General Palaeontology, Systematics and Evolution (Vertebrate Palaeontology)

Assessing astragalar morphology and biomechanics in western Palaearctic *Bison* populations with geometric morphometrics

Essai d'évaluation de la morphologie et de la biomécanique astragalienne des populations de bisons du Paléarctique occidental par morphométrie géométrique

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ABSTRACT

Having arrived 1.8 Ma ago, bison prevailed in the bovid assemblages of the European sub-continent for more than 1.5 Ma. The current geometric morphometric study outlines a framework of ecomorphological differences among several *Bison* populations of the western Palaearctic, shown by inferences from the tibial and tarsal joint surfaces of their astragalus. Given the principal biomechanical role of this element in the locomotion mechanism, its anatomical features could be linked to diverse functional aspects. In terms of morphological affinity, it is possible to attribute the studied fossil *Bison* astragalar material to several morphological trends. Shape variation is not explained by size differences and is possibly associated with an open-close habitat gradient, as indicated by the presence of expanded or compressed astragali, respectively. This intragroup spatial and temporal phenotypic diversity among the examined populations could indicate a biogeographic segregation influenced by regional climatic and landscape heterogeneity in the European territory during Pleistocene. Furthermore, a relation to habitat-specific locomotor ecology could be supported, revealing forms with increased cursoriality, operating in open biomes and closed-country dwellers as well.

RÉSUMÉ

Apparu il y a 1,8 Ma, le bison a régné dans les assemblages bovins du sous-continent Européen pendant plus de 1,5 Ma. La présente étude morphométrique géométrique propose un cadre de différences écomorphologiques entre plusieurs populations de *Bison* du Paléarctique occidental, révélées par les inférences des surfaces tibiales et tarsiennes de leur astragale. Compte tenu du rôle biomécanique principal de cet élément dans le mécanisme de locomotion, ses caractéristiques anatomiques pourraient être liées à divers aspects fonctionnels. En termes d'affinité morphologique, il est possible d'attribuer plusieurs tendances aux astragales fossiles des *Bisons* étudiés. La variation de forme n'est pas expliquée par une différence de taille et pourrait être associée à un gradient d'habitats ouverts à fermés,

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comme l'indique la présence d'astragales respectivement étirées ou compressées. Cette diversité phénotypique spatiale et temporelle pourrait indiquer une ségrégation biogéographique, influencée par l'hétérogénéité climatique et paysagère régionale, sur le territoire européen pendant le Pléistocène. De plus, une relation avec l'écologie locomotrice propre à l'habitat pourrait être soutenue, révélant des formes ayant une cursorialité accrue, opérant aussi bien dans des biomes ouverts que dans des milieux fermés.

1. Introduction

The relatively abundant and well-preserved fossil record of bovids has repeatedly served as a valuable tool in palaeoecological studies that depend on detecting the relation between morphology and specialized function, providing an illumination of shape variation through time and space (e.g., Barr, 2014, 2015; DeGusta & Vrba, 2003; Kappelman, 1988; Klein et al., 2010; Plummer et al., 2008; Wilson & Reeder, 1993). Several representatives of the genus *Bison* in the Bovinae subfamily have been a predominant component of the Pleistocene herbivore mammal faunas, as shown from numerous fossil sites in the European subcontinent since the beginning of the Late Villafranchian (~1,8 Ma). During the 1.8–1.0 Ma interval, several early members of the genus *Bison* show a wide European distribution, being present in the northern part of the Mediterranean basin and in central, southeastern and western Europe. The first advanced bison or often called "true bison" appeared at the subsequent early/middle Pleistocene transition. The short-horned woodland wisent *Bison schoetensacki* Freudenberg 1914 is known from several German, French, British and Italian sites (Breda et al., 2010; Brugal, 1995; Kahlke et al., 2011; Moullé, 1992; Sala, 1986). It persisted until the middle Middle Pleistocene, shortly after the appearance of the larger broad horn-cored *B. priscus* Bojanus 1827. The latter taxon, often called steppe bison, survived throughout the Late Pleistocene and spread further north and across a vast geographic region of the western Palaearctic, reaching northern Eurasia and even invading mid-latitude northern America (Kahlke, 1999; Shapiro et al., 2004). A phenotypic diversity, driven by climatic fluctuations and landscape-vegetational heterogeneity, is possibly imprinted in the limb structure among primitive and more evolved forms, as previous studies have already partly shown (e.g., Maniakas and Kostopoulos, 2017). Here, a morphological framework for interpreting part of the possible spatial and temporal variation in the astragalar morphological and biomechanical features of several Western Palaearctic bison is attempted. Ecomorphological patterns, in terms of functionally related habitat signals, are investigated and visualized via the application of geometric morphometrics.

