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Taxonomic and phylogenetic signals in Bovini cheek teeth: towards new biosystematic markers to explore the history of wild and domestic cattle

Thomas Cucchi^{1,*,#}, Barbara Stopp^{2,#}, Renate Schafberg³, Joséphine Lesur¹, Alexandre Hassanin⁴, Jörg Schibler²,

¹ CNRS, Muséum national d'Histoire naturelle, Sorbonne Universités, UMR 7209, Archéozoologie, Archéobotanique: Sociétés, Pratiques et Environnements, 75005 Paris, France

² IPAS - Integrative Prehistory and Archaeological Science, University of Basel, 4051 Basel, Switzerland

³ Martin-Luther-University Halle-Wittenberg, Museum of Domesticated Animals “Julius Kühn”, 06108 Halle, Germany

⁴ CNRS, Muséum national d'Histoire naturelle, Sorbonne Universités, UMR 7205, Institut de Systématique, Évolution, Biodiversité, 75005 Paris, France

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* Corresponding author: thomas.cucchi@mnhn.fr

Abstract

Domestic cattle have contributed both to the rise of civilizations and the global loss of biodiversity, but the timing and mechanism of their domestication history remain to be fully understood. Palaeogenetics, which can now explore the target of human selection in the genome, have revolutionized our understanding of cattle domestication. However, biometric approaches of bone remains are still required as a prerequisite for targeted paleogenetic studies to document the taxonomic diversity of wild progenitors and the emergence of domestic morphotypes. But so far, biometric markers of cattle domestication have proven limited in their capacity to disentangle human intentionality from other biotic and abiotic factors.

Using a two-dimensional geometric morphometric approach (GMM), we assessed the taxonomic and phylogenetic signals of the enamel folding pattern of occlusal surfaces (EFPOS) in the maxillary and mandibular molars of wild and domestic species of the tribe Bovini, including ancient cattle breeds and archaeological aurochs and domestic cattle. The phylogenetic signal was assessed using a mitochondrial genome phylogeny across 11 wild taxa of the tribe Bovini. We found that EFPOS could accurately identify both the wild and domestic species of the Bovini taxa as well as shape differentiation among aurochs and modern and archaeological cattle. The phylogenetic differentiation among aurochs and both taurine and zebu cattle is strong, but the overall phylogenetic signal among the tribe Bovini is blurred by genetic introgression between wild and domestic *Bos* species in south-east Asia.

These results strongly suggest that the GMM analysis of dental traits are relevant markers that can be used before the implementation of targeted paleogenomic analyses as a mean to document the diversity and distribution of wild progenitors of domestic forms, identify the emergence of the earliest regional morphotype and their trajectory towards modern breeds.

Key words: Bovini, domestication, zooarchaeology, geometric morphometrics, teeth, phylogenetic signal, taxonomy

1. Introduction

The domestication of aurochs in several parts of Eurasia had a dramatic effect on human history. Their domestic forms became the symbols of prestige, leadership and wealth, while their strength intensified agricultural production, driving the rise of cities and civilisations (Fagan 2015). Nowadays, they provide nearly 30% of the world's meat and over 87% of the world's milk (Scherf, 2000). They are also one of the main sources of unsustainability in our food production system, since 26% of the ice-free land on our planet is used for grazing livestock and a third of cropland is used for livestock feed production (www.fao.org/nr/sustainability/sustainability-and-livestock). Aurochs domestication also played a major role in the evolution of the tribe Bovini (Figure 1). Two domestic forms stemmed from aurochs: common cattle (*Bos primigenius taurus*) and zebu cattle (*Bos primigenius indicus*), one from the yak: the domestic yak (*Bos mutus grunniens*), one from the gaur: the gayal (*Bos gaurus frontalis*), and one from the Asian water buffalo: the river buffalo (*Bubalus arnee bubalis*) (Hassanin, 2014). Currently, due to habitat loss, these wild ancestors, if not extinct or endangered, have been reduced to small pockets of populations in confined areas (Figure 1). The worldwide dispersal of common and zebu cattle induced adaptations to highly diverse environments, such as lowlands and highlands or humid and arid conditions, driving the emergence of different phenotypes (e.g. Felius et al., 2011). This variety of characteristics evolved over thousands of years, but the emergence and development of well-defined, genetically isolated breeds was mainly established from the Industrial Revolution onwards (Ajmone-Marsan et al., 2010).

So far, the domestication of aurochs is the most documented history from archaeological and genetic evidence. The earliest evidence (10,500 BP) of the human control of aurochs based on male body size reduction comes from the northern Fertile Crescent (Helmer et al. 2005, Peters et al 2005). Since common or taurine cattle have a potential progenitor in the form of European aurochs, it is still debated how much local European aurochs have contributed to the genetic make-up of modern cattle (e.g. Cymbron et al., 2005; Beja-Pereira et al., 2006; Tresset et al., 2009; Schibler et al., 2014; Scheu et al., 2015; Upadhyay et al., 2017). The second domestication centre led to the origin of zebu cattle, also referred to as humped cattle. A general consensus stemming from archaeological (Meadow, 1996) and genetic evidence (e.g. Bradley and Magee, 2006) considers the Indus Valley as the most likely centre of domestication for this animal. However, other regions in the Indian subcontinent are also considered potential domestication centres (Chen et al., 2010). The third domestication centre for aurochs has been claimed as Africa (Pérez-Pardal et al., 2010), but

genetic evidence suggests that the first domestic cattle here were actually taurine cattle from the Middle East, with an admixture from local African aurochs (Pitt et al., 2019). In mainland and island south-east Asia, the domestication history of the yak in the Tibetan Plateau or the Asian water buffalo, the gaur and the banteng, mainly relies on genetic and genomic investigations from current wild and domestic populations (Bradley 2006). Although genetic and paleogenetic studies have provided huge insights into the broad origin and trajectories of taurine and zebu cattle, a refined understanding of the timing and mechanisms of their emergence remains to be undertaken in south-western Asia (Arbuckle et al. 2016). In south-east Asia, studies on the domestication process of wild cattle through archaeological evidence is progressing, with the first ancient mtDNA sequences just being published (Siripan et al. 2019).

Along with genetic markers, documenting the domestication pathway of wild cattle could greatly benefit from more powerful morphometric tools to document, from the fossil record, the distribution range of wild cattle species and sub-species before the domestication process was initiated, and identify intraspecific morphological changes induced by the domestication process. The morphological markers used to identify cattle domestication from aurochs and subsequent livestock diffusion have so far relied on body-size reduction (Peters et al. 2005; Helmer et al., 2005; 2015, Arbuckle, 2014). This body-size reduction, used as domestication syndrome (Hemmer, 1990), is a pleiotropic consequence of the selection for behaviours facilitating adaption to the human environment (Trut, 2009; Wilkins et al., 2014). However, difficulty in disentangling human intentionality from other potential biotic and abiotic factors (Maurer et al., 1992) makes it a poor phenotypic marker of early domestication (Helmer et al. 2005; Vigne, 2011; Zeder, 2015).

