (Table 5). These averages indicate a clear separation in prism density among taxa, while differences related to tooth type appear comparatively limited within each species.

Enamel prism number does not represent an independent taxonomic marker and was therefore not used for classification or inferential testing. Instead, it provides an internal control demonstrating that SEM sampling was consistent within teeth and broadly comparable across taxa. The relatively low intra-tooth variability in prism counts supports the robustness of the acquisition protocol and validates the use of tooth-level summary statistics for subsequent morphometric analyses.

The observed differences in enamel prism dimensions among taxa indicate that these variables capture systematic variation associated with taxonomic grouping. Although similar morphometric descriptors have been widely applied to dentinal tubules in previous studies, their application to enamel prisms remains comparatively underexplored (i. e., Castilhos Ruschel and Chevitaese (2002); Gevkaliuk et al. (2023)). The present results demonstrate that these descriptors can be effectively transferred to enamel microstructures, retaining strong taxonomic signal. The larger absolute dimensions recorded for *Bos taurus* reflect broader structural scaling, whereas *Sus* sp. and medium-sized herbivores occupy partially overlapping but distinguishable morphometric ranges (Supplementary Material 3-6).

The strongest discriminators are descriptors linked to maximum diameter (Dmax) and area, suggesting that prism scale (and scale-related geometry) carries major taxonomic information. Ratios involving Dmax and Perim are also highly discriminative, implying that both size and shape properties contribute to separation. The projection of original variables onto canonical axes confirms that prism size is the primary diagnostic feature for large bovids, whereas the relationship between area and perimeter provides a secondary rule for distinguishing suids from smaller herbivores (Fig. 7 a). Regarding the 'M-S herb. sp.' group, the dedicated PCA highlights that its apparent 'mixed' nature in the general model actually hides significant internal trends. The separation observed between molars and premolars, as well as slight variations between sites, suggests that the high variance in this group is not mere 'noise' but reflects the diverse composition of the medium-small herbivore category (Fig. 7b). This underlines the importance of considering tooth position and site-specific factors when attempting finer taxonomic resolutions within this group.

ToothType effects were not significant in the present dataset. Two interpretations are plausible: prism morphometrics may be relatively conserved across tooth classes within these taxa; sampling may be unbalanced across species and tooth-type combinations (not all combinations observed), limiting power to detect ToothType and interaction effects. This is consistent with the warnings encountered during model estimation and should be addressed in future sampling if within-taxon dental variation is of interest.

A key limitation is class imbalance, particularly the small number of *Bos taurus* teeth ($n = 6$). While balanced accuracy = 1.0 is reassuring, expanding *Bos taurus* sampling would strengthen external validity.

Despite this, the present analysis supports the feasibility of using enamel prism morphometrics as a taxonomic proxy for distinguishing these *taxa* from dental tissue alone. This approach is particularly promising for fragmentary assemblages where traditional morphological identification is challenging.

5. Conclusion

This study proposes an operational protocol for SEM-based analysis of enamel prisms in archaeological teeth, designed to maximize comparability across specimens while minimizing intervention on fragile materials. By combining standardized image acquisition, prism counting, and the extraction of a consistent set of morphometric descriptors (Dmax, Dmin, perimeter, area) together with derived shape ratios, the approach provides a replicable framework for generating quantitative microstructural data from enamel surfaces that are often already readable in archaeological contexts. Within this framework, enamel prism morphometrics proved informative for taxonomic discrimination at the tooth level, showing a strong and consistent separation among the main taxonomic groups considered. Both absolute measures and size-normalized ratios—especially Dmax/Dmin and Perim/Area—capture interspecific differences that can support TI when macroscopic criteria are ambiguous or when remains are fragmentary. Although further work is needed to validate performance under different preservational conditions and to strengthen inferences through larger and more balanced samples, the results indicate that enamel prisms represent a useful microstructural proxy for taxonomic attribution and that the proposed protocol can be integrated as a practical tool within the broader methodological toolkit of zooarchaeology.

CRedit authorship contribution statement

O. Larentis: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **A. Fontana:** Data curation, Formal analysis, Methodology, Visualization, Writing – review & editing. **S. Grimaldi:** Project administration, Resources, Visualization, Writing – review & editing. **A. Santaniello:** Data curation, Resources, Software, Visualization. **A. Pedrotti:** Project administration, Resources, Visualization, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Omar Larentis reports article publishing charges was provided by University of Trento. The other 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.jas.2026.106640>.