2. Material and methods

2.1. Biomechanical background of the astragalus

The skeletal postcranial element employed in this analysis is the bovid astragalus. Astragali are the most commonly preserved bones in the fossil record due to their favorable taphonomic properties (Barr, 2015), providing a quite robust statistical sample. In terms of functional morphology, this limb component is strongly involved in the locomotion mechanism and thus, its morphological properties would be linked to diverse locomotor aspects, at least to some extent, as have been suggested by some recent studies of taxon-free habitat reconstructions (Barr, 2014, 2015; DeGusta and Vrba, 2003; Plummer et al., 2008, 2015). Research on astragalar ecomorphology has been merely based on multivariate analyses of raw (e.g., DeGusta and Vrba, 2003; Plummer et al., 2008, 2015) and size-corrected measurements (e.g., Barr, 2014, 2015). This multi-levelled bone is considered as a principal part of a rather complex structure (Fig. 1a), i.e., the double-trochleated hinge joint between the metatarsus and the tibia that functions in the plantar and dorsal flexion of the foot (Barr, 2014). This complex also includes the calcaneus, the cubonavicular bone and the distal tibia, transporting the animal's weight via a "cam-like mechanism" (sensu Schaeffer, 1947) or the "four-bar linkage hock joint system" described by Alexander and Bennett (1987). The latter is involved in the motion to the anteroposterior direction across the upper ankle joint between the tibia and the astragalus and the transverse tarsal joint between the astragalus and the cubonavicular under the stabilizing action of the lower ankle joint between the astragalus and the calcaneus (Schaeffer, 1948). However, the functional role of the astragalus and its relation to habitat preference is debatable. A recent study (Klein et al., 2010) suggested that this skeletal element contributes with rather limited power in ecomophological studies, as the observed shape variation is mainly affected by differences in body size and phylogeny. Other authors (e.g., Barr, 2014, 2015), while controlling for body size, have linked its morphology to a habitat-specific locomotion under several functional hypotheses.

2.2. Studied localities and species

The studied fossil material studied, consists of 83 astragali, representing populations of various taxa of the genus *Bison* from the early, middle and late Pleistocene of southwestern, southeastern and central-northern European region (Fig. 2). Specimens of Early Pleistocene bison were obtained from three key localities, which bear fossil mammal assemblages with similar faunal characteristics. *Bison* astragalar remains, referred to *Bison* sp., were derived from the localities of Venta Micena, located at the margins of the Guadix-Baza basin in southeastern Spain (Moyà-Solà, 1987) and Apollonia, in Mygdonia basin in northern Greece (Kostopoulos, 1997). Both are dated between 1.2–1.6 Ma with Venta Micena considered slightly older than Apollonia
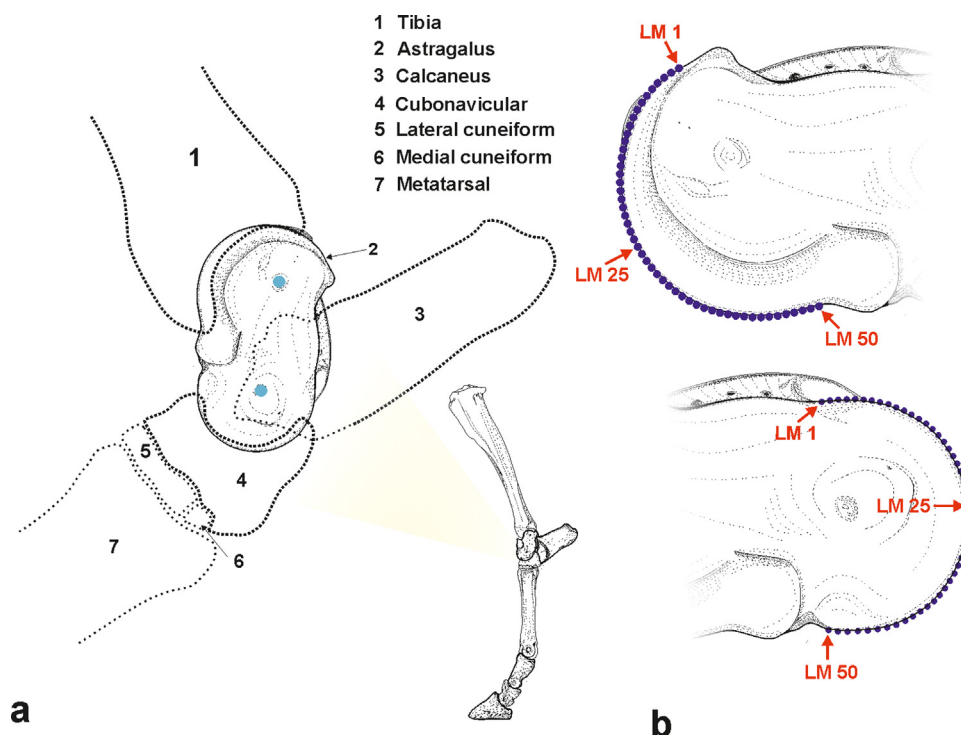
Fig. 1. a: schematic drawing of the position of the bovid astragalus and other elements involved in the "cam-like mechanism" joint system (sensu Schaeffer, 1947) in bovid hindlimb, served for the plantar and dorsal flexion of the foot; **b**: curve and semilandmarks configuration description on the proximal and distal medial trochlear margins of the astragalus.