The geometric morphometrics (GMM) revolution in the study of biological forms (Zelditch et al. 2012) have provided an important breakthrough to track phenotypic responses associated with both the process of domestication and its geographical origins in the archaeological record (Cucchi et al., 2011; Larson, Dobney & Cucchi 2011; Evin, Dobney & Cucchi et al. 2017). Mainly applied on suid teeth, GMM studies have contributed to reach new insights into pig domestication and dispersal in Island South East Asia (Larson et al. 2007; Cucchi et al. 2009), China (Cucchi et al., 2011, 2016) and Europe (Otonari et al., 2013; Evin et al., 2015; Balasse et al., 2019), as well as into the emergence of landrace breeds in Gaul (Duval et al., 2015; 2018) and Bronze-Age Switzerland (Bopp-Ito et al., 2018). The GMM approach of the enamel folding pattern of the occlusal surfaces (EFPOS hereafter) has been used to distinguish Bovidae from South Africa (Brophy et al., 2014), explore diversity in Gallic-

Roman domestic cattle (Nuviala, Laffont & Montuire 2014) and assess taxonomic and phylogenetic signals in *Equus* mandibular teeth among extant wild, domestic and hybrid taxa (Cucchi et al., 2017).

In this study we would like to further assess the biosystematic resolution of EFPOS variation in Bovini. The first objective is to test whether EFPOS can reach an accurate taxonomic identification at the genus and species levels to document the diversity in wild cattle before the emergence of agriculture. The second is to test whether genetic isolation can lead to observable intraspecific phenotypic divergence. And finally, we tested the extant of phylogenetic signal in EFPOS variation to assess its potential in tracking genetic relationship. To provide a proof of concept useful across the wide geographic range where these questions apply, we have chosen to test these biosystematic signals from three molars (M^2 , M^3 and M_3) commonly found in archaeological deposits and over two subtribes of Bovini: the Bubalina (*Bubalus* and *Syncerus*) and the Bovina (*Bos* and *Bison*) (Hassanin et al., 2013), which covers most of the current diversity in wild and domestic cattle. We assessed which molar would perform best in identifying the genus and species of the *Bovini* tribe and populations within *Bos p. taurus*, taking into account the age and different parameters of the EFPOS quantification. We assessed the phylogenetic signal using a mtDNA genome phylogeny from modern specimens contrasted with the interspecific morphospace of each molar.

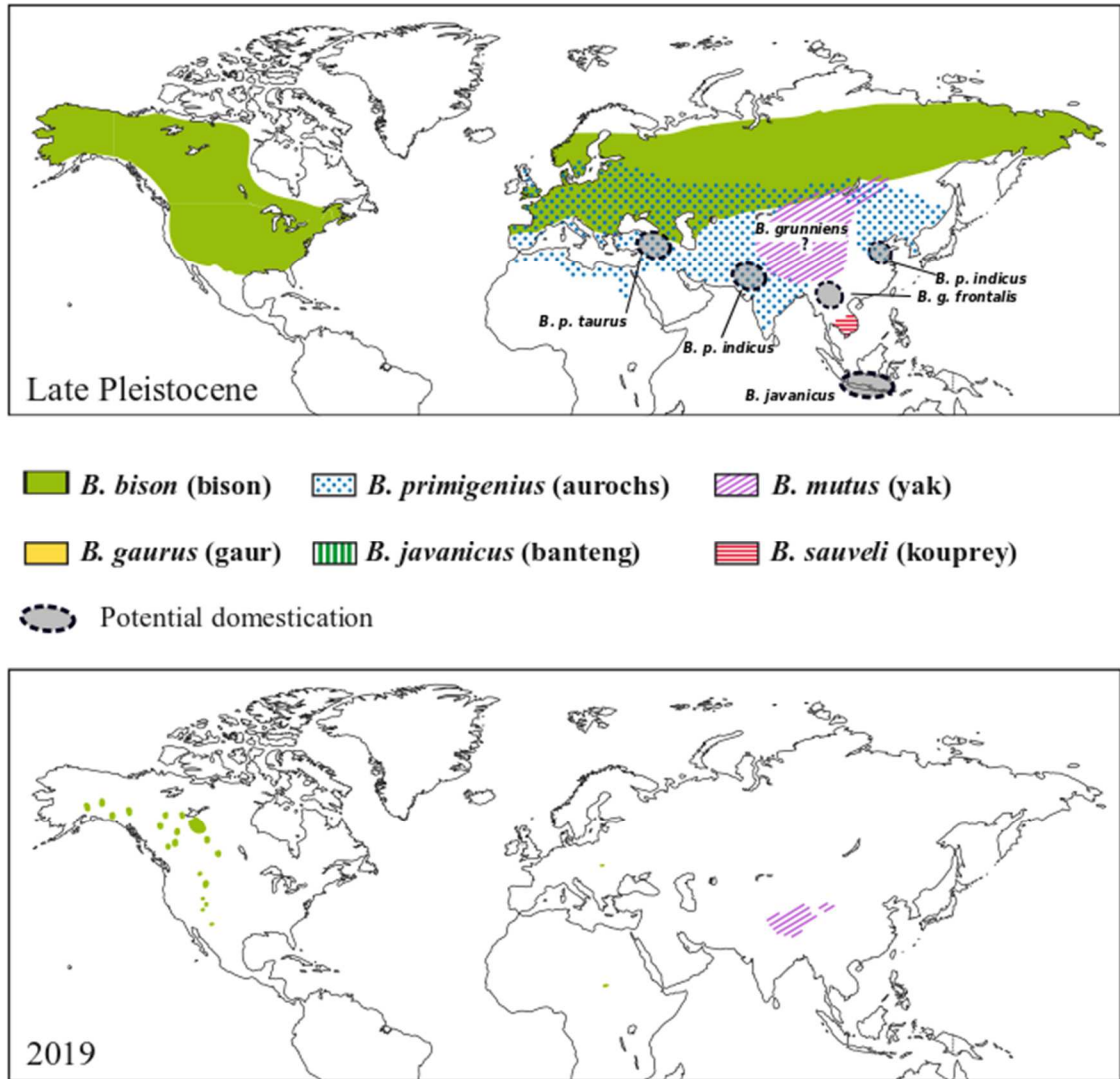


Figure 1: Former range (Late Pleistocene) and current geographic distribution of wild species of *Bovina* after Hassanin 2015 with localisation of their potential domestication centres after Irving-Pease et al. 2018.

2. Materials and methods

Specimens analysed include three extant species of the subtribe Bubalina: the Asian water buffalo (*Bubalus arnee*), the lowland anoa (*Bubalus depressicornis*) and the African buffalo (*Syncerus caffer*); two extant species of Bison: the American bison (*Bison bison*), and the European bison (*Bison bonasus*); four wild species of the genus *Bos*: the yak (*Bos mutus*), the gaur (*Bos gaurus*), the banteng (*Bos javanicus*) and the extinct aurochs (*Bos primigenius*); and two domestic species: common cattle (*Bos primigenius taurus*) and zebu cattle (*Bos primigenius indicus*). The modern breeds of common cattle used in this study (Hollaender, Jersey, Shorthorn and Wilstermarsch) are from the Museum of Domesticated Animals “Julius

Kühn” in Halle, which collected the specimens in the late 19th and early 20th century. These specimens belong to the so-called ancient breeds or little developed country breeds. Wilstermarsch and Hollaender belong to the Pied Lowland/Northwestern Lowland subunit, whereas Shorthorn and Jersey belong to the British/Celtic/North European subunit (Felix et al., 2011). Jersey and Hollaender are two milk breeds, while Wilstermarsch and Shorthorn are dual-purpose/meat breeds. These different selective purposes are observable from their skull morphotypes: milk breeds have longer and narrower skulls, whereas meat animals have shorter and more compact ones (Duerst, 1931; Haring, 1949). We also included two archaeological samples of domestic cattle (*Bos p. taurus*), one from the late Iron Age site of Basel-Gasfabrik (BS, Switzerland, c. 150-80 BC) and one from the Bronze Age site of Savognin-Padnal (GR, Switzerland, c. 1950-1050 BC).