References

- Bagolini, B., 1972. Maso pasquali - riparo gaban (trento). In: Aa, Vv (Ed.), *Proceedings of the XV Scientific Reunion of the IIPP*, 27–29 October 1972. All'Insegna del Giglio, Firenze, pp. 91–94.
- Bagolini, B., 1990. Cultura dei vasi a bocca quadrata. Il sepolcreto neolitico della Vela di Trento. In: Aa, Vv (Ed.), *Die Ersten Bauern*, 2. Schweizerisches Landesmuseum Zürich, Zurich, pp. 225–231.
- Bagolini, B., Bergamo Decarli, G., Bertoldi, L., Postal, L., 1973. Riparo Gaban. *PreistAlp* 9, 245–246.
- Bagolini, B., Biagi, P., 1977. Le più antiche facies ceramiche dell'ambiente montano padano. *Rivista di Scienze Preistoriche* 21 (XXXII), 219–233.
- Barfield, L.H., 1970. La stazione neolitica de “La Vela” presso Trento. *Considerazioni sulle tombe a cista nel Trentino Alto Adige*. STSN 47, 35–55.
- Barone, R., 2003. Anatomia comparata dei mammiferi domestici. In: Bortolami, R., Callegari, E. (Eds.), *Osteologia*, 5. Edagricole, Bologna.
- Baxter, M.J., 2003. *Statistics in Archaeology*. Arnold, London.
- Bergman, G., 1963. Microscopic demonstration of liquid flow through human dental enamel. *Arch. Oral Biol.* 8, 233–234. [https://doi.org/10.1016/0003-9969\(63\)90061-X](https://doi.org/10.1016/0003-9969(63)90061-X).
- Berkovitz, B., Shellis, P., 2023. *The teeth on non-mammalian vertebrates. Form, Function, Development and Growth*. Academic Press, London.
- Borovskii, E.V., Leontiev, V.K., 1991. *Biology of the Oral Cavity*. Meditsina, Moscow.
- Boyd, B., 2017. Archaeology and human-animal relations: thinking through anthropocentrism. *Annu. Rev. Anthropol.* 46 (1), 299–316. <https://doi.org/10.1146/annurev-anthro-102116-041346>.
- Brewer, D.J., 1992. Zooarchaeology: method, theory, and goals. *J. Archaeol. Method Theor* 4, 195–244.
- Brits, D., Steyn, M., LÁbbé, E.N., 2014. A histomorphological analysis of human and non-human femora. *Int. J. Leg. Med.* 128, 369–377. <https://doi.org/10.1007/s00414-013-0854-3>.
- Brown, S., Douka, K., Collins, M.J., Richter, K.K., 2020. On the standardization of ZooMS nomenclature. *J. Proteonomics* 235, 104041. <https://doi.org/10.1016/j.jprot.2020.104041>.
- Buckley, M., Collins, M., Thomas-Oates, J., Wilson, J.C., 2009a. 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 <https://doi.org/10.1010.1002/rcm.4316>.
- Buckley, M., Collins, M., Thomas-Oates, J., Wilson, J.C., 2010. Erratum: species identification by analysis of bone collagen using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry. *Rapid Commun. Mass Spectrom.* 24, 3372. <https://doi.org/10.1002/rcm.4745>.
- Buckley, M., Harvey, V.L., Chamberlain, A.T., 2017. Species identification and decay assessment of late Pleistocene fragmentary vertebrate remains from Pin Hole Cave (Creswell Crags, UK) using collagen fingerprinting. *Boreas* 46, 402–411 <https://doi.org/10.1010.1111/bor.12225>.