Fig. 1. a : Schémas de la position de l'astragale bovin et d'autres éléments impliqués dans le système articulaire (sensu Schæffer, 1947) du membre postérieur des bovidés, servant à la flexion plantaire et dorsale du pied ; **b** : configuration de la courbure et des semilandmarks sur les marges trochléaires médiales proximales et distales de l'astragale.

(Kahlke et al., 2011; Kostopoulos, 1997; Martínez-Navarro et al., 2011; Moyà-Solà, 1987). Untermassfeld in Thuringia of central Germany is correlated to the Jaramillo event, dated at ∼1.05 Ma (Kahlke, 2006; Kahlke et al., 2011). The *Bison* form of this locality is assigned to the species *Bison menneri* Sher 1997. Material of the middle Pleistocene *Bison schoetensacki* comes from two roughly contemporaneous localities, Isernia La Pineta in southern Italy (dated at 0.62 Ma; Coltorti et al., 2005) and Süssenborn in Thuringia of central Germany (dated to ∼0.65 Ma; Kahlke et al., 2011). The samples of the late Pleistocene *Bison priscus* specimens, attributed to the Last Interglacial period and dated approximately to ∼0.1 Ma (Currant and Jacobi, 2001; Flerov, 1977; Lewis et al., 2011), are derived from the localities of Taubach in central Germany and several fossil sites of the rich fossiliferous Joint Mitnor Cave Mammal Assemblage Zone in the south-southwestern part of England (Barrington, Joint Mitnor Cave, Waterhall Farm) (Fig. 2). A comparative dataset for the modern European bison, *Bison bonasus* (*n* = 8) from the population in the National Park of Bialowieza Primeval Forest (Eastern Poland) was provided by G. Konidaris (University of Tübingen).