Due to a nomenclature problem (e.g. Gentry et al., 2004; Roskov et al., 2018), we made no distinction between the wild and domestic forms of yak (*Bos mutus*), gaur (*Bos gaurus*), banteng (*Bos javanicus*) or Asian water buffalo (*Bubalus arnee*) for this analysis.

Status	Species	M ³	M ²	M ₃
modern wild taxa	<i>Bison bison</i>	7	10	13
	<i>Bison bonasus</i>	14	9	10
	<i>Bos gaurus</i>	13	14	6
	<i>Bos mutus</i>	2	3	-
	<i>Bos javanicus</i>	15	12	3
	<i>Bubalus arnee</i>	12	11	4
	<i>Bubalus depressicornis</i>	2	4	5
	<i>Syncerus caffer</i>	21	28	11
modern domestic taxa	<i>Bos primigenius indicus</i> (zebu cattle)	5	5	6
	<i>Bos primigenius taurus</i> (common cattle)			
	Hollaender	13	12	10
	Jersey	8	7	8
	Shorthorn	8	10	10
	Wilstermarsch	5	5	5
archaeological samples	<i>Bos primigenius primigenius</i>	21	21	18
	<i>Bos primigenius taurus</i>			
	Basel-Gasfabrik (late Iron Age)	17	15	9
	Savognin-Padnal (Bronze Age)	15	12	7

total sample size	178	178	125
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Tab. 1: Sample size for the modern and archaeological *Bovini* used in this study. Maxillary molars: M³, M²; mandibular molar: M₃.

Our morphometric study focused on the maxillary second and third molars (M², M³) and the mandibular third molar (M₃) (fig. 2a, 2b). Both maxillary and mandibular third molars provide important advantages when dealing with archaeological material since they are easily distinguishable from the other teeth. Understanding the effect of attrition through ageing over the variation of the EFPOS was pivotal for this study. This effect has been proven null or negligible on the EFPOS of horses' cheek teeth (Seetah et al., 2014), and CT analysis on the third maxillary and mandibular molars of African bovids (Brophy et al., 2014) also indicate a negligible effect. To investigate whether this observation is also valid for bovine teeth, we only considered teeth with full abrasion for this study. To obtain a relative age determination for the modern and archaeological specimens, we used the wear stage recording systems accessible from the IPAS website (<https://duw.unibas.ch/de/ipna/forschung/archaeobiologie/archaeozoologie/methodik/>). With this system, each tooth is given a numerical score which corresponds to its eruption and attrition stages, combining the age systems of Barone 1986, Becker/Johansson 1981, Ducos 1968, Habermehl 1975 and Grigson 1982.

2.1 Geometric morphometric approach to *Bovini* molar's EFPOS

Following previous studies on equines (Cucchi et al., 2017), we quantified the EFPOS of bovine molars with a 2D GMM approach combining landmarks of type 3 on the main enamel folds and semilandmarks on enamel curves between each landmark. For all three molars, we collected 7 landmarks (1–7) on the maximum curvature of the enamel folds at the junction between dentine and enamel, (fig. 2AB). The semilandmarks were then sampled equidistantly on the curves drawn between each landmark, at the junction between dentine and enamel (tab. 2).

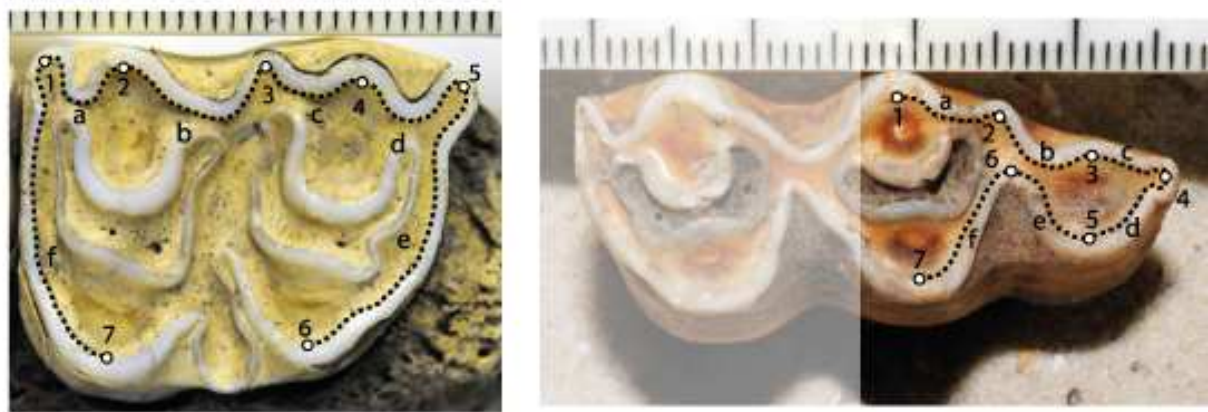


Fig. 2: A: Landmarks (1–7) and semilandmarks (a–f) used for the maxillary M^2 and M^3 (here a M^3 of an archaeological *Bos p. taurus* from the Basel-Gasfabrik sample). B: Landmarks (1–7) and semilandmarks (a –f) used for the M_3 (here a M_3 of an archaeological *Bos p. taurus* from the Basel-Gasfabrik sample). Scale in mm.

We performed a Generalised Procrustes Superimposition (GPS) on all the 2D Cartesian point coordinates of the EFPOS outline configurations to remove the effects of position, size and orientation. The semilandmarks were slid using the Bending Energy method (Bookstein 1996). The GPS produces Procrustes coordinates which can be used as EFPOS' shape parameter. GPS also computes the centroid size of the EFPOS as the square root of the sum of the squared distances from the landmarks and semilandmarks to the centroid of the enamel outline (Bookstein, 1996; Zelditch et al., 2012).

	a	b	c	d	e	f	Total
M^3	23	28	20	22	38	44	175
M^2	19	26	20	21	34	34	154
M_3	21	22	16	15	17	22	113

Tab. 2: Number of semilandmarks (SLM) for each tooth and each curve (a–f). For the position of the curves see fig. 1a/b.

The points and curves digitization were computed from the images using tpsDig V2.17 and the GPS with tpsRelw v1.53 (Rohlf, 2016).

2.2 Assessing taxonomic signals in bovine molars EFPOS size and shape while accounting for age effect and allometry

Differences in centroid size variation of the EFPOS among bovine taxa were tested for the

three molars using Analysis of Variance (ANOVA) with Bonferroni correction for pairwise comparisons (SI1). EFPOS size differences between bovine species, while accounting for size varying with age, were tested for each molar with a single factor Analysis of Covariance (ANCOVA).