- Buckley, M., Kansa, S.W., Howard, S., Campbell, S., Thomas-Oates, J., Collins, M., 2009b. Distinguishing between archaeological sheep and goat bones using a single collagen peptide. *J. Archaeol. Sci.* 37 (1), 13–20. <https://doi.org/10.1016/j.jas.2009.08.020>.
- Buckley, M., Collins, M., 2011. Collagen survival and its use for species identification in holocene-lower Pleistocene bone fragments from British archaeological and paleontological sites. *Antiqua* 1 (1), e1.
- Caccia, G., Magli, F., Tagi, V.M., Porta, D.G., Cummaudo, M., Márquez-Grant, N., Cattaneo, C., 2016. Histological determination of the human origin from dry bone: a cautionary note for subadults. *Int. J. Leg. Med.* 130, 299–307. <https://doi.org/10.1007/s00414-015-1271-6>.
- Castillhos Ruschel, H., Chevatarese, O., 2002. Density and diameter of dentinal tubules of first and second primary human molars - comparative scanning electron microscopy study. *J. Clin. Pediatr. Dent* 26, 297–304. <https://doi.org/10.17796/jcpd.26.3.t5344598326r5687>.
- Corradini, B., Gianfreda, D., Ferri, G., Ferrari, F., Borciani, I., Santunione, A.L., Cecchi, R., 2024. Forensic species identification: practical guide for animal and plant DNA analysis. *Int. J. Leg. Med.* 138, 2271–2280. <https://doi.org/10.1007/s00414-024-03284-2>.
- Crescimanno, A., Stout, S.D., 2011. Differentiating fragmented human and nonhuman long bone using osteon circularity. *J. Forensic Sci.* 57 (2), 287–294. <https://doi.org/10.1111/j.1556-4029.2011.01973.x>.
- Croze, M., Paladin, A., Zingale, S., Alemanno, S., Nicolis, F., Mottes, E., et al., 2025. Genomic diversity and structure of prehistoric alpine individuals from the Tyrolean Iceman's territory. *Nat. Commun.* 16, 6431. <https://doi.org/10.1038/s41467-025-61601-8>.
- Cummaudo, M., Cappella, A., Giacomini, F., Raffone, C., Márquez-Grant, N., Cattaneo, C., 2019. Histomorphometric analysis of osteocyte lacunae in human and pig: exploring its potential for species discrimination. *Int. J. Leg. Med.* 133, 711–718. <https://doi.org/10.1007/s00414-018-01989-9>.
- Degasperis, N., Endrizzi, L., Mottes, E., Nicolis, F., 2011. New evidence of Ancestral Landscape in Trentino in the Copper and Bronze Ages: the ritual sites of Cles-Campi Neri and La Vela di Trento. In: Aa, Vv (Ed.), *Ancestral Landscape: Burial Mounds in the Copper and Bronze Ages (Central and Eastern Europe - Balkans - Adriatic - Aegean, 4th-2nd millen-nium B.C.)*, vol. 58. TMO, pp. 511–522.
- Dominguez, V.M., 2025. Human versus non-human. Bone microstructure in species differentiation and experimental research. In: Stout, S.D., Crowder, C. (Eds.), *Bone Histology a Biological Anthropological Perspective*. Taylor & Francis, Boca Raton, pp. 248–269.
- Driver, J.C., 2011. Identification, classification and zooarchaeology. *Ethnobiol. Lett.* 2, 19–39. <https://doi.org/10.14237/ebi.2.2011.32>.