EFPOS shape differences between bovine species, while accounting for shape covarying with age and centroid size (allometry), were tested for each of the three molars with a single factor Multivariate Analysis of Covariance (MANCOVA). The MANCOVA assessed whether age and allometry effects differ significantly between the bovine species (interaction) and which of the three molars was the most affected by these two effects (R-squared).

We then assessed which of the three phenotypic markers (M^3 , M^2 , M_3) would provide the greatest taxonomic resolution at the genus, species and population level, and which parameters of EFPOS quantification parameters (the shape, the size-free shape, the age-free shape or the form) would perform the best. The size- and age-free shape parameters are the residuals of the multivariate regression between the Procrustes coordinates as dependent variables and respectively the Log-centroid size and the age scores. The Procrustes from space were obtained by combining the Log-centroid size with the Procrustes coordinates (Mitteroecker and Gunz, 2009; Klingenberg 2015).

The taxonomic resolution (genus, species, and populations) for the three molars was assessed using Linear Discriminant Analysis (LDA). Its predicting approach calculated the percentage of cross validated correct classification (% CVC) of each specimen into its taxonomic group, following a 2 fold cross-validation over 10 000 iterations.

The genus factor comprised four groups: *Bos*, *Bison*, *Bubalus* and *Syncerus*. The species factor comprised the 11 specific groups of our dataset (tab. 1). The population factor was characterized by the 4 modern breeds and 2 archaeological populations (tab. 1). To avoid spurious results of the LDA induced by the high dimensionality of the shape parameters compared to the number of observation, we performed Principal Component Analysis (PCA) for each parameter and used only the PC scores explaining 99% of the total variance as variables in the LDA analysis. On average, 30 PC scores were enough to reach this threshold.

2.3 Assessing the phylogenetic signal in bovine molars EFPOS

To assess the phylogenetic signal of the three molars morphospace, we used the sequences of the complete mitochondrial genome of Bovini, extracted from the NCBI (National Center for Biotechnology Information) for the 11 following taxa (the accession numbers are indicated between brackets): domestic Asian buffalo – *Bubalus arnee bubalis* (JN632607), anoa –

Bubalus depressicornis (EF536351), African buffalo – *Syncerus caffer* (EF536353), American bison – *Bison bison* (JN632601), European bison – *Bison bonasus* (JN632602), domestic yak – *Bos mutus grunniens* (EF494179), domestic cattle – *Bos primigenius taurus* (V00654), zebu – *Bos primigenius indicus* (AY126697), gaur – *Bos gaurus* (JN632604), banteng – *Bos javanicus* (JN632606) and aurochs – *Bos primigenius primigenius* (GU985279).

The sequences were aligned on SeaView version 4 (Gouy et al., 2010). Our final alignment contained 16,284 characters. A Bayesian tree was constructed with MrBayes v3.2.1 (Ronquist et al., 2012) and the GTR+I+G model selected under jModelTest 2.1.7 (Darriba et al., 2012) using the Akaike information criterion. The posterior probabilities were calculated using four independent Markov chains run for 10,000,000 Metropolis-coupled MCMC generations, with tree sampling every 1000 generations and a burn-in of 25%.

To estimate the strength of the phylogenetic signal relative to a Brownian motion model of evolution, we used the *Kmult* method (Adams, 2014), a multivariate generalization of the *K*-statistic (Blomberg et al. 2003). Under a Brownian motion model of evolution the expected *Kmult* value is 1.0. Observed *Kmult* values were evaluated by comparing with the permuted *K*-value from randomized shape data relative to the tree topology of the molecular phylogeny. To visualise the association between the molecular phylogeny and the dental shape space we created a phylomorphospace, which fitted the shape changes from the bovini taxa onto the tree topology using a squared change parsimony approach (Klingenberg and Gidaszewski, 2010).

Morphometric statistics were performed with R (RCoreTeam, 2016), and the libraries MASS (Venables & Ripley 2002) and geomorph (Adams and Otárola-Castillo, 2013).

3. Results

3.1 EFPOS size and shape differences among Bovini accounting for age and allometric effect

The ANCOVA found significant overall differences in the size variation of the EFPOS among the Bovini species (modern and archeological *Bos p. taurus* have been separated) for each tooth (Figure 3, tab. 3, See SI1 for taxa pairwise comparisons for each molar). All three molars displayed the same pattern of size differentiation among taxa, although the

differentiations had a greater resolution for the maxillary molars. *Bos p. primigenius* is significantly bigger than all the other species, while *Bubalus depressicornis* is the smallest. After *Bos p. primigenius*, the bison's M^3 and M^2 were significantly larger than those of other genera. We also observed that Iron Age common cattle were significantly smaller than modern cattle breeds. In most cases there was an overlap in the size range of the remaining taxa.

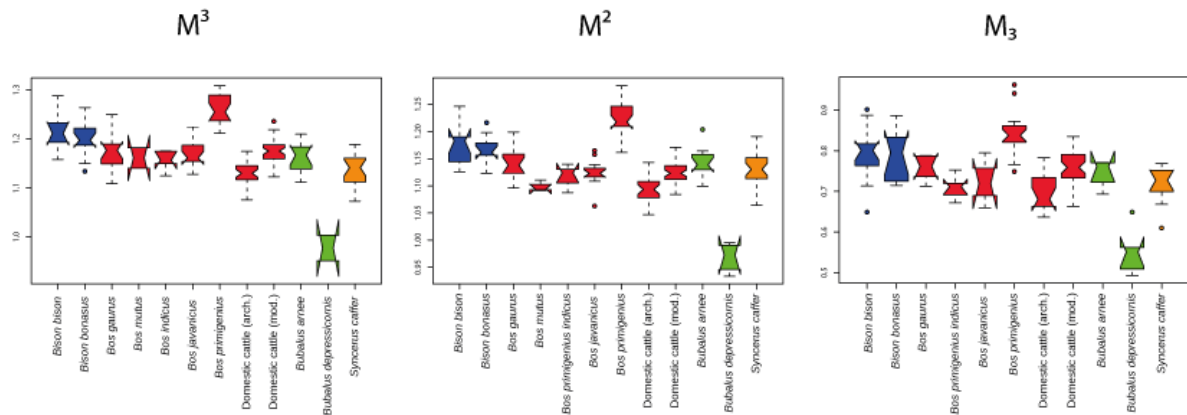


Fig. 3: Box plot of the M^3 , M^2 and M_3 centroid sizes (log) from the tribe Bovini, including modern and archaeological domestic cattle (*Bos p. taurus*).

The size differences among Bovini species are reflected in the strong taxonomic signal in the size variation of the EFPOS which is above 50% (R-squared) for all three molars, while an age effect below 10% was only significant for the third maxillary and mandibular molars (tab.3), common among the species (tab. 3: Age*Taxa).