- Elnaggar, A., Osama, A., Anwar, A.M., Ezzeldin, S., Abou Elhassan, S., Ebeid, H., Leona, M., Magdeldin, S., 2022. Paleoproteomic profiling for identification of animal skin species in ancient Egyptian archaeological leather using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). *Herit. Sci.* 10, 182. <https://doi.org/10.1186/s40494-022-00816-0>.
- Enax, J., Pandrich, P., Wiesche, E.S.Z., Eppe, M., 2024. The remineralization of enamel from saliva: a chemical perspective. *Dent. J.* 12 (11), 339. <https://doi.org/10.3390/dj12110339>.
- Gevkaliuk, N.O., Martys, I.M., Mykhailiuk, V.M., Pynda, M.Y., Pudiak, V.Y., Krupei, V.Y., 2023. Quantity and diameter of dentinal tubules of human teeth and teeth of experimental animals according to scanning electron microscopy data. *Reg. Mechan. Biosyst.* 14 (4), 609–616. <https://doi.org/10.15421/022388>.
- Gibaja, J.F., Santaniello, F., Grimaldi, S., Zambaldi, M., Mazzucco, N., Pedrotti, A., 2025. Technological and functional analyses of late Mesolithic and early Neolithic lithic assemblages from Riparo Gaban (Trento, Italy). *J. Archaeol. Sci.: Rep.* 62, 105031. <https://doi.org/10.1016/j.jasrep.2025.105031>.
- Glass, G.V., Peckham, P.D., Sanders, J.R., 1972. Consequences of failure to meet assumptions underlying fixed effects analyses of variance and covariance. *Rev. Educ. Res.* 42, 237–288. <https://doi.org/10.2307/1169991>.
- Gobalet, K.W., 2001. A critique of faunal analysis; inconsistency among experts in blind tests. *J. Archaeol. Sci.* 28 (4), 377–386. <https://doi.org/10.1006/jasc.2000.0564>.
- Gordon, C.C., Buikstra, J.E., 1981. Soil pH, bone preservation, and sampling bias at mortuary sites. *Am. Antiq.* 46 (3), 566–571. <https://doi.org/10.2307/280601>.
- Grealy, A.C., McDowell, M.C., Scofield, P., Murray, D.C., Fusco, D.A., Haile, J., Prideaux, G.J., Bunce, M., 2015. A critical evaluation of how ancient DNA bulk bone metabarcoding complements traditional morphological analysis of fossil assemblages. *Quat. Sci. Rev.* 128, 37–47. <https://doi.org/10.1016/j.quascirev.2015.09.014>.
- Green, E., 2024. Fragmented analysis, fragmented interpretation: the necessity of integrated faunal and human analysis for identifying and understanding ritual contexts. *J. Archaeol. Sci.: Rep.* 58, 104697. <https://doi.org/10.1016/j.jasrep.2024.104697>.
- Greenlee, D.M., Dunnell, R.C., 2009. Identification of fragmentary bone from the Pacific. *J. Archaeol. Sci.* 37 (5), 957–970. <https://doi.org/10.1016/j.jas.2009.11.029>.
- Gueriau, P., Bernard, S., Bertrand, L., 2016. Advanced synchrotron characterization of paleontological specimens. *Elements* 12 (1), 45–50. <https://doi.org/10.2113/gselements.12.1.45>.
- Hillier, M.L., Bell, L.S., 2007. Differentiating human bone from animal bone: a review of histological methods. *J. Forensic Sci.* 52 (2), 249–263. <https://doi.org/10.1111/j.1556-4029.2006.00368.x>.
- Hillson, S., 2005. *Teeth*. Cambridge University Press, Cambridge.
- Huisman, H., Ismail-Meyer, K., Sageid, B.M., Josten, I., 2017. Micromorphological indicators for degradation processes in archaeological bone from temperate European wetland sites. *J. Archaeol. Sci.* 85, 13–29. <https://doi.org/10.1016/j.jas.2017.06.016>.
- Hurlbert, S.H., 1984. Pseudoreplication and the design of ecological field experiments. *Ecol. Monogr.* 54, 187–211. <https://doi.org/10.2307/1942661>.
- Imai, T., Hattori, S., Uesugi, K., Hoshino, M., 2023. High-energy synchrotron-radiation-based X-ray micro-tomography enables non-destructive and micro-scale palaeohistological assessment of macro-scale fossil dinosaur bones. *J. Synchrotron Radiat.* 30 (3), 627–633. <https://doi.org/10.1107/s1600577523001790>.
- Ippolitov, Y.A., Ippolitov, I.Y., Seredin, P., 2014. Morphology of the human dental enamel. *Indian J. Dent.* 5, 135–139. <https://doi.org/10.1016/j.ijd.2014.03.004>.
- Isaakidou, V., 2020. Zooarchaeology. In: Hollander, D., Howe, T. (Eds.), *A Companion to Ancient Agriculture*. John Wiley & Sons, Hoboken, pp. 37–54. <https://doi.org/10.1002/9781118970959.ch3>.