M^3	Df	SS	MS	R-Squared	F	Z	Pr (>F)	
Age	1	0.04363	0.043632	0.08556	43.5365	2.1454	0.001	**
Taxa	10	0.29316	0.029316	0.57487	29.2515	6.6617	0.001	**
Age*Taxa	9	0.00580	0.000644	0.01137	0.6427	-0.6191	0.753	ns
M^2	Df	SS	MS	R-Squared	F	Z	Pr (>F)	
Age	1	0.00148	0.001481	0.00312	1.9206	0.8700	0.168	ns
Taxa	10	0.33697	0.033697	0.71006	43.7088	7.3857	0.001	**
Age*Taxa	10	0.00968	0.000968	0.02040	1.2558	0.6748	0.266	ns
M_3	Df	SS	MS	R-Squared	F	Z	Pr (>F)	

Age	1	0.03879	0.038795	0.05184	20.25	1.9168	0.001	**
Taxa	9	0.46190	0.051323	0.61718	26.79	6.6697	0.001	**
Age*Taxa	9	0.02931	0.003257	0.03917	1.70	1.1925	0.107	ns

Tab. 3: Results of the single factor ANCOVA test for age effect on the size variation of the M^3 , M^2 and M_3 (Age) and the size differences among Bovini taxa (Taxa) while accounting for size covarying with age (Age*Taxa).

MANCOVA found significant overall EFPOS shape differences among the Bovini species for the three molars (tab. 4: "Taxa"). Patterns of shape variation differ according to the molars (figure 4), but most of the variation was triggered by the differentiation between the *Bubalina* and *Bovina* species. Within the *Bovina* species, the Bison species separate from the *Bos* species. Within the *Bos* species, *Bos p. indicus* separate from *Bos p. primigenius* and *Bos p. taurus*, and *Bos javanicus* and *Bos gaurus* separate significantly, except for the M_3 .

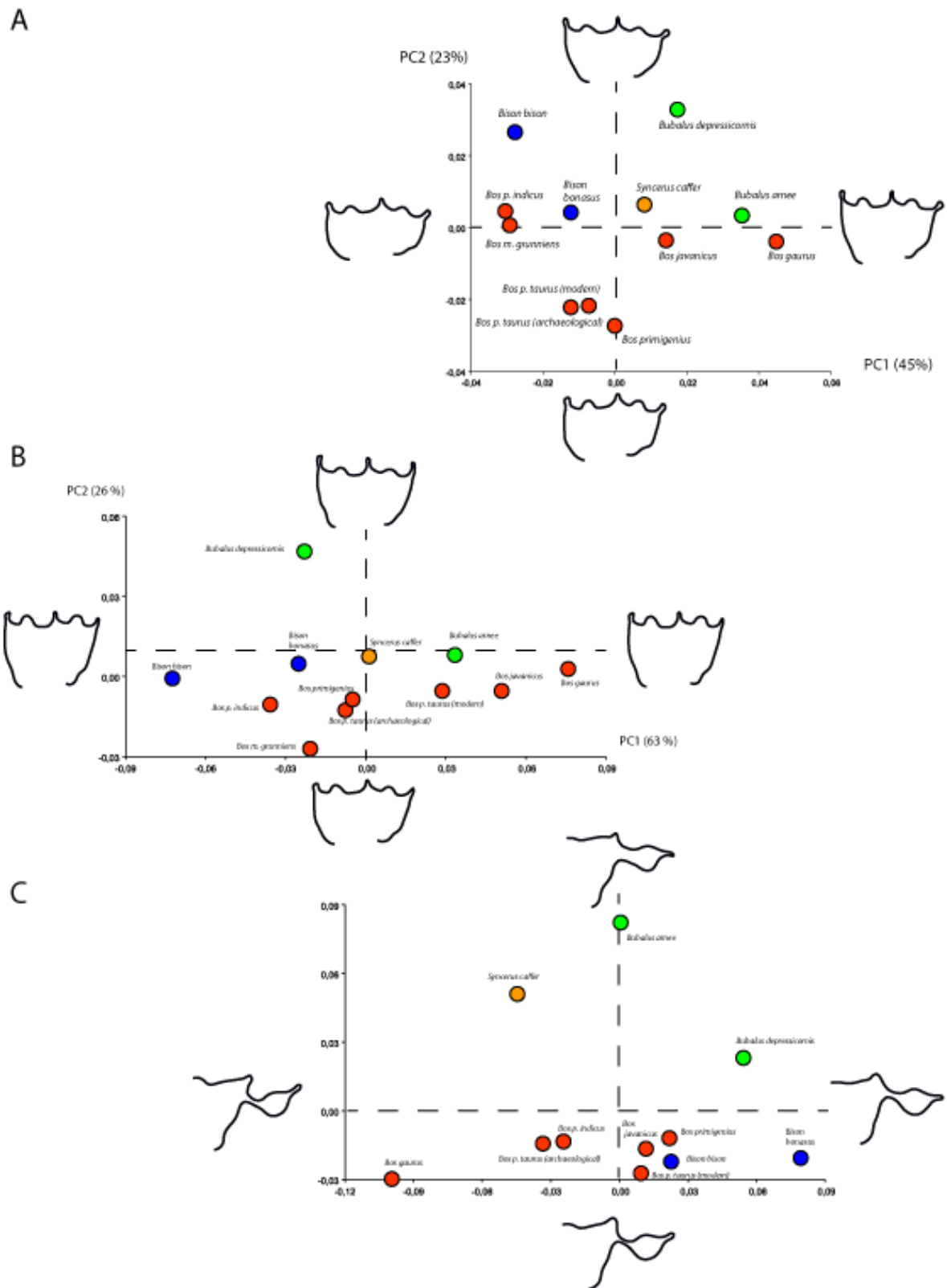


Figure 4: Patterns of shape variation among the Bovini species mean shape for M^3 (A), M^2 (B) and M_3 (C). Shape deformation along the PCs axes are displayed using the shape of specimens at the ends of the range of variability along PC1 and PC2.

333

334 For both third molars, the age related attrition effect accounted for 10% and 12% of the
 335 EFPOS shape variation, while for the second maxillary molar the age effect reach 32% (tab.
 336 4). It was reduced to around 18% by eliminating the youngest and oldest age groups. A
 337 common, and almost common, age effect among species for the third maxillary lower and
 338 mandibular molars for the M^2 (tab.4) was found. We then obtained age-free shape variables
 339 using the residuals of the multivariate regression.

340

M^3	Df	SS	MS	R-Squared	F	Z	Pr (>F)	
Age effect	1	0.12673	0.126734	0.120641	29.579	6.4949	0.001	**
Taxa	10	0.16502	0.016502	0.157089	3.8515	6.2211	0.001	**
Age*Taxa	9	0.04322	0.004802	0.04141	1.1208	0.5946	0.276	ns
M^2	Df	SS	MS	R-Squared	F	Z	Pr (>F)	
Age effect	1	0.45424	0.45424	0.32858	117.1079	6.6222	0.001	**
Taxa	10	0.22299	0.02230	0.16130	5.7489	5.9444	0.001	**
Age*Taxa	10	0.06909	0.00691	0.04998	1.7813	2.0784	0.017	*
M_3	Df	SS	MS	R-Squared	F	Z	Pr (>F)	
Age effect	1	0.25647	0.256474	0.098254	17.5115	5.7224	0.001	**
Taxa	9	0.51961	0.057735	0.199060	3.9420	6.6318	0.001	**
Age*Taxa	9	0.16458	0.018287	0.063051	1.2486	1.1209	0.133	ns

341 Tab. 4: Results of the single factor MANCOVA test for age effect on the shape variation of
 342 the M^3 , M^2 and M_3 , (Age effect) and the shape differences among Bovini species (Taxa) while
 343 accounting for shape covarying with age (Age*Taxa) , where"" denotes interaction.

344

345 The MANCOVA found significant but very weak allometric effects in the M^3 and M_3 shape
 346 variation, explaining less than 5% of the shape variation (tab. 5). Furthermore, this allometric
 347 effect did not interact with the taxonomic signal, which suggests a common allometric effect
 348 on molar shape that does not affect species differentiation. We obtained size-free shape
 349 variables using the residuals of the multivariate regression.

350

M^3	Df	SS	MS	R-Squared	F	Z	Pr (>F)	
Allometry	1	0.02365	0.0236487	0.022512	4.9849	3.1304	0.003	**
Taxa	10	0.17921	0.0179213	0.170598	3.7776	6.1584	0.001	**
Allometry*taxa	10	0.06013	0.0060126	0.057235	1.2674	1.1413	0.128	ns

M ²	Df	SS	MS	R-Squared	F	Z	Pr (>F)	
Allometry	1	0.00756	0.0075614	0.00547	1.2441	0.6824	0.256	ns
Taxa	10	0.28292	0.0282919	0.20465	4.6550	5.2857	0.001	**
Allometry*taxa	10	0.09522	0.0095223	0.06888	1.5668	1.6533	0.041	*
M ₃	Df	SS	MS	R-Squared	F	Z	Pr (>F)	
Allometry	1	0.11535	0.115347	0.044189	7.9699	4.0634	0.001	**
Taxa	9	0.60338	0.067042	0.231150	4.6323	7.3664	0.001	**
Allometry*taxa	9	0.24170	0.026855	0.092593	1.8556	2.9532	0.002	**

Table 5: Results of single factor MANCOVA test for the allometric effect over shape variation of the M³, M² and M₃ as well as shape differences between Bovini species (Taxa) while accounting for shape covarying with size (Log CS), where “*” denotes interaction.

3.3 Taxonomic signals in Bovini cheek teeth shape and form

The taxonomic resolution from the genus to the population levels differed among the phenotypic markers and their morphometric variables, according to the percentage of CVC (tab. 6). Maxillary molars performed better than the mandibular ones in separating the taxa to the genus and species level, which is not surprising since only half of the M₃ could be quantified. The M² shape was the most efficient at discriminating the different genera in bovines with almost 90% correct classifications, closely followed by both third molars. Discrimination of species was still high for the M³ and M² shapes with more than 75% of correct classifications for the M³, but was enhanced in all three molars when using the form variables with M², reaching nearly 85% CVC. For population discrimination the CVC dropped below 50%, with the M₃ providing the best signal (48%). Finally, correcting for age or size effects did not significantly improve the discrimination of genus and species in bovines.

	Shape			Size-free shape		Age-free shape		Form		
	Genus	Species	Population*	Genus	Species	Genus	Species	Genus	Species	Population*
M ³	88.03	75.5	44.12	89.06	75.52	87.5	73.43	88.51	82.82	38.24
M ²	89.95	72.48	30.3	89.42	73.02	89.95	74.61	88.36	84.65	30.3
M ₃	84.18	66.18	48.38	84.89	53.96	84.89	53.24	87.77	66.91	38.71

Tab. 6: Percentage of correct classification after cross validation for M³, M², and M₃ at different taxonomic levels: genus, species and population and according to four phenotypic parameters: Shape, Size-free shape, Age-free shape and Form. *: analysis performed on extant populations of *Bos p. taurus* only.

CVA shows significant divergence (MANOVA: $Pillai=1.6655$, $F=2.9535$, $p<0.0001$) between the ancestral wild shape of the Aurochs and the domestic phenotype of common and zebu cattle, between common and zebu cattle and between modern and archaeological common cattle, with zebu cattle showing the greatest divergence (Figure 5A). The CVC is too low to accurately separate the different populations of modern cattle based on their molar shape (Tab. 6). However, we found significant differentiation patterns (MANOVA: $Pillai=2.423$, $F=1.558$, $p=0.001545$) among modern and archaeological common cattle (Figure 5B), where Iron Age and Bronze Age cattle from Switzerland are within the variation range of modern breeds. In this morphospace, Jersey ancient breeds is the most divergent phenotype. Also, Iron Age and Bronze Age cattle displayed greater phenotypic similarities with Hollaender ancient breeds (SI 2).