- Jensen, T.Z.T., Sjöström, A., Fischer, A., Rosengren, E., Lanigan, L.T., Bennike, O., Richter, K.K., Gron, K.J., Mackie, M., Mortensen, M.F., Sørensen, L., Chivall, D., Iversen, K.H., Taurozzi, A.J., Olsen, J., Schroeder, H., Milner, N., Sørensen, M., Collins, M.J., 2020. An integrated analysis of maglemose bone points reframes the early Mesolithic of Southern Scandinavia. *Sci. Rep.* 10 (1), 17244. <https://doi.org/10.1038/s41598-020-74258-8>.
- Kirby, D.P., Buckley, M., Promise, E., Trauger, S.A., Holdcraft, T.R., 2013. Identification of collagen-based materials in cultural heritage. *Analyst* 138 (17), 4849–4858. <https://doi.org/10.1039/c3an00925d>.
- Koenigswald, W.V., Clemens, W.A., 1992. Levels of complexity in the microstructure of mammalian enamel and their application in studies of systematics. *Scanning Microsc.* 6 (1), 16.
- Kozłowski, S.K., Dalmeri, G., 2000. Riparo gaban: the Mesolithic layers. *Preistoria Alp.* 36, 3–42.
- Kuntze, M., Souza, B.M., Schmidt, T., Almeida, J., Bortoluzzi, E., Felipe, W., 2020. Scanning electron microscopy evaluation of dentin ultrastructure after surface demineralization. *J. Conserv. Dent.* 23 (5), 512. https://doi.org/10.4103/jcd.jcd_102_19.
- Lachenbruch, P.A., Mickey, M.R., 1968. Estimation of error rates in discriminant analysis. *Technometrics* 10, 1–11. <https://doi.org/10.2307/1266219>.
- Lagace, F., Schmitt, A., Martrille, L., Benassi, J., Adalian, P., 2020. Using histomorphometry for human and non-human distinction: a test of four methods on fresh and archaeological fragmented bones. *Forensic Sci. Int.* 313, 110369. <https://doi.org/10.1016/j.forsciint.2020.110369>.
- Larentis, O., 2023. Human, all too human: differentiating non-human from human bones in protohistoric cremation contexts from Northern Italy. *Heritage* 6, 647–661. <https://doi.org/10.3390/heritage6010034>.
- Lazic, S.E., 2010. The problem of pseudoreplication in neuroscientific studies: is it affecting your analysis? *BMC. Neurosciences* 11, 5. <https://doi.org/10.1186/1471-2202-11-5>.
- LeFebvre, M.J., Sharpe, A.E., 2017. Contemporary challenges in zooarchaeological specimen identification. In: Glovas, C.M., LeFebvre, M.J. (Eds.), *Zooarchaeology in Practice*. Springer, Princeton, pp. 35–57. https://doi.org/10.1007/978-3-319-64763-0_3.
- Mao, F., Wang, Y., Meng, J., 2015. A systematic study on tooth enamel microstructures of *lambdopsalis bulla* (Multituberculata, Mammalia) implications for multituberculata biology and phylogeny. *PLoS One* 28 (10), e0128243. <https://doi.org/10.1371/journal.pone.0128243>.
- Marsico, C., Grimm, J.R., Renteria, C., Guillen, D.P., Tang, K., Nikitin, V., Arola, D.D., 2024. Characterizing the microstructures of mammalian enamel by synchrotron phase contrast microCT. *Acta Biomater.* 178, 208–220. <https://doi.org/10.1016/j.actbio.2024.02.038>.
- Martiniaková, M., Grosskopf, B., Omelka, R., Dammers, K., Vondráková, M., Bauerová, M., 2007. Histological study of compact bone tissue in some mammals: a method for species determination. *Int. J. Osteoarchaeol.* 17 (1), 82–90. <https://doi.org/10.1002/oa.856>.
- Martiniaková, M., Grosskopf, B., Omelka, R., Vondráková, M., Bauerová, M., 2006. Differences among species in compact bone tissue microstructure of mammalian skeleton: use of a discriminant function analysis for species identification. *J. For. Sci.* 51 (6), 1235–1239. <https://doi.org/10.1111/j.1556-4029.2006.00260.x>.
- Matthies, H., 2004. In situ measurement of soil pH. *J. Archaeol. Sci.* 31 (10), 1373–1381. <https://doi.org/10.1016/j.jas.2004.03.005>.
- MinC, 2022. I Resti Scheletrici Umani: Dallo Scavo, Al Laboratorio, Al Museo. Ministero Della Cultura.
- Mottes, E., 2007. *Spirali del tempo, meandri del passato. Gli Scavi Archeologici a La Vela Di Trento Dal 1960 Al 2007*. Comitato Pro Vela, Trento.
- Mottes, E. (Ed.), 2022. *Vasi a Bocca Quadrata. Evoluzione Delle Conoscenze E Nuovi Approcci Interpretativi*. Provincia autonoma di Trento, Trento.