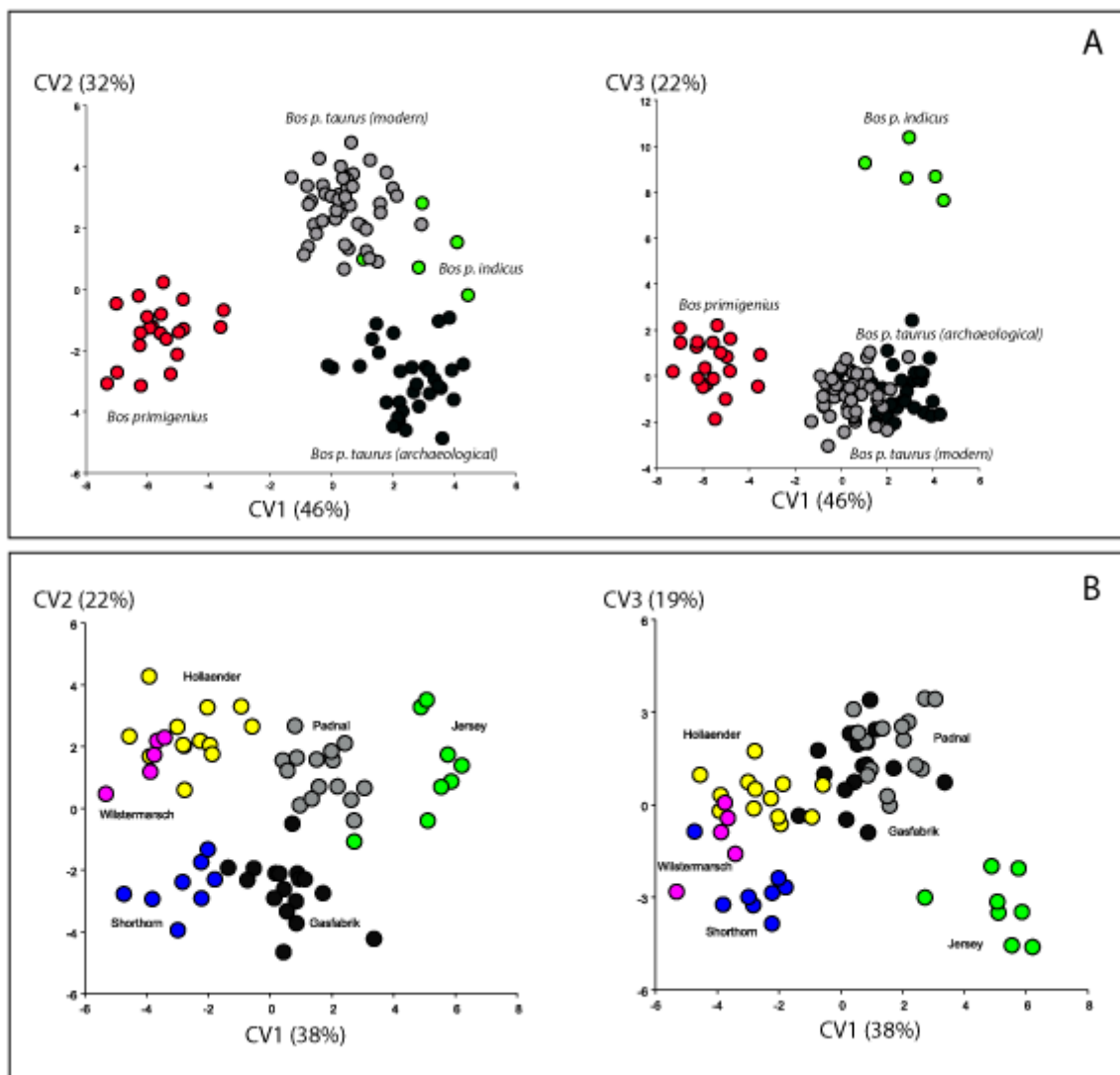


Fig. 5: Patterns of M^3 shape differentiation within the first three canonical factors of the CVA (A) among wild extinct aurochs (*Bos primigenius*) and modern and archaeological domestic cattle (*Bos p. taurus*) and (B) among modern and archaeological common cattle.

3.4 Phylogenetic signal in Bovini molar shape.

The *Kmult* statistics found a low but significant phylogenetic signal in M^3 (*Kmult* observed = 0.206; P-value = 0.009) and M_3 (*Kmult* observed = 0.2781; P-value = 0.003) but not in the M^2 (*Kmult* observed = 0.17; P-value = 0.18). Both third molars phylomorphospace captured the phylogenetic signal separating the Bovinae from the Bubalina and *Bos* from *Bison* species within the Bovina (fig. 6). The low phylogenetic signal in M^3 is driven by the phenotypic relationships of the cattle of south-east Asia – the banteng (*Bos javanicus*) and the gaur (*Bos gaurus*) within the Bubalina, and greater dental shape similarity between the yak (*Bos mutus*) and the humped cattle (*Bos p. indicus*) than with the American bison (*Bison bison*), both being incongruent with the mitochondrial phylogeny (fig. 6A). For the M_3 (figure 6B), the low phylogenetic signal is due to greater phenotypic similarities of *Bos p. primigenius* and the common cattle (*Bos p. taurus*) with the American bison (*Bison bison*), than with the European one (*Bison bonasus*), as well as a greater similarity between the bateng (*Bos javanicus*) and the common cattle (*Bos p. taurus*) than with the gaur (*Bos gaurus*).

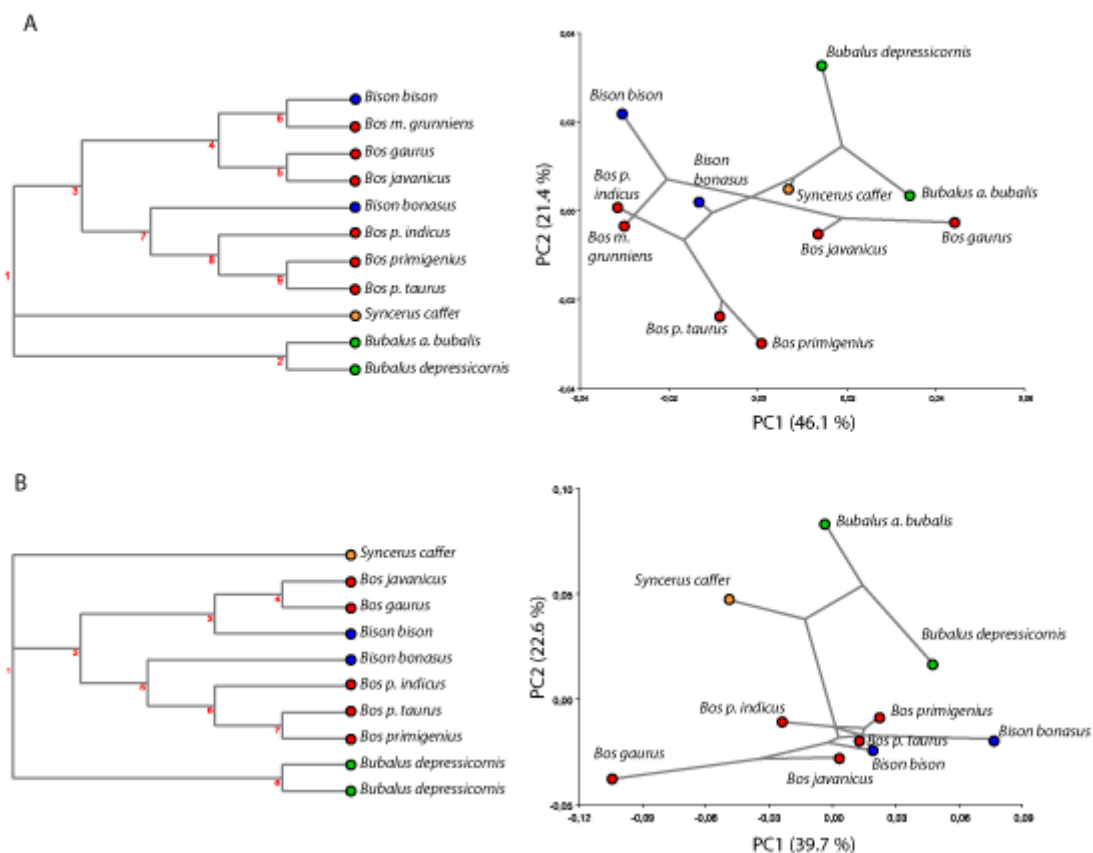


Fig. 6: Bovini MtDNA genome phylogeny and dental shape Phylomorphospace of M^3 (A) and

M₃ (B).

4. Discussion

4.1. Identifying the diversity of the wild progenitors and the domestication process in *Bos* species

Our study has shown that both the EFPOS size and shape variations of the maxillary molars provided reliable taxonomic resolution at the genus and species levels. The resolution to species was not affected by the age effect, or only slightly for the M² shape, and could be increased when using the form parameter, bringing together the taxonomic resolution of both size and shape. These results are congruent with previous studies on South African Bovids (Brophy et al. 2014) and equids (Cucchi et al., 2017) in rejecting the general idea that hypsodont teeth from ruminants are not good taxonomic characters because of the attrition blurring process. As expected the second molar was the most affected by attrition, leading to potential spurious identification. Therefore, we suggest to implementing taxonomic studies on the M³.

Our study also showed that both maxillary and mandibular third molar shape variations had a significant phylogenetic signal among Bovini species, finding phenotypic relationships congruent with the most recent phylogeny (Hassanin, 2014; Hassanin, 2015). Both molars captured the genetic relationship between aurochs (*Bos p. primigenius*) and the two lines of domestic cattle (*Bos p. taurus / indicus*), between the two species of bison (*Bison bison* and *Bison bonasus*) and the yak (*Bos mutus*), and between gaur (*Bos gaurus*) and banteng (*Bos javanicus*). However, the strength of this signal was weakened by the phenotypic relationship between the south-east Asian cattle (*Bos gaurus* and *Bos javanicus*) and the *Bubalina*, as well as the one between *Bos mutus* and *Bos p. indicus* for the M³ and the phenotypic proximity between *Bos javanicus* and *Bos p. taurus* for the M₃. Since we could not make a distinction between the wild and domestic forms of yak (*Bos mutus*), gaur (*Bos gaurus*), and banteng (*Bos javanicus*) for this analysis, it is likely that this incongruence between genotype and phenotype is the consequence of genetic introgression among *Bos* species (Hassanin 2015, Wu et al. 2018).

The high taxonomic resolution up to the species level, the lack of significant impact of attrition in this resolution and the strength of the phylogenetic signal recorded in the M³ clearly suggest that this molar should be preferred for biosystematic studies of Bovini dental series from fossil and archaeological contexts. Since the wild ancestors of domestic cattle are

either extinct (aurochs) or reduced to very small pockets of range distribution, this biosystematic power would prove extremely valuable to document the diversity and distribution of the wild progenitors of domestic forms, from the Pleistocene and early Holocene archaeological records. Applied to all the available dental series of Bovini in the old world, this marker would help illustrate the natural geographic distribution of aurochs variants and test the relevance of the three different sub-species proposed by zooarchaeologists (Clutton-Brock 1989). In south-east Asia, these studies would help map the distribution of the four wild species of *Bos* and understand their geographic diversity and sympatry.

The discrimination and the phylogenetic signal recorded in the M³ between the wild and domestic European forms of Bovini (*Bison bonasus*, *Bos p. primigenius* and *Bos p. taurus*, *Bos p. indicus*) clearly suggest that GMM on M³ could trace the phylogenetic histories of domestic cattle in the core area of the Fertile crescent. However, this approach alone could prove unable to identify the early process of domestication, when high gene flow between wild and domestic populations would prevent the expression of genetically induced phenotypic divergence (Irving-Pease et al. 2018, Vigne 2015). Unless corralling and reproductive control were strong enough to foster the adaptive phenotypic change induced by the anthropic environment (Zohary et al. 1998).

The biosystematic resolution of the M³ could also prove useful to document the subsequent dispersal of domestic forms outside the distribution range of their wild ancestor. For example, the possibility of identifying the two domestic cattle (*Bos p. taurus* and *indicus*) and their relationships with their potential wild progenitor could help provide new clues on the development of herding in Africa, imported from the Near East during the 7th millennium BC (Linseele, 2013; Lesur et al., 2014). According to the latest understanding, the first domestic bovine in Africa are considered to be taurine cattle – zebu seem to occur later, during the 2nd millennium BC – but we still do not know where it entered the continent, although genetic data suggest it happened through the Horn of Africa (Hanotte et al., 2002; Gifford-Gonzalez and Hanotte, 2011).

The scarcity of evidence for zebu in archaeological contexts partly lies in the fact that only limited morphological criteria have been established to identify zebu from taurine specimens (Grigson, 1976; Grigson, 1980). However, these criteria are not especially useful for archaeological material because the study was based on cranial parts that are seldom preserved in ancient contexts. For the moment, only a few studies have suggested the ancient presence of zebu in Africa, and only from the 1st millennium BC (Chaix, 2011; Chaix, 2013). The GMM approach of EFPOS, therefore, provides a new method to address this issue.

However, the remaining difficulty lies in the fact that hybridization between the two species may have occurred very early in Africa (Hanotte et al., 2002; Lesur, 2017). The method now needs to integrate hybridised African specimens to check if we can also discriminate them.

4.2. Identifying the emergence of early cattle morphotype in archaeology

Identifying the emergence of early domestic cattle breeds due to human selective pressure, for traits such as greater milk yield, is in the realm of palaeogenomic studies (Bradley & Magee 2006, Irving- Pease et al. 2018) but not of morphometrics. Indeed, morphometric changes in skeleton bones cannot be directly related with a specific selective pressure (Zeder 2015), such as meat or milk yield. However, documenting the origin and pace of morphometric changes over a large dental series from the 4th millennium BC in the Near and Middle East and from the 3rd millennium BC in Europe until Antiquity, would allow us to pinpoint the timing of the emergence of regional morphotypes due to neutral genetic shifts fostered by geographic isolation, as has already been performed for pigs (Duval et al. 2014, 2018). Once these key locations and periods are identified, targeted paleogenomic analysis could then be more efficiently implemented to explore selective traits within these regional morphotypes.

Our study found clear M³ shape divergence between aurochs, Bronze and Iron Age Switzerland cattle and modern cattle breeds, suggesting that regional morphotypes are clearly attested in this region from the Bronze Age. Furthermore, these archaeological cattle morphotypes show greater phenotypic similarity towards modern cattle breeds than to their aurochsen progenitor, suggesting that great selective breeding in cattle was already in place from the 2nd millennium BC in Europe. But was that selection directed towards adaptation to mountainous regions or towards greater meat or milk production? Only paleogenomic analyses would be able to provide a definite answer. The population signal in the molar shape variation of *Bos p. taurus* is too weak to make safe taxonomic identification. However, phenotypic proximities between the Bronze Age and Iron Age morphotypes with the Hollaender breed (Felius et al. 2011), suggest that these Bronze and Iron Age morphotypes may have been selected for milk production. Other potential explanations could be the genetic consequence of different regional connections between the two sites. Archaeological finds evidence that the Padnal people had connections with the north of Italy and with the north and east of Switzerland (Rageth, 1986). The Basel-Gasfabrik people, on the other hand, had more connections with the west and south of France as well as with the Mediterranean (Spichtig, 2005). This hypothesis will have to be tested with a paleogenetic approach and a large

comparative molar shape analysis of the Bronze and Iron Age domestic cattle sampled from Switzerland and Italy.

CONCLUSION

Our understanding of the origin and trajectories of wild cattle domestication have been mainly driven by the advance in genetic studies of modern and ancient *Bos* species, but zooarchaeologists still need powerful biometric markers to document the timing and mechanisms of cattle domestication in the old world and implement targeted biomolecular studies.

Our GMM approach of the taxonomic and phylogenetic signals encapsulated in the variation of molar morphology of wild and domestic species of the tribe Bovini found that M^3 shape variation could accurately separate the genera and species of both wild and domestic modern specimens of the tribe Bovini with high statistical accuracy, as well as their phylogenetic relationships. But recent genetic introgression between wild and domestic *Bos* species in eastern Asia have partly blurred this phylogenetic signal. The taxonomic resolution at a population level does not reach such a reliable probabilistic classification. However, significant differentiation patterns among aurochs and modern and ancient cattle breeds clearly suggest that genetic divergence indirectly triggered by intentional breeding selection can be deciphered from the archaeological record.

This proof of concept strongly suggest that GMM analysis of M^3 applied on *Bos* remains from Pleistocene and Holocene dental series and through Eurasia would be a useful pre requisite for targeted bio molecular studies for three main reasons: (1) to document the diversity and distribution of wild progenitors of domestic forms; (2) to identify where and when the first domestic phenotype emerged; and (3) to trace the emergence of the earliest regional morphotype and their trajectory towards modern breeds.

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Authors contributions:

TC and BS designed the study, BS collected the morphometric and Age data, TC and BS analyzed and interpreted the data, TC and BS wrote the paper in collaboration with RS, AH, JL and JS.

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