- Newman, M.E., Parboosingh, J.S., Bridge, P.J., Ceri, H., 2002. Identification of archaeological animal bone by PCR/DNA analysis. *J. Archaeol. Sci.* 29 (1), 77–84. <https://doi.org/10.1006/jasc.2001.0688>.
- Nikita, E., 2017. *Osteoarchaeology: a Guide to the Macroscopic Study of Human Skeletal Remains*. Academic Press, London.
- O'Brien, H.N., 2019. Human vs. non-human bone: a non-destructive histological method. *Grad. Stud. Theses, Diss. Prof. Pap.*, 11373. <https://scholarworks.unt.edu/etd/11373>.
- Oma, K.A., Goldhahn, J., 2020. Introduction: human-animal relationships from a long-term perspective. *Colloids Surf., A* 28, 11–22. <https://doi.org/10.37718/csa.2020.01>.
- Pashley, D.H., Ciucchi, B., Sano, H., Horner, J.A., 1993. Permeability of dentin to adhesive agents. *Quintessence Int.* 24, 618–631.
- Pedrotti, A., 1990. L'abitato neolitico de "La Vela" di Trento. In: Aa, Vv (Ed.), *Die Ersten Bauern, vol. 1. Schweizerisches Landesmuseum Zürich, Zürich*, pp. 219–224.
- Pedrotti, A., 1998. Il gruppo Gaban e le manifestazioni d'arte del primo Neolitico. In: Pessina, A., Muscio, G. (Eds.), *Settemila Anni Fa Il Primo Pane. Ambienti E Culture Delle Società Neolitiche. Arti grafiche friulane, Udine*, pp. 125–131.
- Pedrotti, A., 2001. Il neolitico. In: Lanzinger, M., Marzatico, F., Pedrotti, A. (Eds.), *Storia Del Trentino. La Preistoria E La Protostoria. Il Mulino, Bologna*, pp. 119–181.
- Pedrotti, A., 2009. Il riparo Gaban (Trento) e la neolitizzazione della valle dell'Adige. In: Kruta, V., Poppi, L., Licka, M., Magni, E. (Eds.), *Antenate Di Venere 27000-4000 a. Skira, Ginevra Milano*, pp. 38–47.
- Perdigão, J., van Meerbeek, B., Lopes, M.M., Ambrose, W.W., 1999. The effect of a re-wetting agent on dentin bonding. *Dent. Mater.* 15, 282–295. [https://doi.org/10.1016/s0109-5641\(99\)00049-4](https://doi.org/10.1016/s0109-5641(99)00049-4).
- Porto, S., Flavel, A., Maggio, A., Franklin, D., 2022. Differentiating human from non-human bone fragments through histomorphological assessment of remains from Camposanto cemetery, Italy. *Archaeometry* 65 (1), 213–229. <https://doi.org/10.1111/arcm.12801>.
- Quinn, G.P., Keough, M.J., 2002. *Experimental Design and Data Analysis for Biologists*. Cambridge University Press, Cambridge.
- Regal, P., Cepeda, A., Fente, C. Development of an LC-MS/MS method to quantify sex hormones in bovine milk and influence of pregnancy in their levels. *Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess.* 29(5), 770–779. <https://doi.org/10.1080/19440049.2011.653989>.
- Reitz, E.J., Wing, E.S., 1999. *Zooarchaeology*. Cambridge University Press, Cambridge.
- Renteria, C., Fernández-Arteaga, J., Grimm, J., Ossa, E., Arola, D., 2021. Mammalian enamel: a universal tissue and diverse source of inspiration. *Acta Biomater.* 136, 402–411. <https://doi.org/10.1016/j.actbio.2021.09.016>.
- Richter, K.K., Codlin, M.C., Seabrook, M., Warinner, C., 2022. A primer for ZooMS applications in archaeology. *Proc. Natl. Acad. Sci. USA* 119 (20). <https://doi.org/10.1073/pnas.2109323119>.
- Rüther, P.L., Husic, I.M., Bangsgaard, P., Gregersen, K.P., Pantmann, P., Carvalho, M., et al., 2022. SPIN enables high throughput species identification of archaeological bone by proteomics. *Nat. Commun.* 13, 2458. <https://doi.org/10.1038/s41467-022-30097-x>.
- Santaniello, F., Grimaldi, S., Pedrotti, A., Gialanella, S., 2016. First evidence of heat treatment during the early Neolithic in northeastern Italy. *Quat. Int.* 402, 80–89. <https://doi.org/10.1016/j.quaint.2015.08.006>.
- Schmid, E., 1972. *Atlas of Animal Bones: for Prehistorian, Archaeologist and Quaternary Geologist*. Elsevier Publishing Company, Amsterdam-London-New York.
- Schmidt, C.W., Quataert, R., Zalala, F., D'Anastasio, R., 2017. Taphonomy of teeth. In: Schotsmans, E.M.J., Márquez-Grant, N., Forbes, S.L. (Eds.), *Taphonomy of Human Remains: Forensic Analysis of the Dead and the Depositional Environment*. John Wiley & Sons, Hoboken, pp. 92–100. <https://doi.org/10.1002/9781118953358.ch7>.

- Sullivan, A., Bird, D., Perry, G., 2017. Human behaviour as a long-term ecological driver of non-human evolution. *Nat. Ecol. Evol.* 1, 65. <https://doi.org/10.1038/s41559-016-0065>.
- Thompson, T.J., 2005. Heat-induced dimensional changes in bone and their consequences for forensic anthropology. *J. Forensic Sci.* 50 (5), 1008–1015.
- Ubelaker, D.H., Wu, Y., 2020. Fragment analysis in forensic anthropology. *Forens. Sci. Res.* 5 (4), 260–265. <https://doi.org/10.1080/20961790.2020.1811513>.
- Underwood, A.J., 1997. *Experiments in Ecology*. Cambridge University Press, Cambridge.
- Ungar, P.S., 2010. *Mammal Teeth: Origin, Evolution, and Diversity*. Johnson Hopkins University Press, Baltimore.
- Wang, C., Fang, Y., Zhang, L., Su, Z., Xu, J., Fu, B., 2021. Enamel microstructural features of bovine and human incisors: a comparative study. *Ann. Anat.* 235, 151700. <https://doi.org/10.1016/j.aanat.2021.151700>.
- Wang, C., Xu, J., Xu, J., Deng, S., Fu, B., Zhang, L., 2023. Effect of the prism-interprisms three-dimension spatial microstructure on the enamel bond strength. *BMC Oral Health* 23 (1). <https://doi.org/10.1186/s12903-023-03599-3>.
- Welker, F., Hajdinjak, M., Talamo, S., Jaouen, K., Dannemann, M., David, F., et al., 2016. Palaeoproteomic Evidence Identifies Archaic Hominins Associated with the Châtelperronian at the Grotte Du Renne. *Proc. Natl. Acad. Sci. USA* 113 (40), 1162. <https://doi.org/10.1073/pnas.1605834113>.
- Welker, F., Soressi, M., Rendu, W., Hublin, J.-J., Collins, M.J., 2015. Using ZooMS to identify fragmentary bone from the Late Middle/Early Upper Palaeolithic sequence of Les Cottés, France. *J. Archaeol. Sci.* 54, 279–286. <https://doi.org/10.1016/j.jas.2014.12.010>.
- Zar, J.H., 2010. *Biostatistical Analysis*. Prentice Hall, Hoboken.
- Zheng, W., Ding, L., Wang, Y., Han, S., Zheng, S., Guo, Q., Li, W., Zhou, X., Zhang, L., 2019. The effects of 8DSS peptide on remineralization in a rat model of enamel caries evaluated by two nondestructive techniques. *JABFM* 17 (1). <https://doi.org/10.1177/2280800019827798>.
- Zougrou, I.M., Katsikini, M., Pinakidou, F., Paloura, E.C., Papadopoulou, L., Tsoukala, E., 2013. Study of fossil bones by synchrotron radiation micro-spectroscopic techniques and scanning electron microscopy. *J. Synchrotron Radiat.* 21 (1), 149–160. <https://doi.org/10.1107/s1600577513025228>.