2.3. Methodology

The geometric analysis dataset was originally derived from 2D photographical documentation of different facets of the astragali sampled (images taken orthogonally to the surfaces), preferably complete and mature specimens, excluding those with obvious pathology (only slightly damaged specimens were considered for analysis). Outlines were used to create separate curves of 2D coordinates along the radii of circles fitting to the condylar margins (Fig. 1b) on the proximal and distal articular surfaces of the medial astragalar side, respectively, with the aid of data acquisition software tpsDig v2.17 (Rohlf, 2013). The analysis of each anatomical region rather than of the astragalus medial side as a single unit was preferred due to the presence of fragmentary material. The recorded curves, either on the right or the left half of the medial side of the astragalus, were resampled from a homologous starting and ending position (related to a combination of Type I and Type III landmarks, sensu Bookstein, 1991) granting the same number of evenly spaced 50 points ("*by length*" option in tpsDig) across all specimens. Afterwards, the operation of converting into an equal number of defined and digitized equispaced landmarks was conducted with the employment of the tpsUtil v1.58 (Rohlf, 2013). On the proximal end, the fist landmark (LM 1) and the last landmark (LM 50) correspond to the most proximo-anterior and the most distoposterior points across the medial margin of the tibial trochlea (Fig. 1b). On the distal end, the first landmark (LM 1) is taken at the most (proximo) anteriorly projecting point, while the last landmark (LM 50) corresponds to the most (disto) posterior point of the transverse medial margin that runs along the tarsal joint surface (Fig. 1b). Those extreme points define
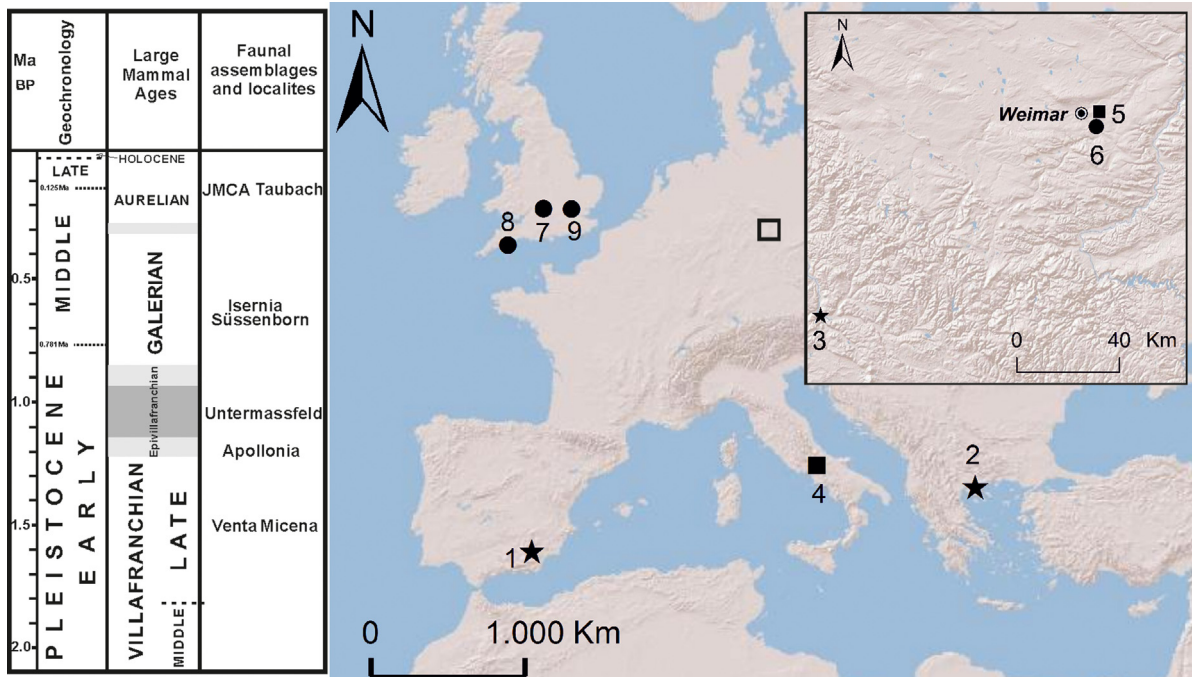
Fig. 2. Time scale and geographic location of selected fossil localites mentioned in the text. Lower Pleistocene localites (dark stars). 1. Venta Micena (Spain). 2. Apollonia (Greece). 3. Untermassfeld (Germany). Middle Pleistocene localities (squares). 4. Isernia La Pineta (Italy). 5. Süssenborn (Germany). Upper Pleistocene localities (circles). 6. Taubach (Germany), Joint Mitnor Cave Mammal Assemblage Zone sites (UK). 7. Barrington. 8. Joint Mitnor Cave. 9. Waterhall Farm.

Fig. 2. Répartition temporelle et géographique des localités fossilifères sélectionnées, mentionnées dans le texte. Localités du Pléistocène inférieur (étoiles noires). 1. Venta Micena (Espagne). 2. Apollonia (Grèce). 3. Untermassfeld (Allemagne); localité du Pléistocène moyen (carrés). 4. Isernia La Pineta (Italie). 5. Süssenborn (Allemagne) ; Llocalités du Pléistocène supérieur (cercles). 6. Taubach (Allemagne). Zone à assemblages mammaliens de la caverne Joint Mitnor (Royaume-Uni). 7. Barrington. 8. Joint Mitnor Cave. 9. Waterhall Farm.

the tangent direction along which, the 48 semilandmarks between them (designated via a 'sliders' file) are allowed to slide with respect to the corresponding curve based on the minimum bending energy criterion (Bookstein, 1997; Gunz and Mitteroecker, 2013). The 50 landmarks and semi-landmarks raw configurations (i.e., their 100 and 100 *x*, *y* coordinates) were transformed, after alignment, rotation and scaling (removal of isometric size effect) with Procrustes superimposition (Kendall, 1984; Rohlf and Slice, 1990; Slice, 2001) in tpsRelw v1.49 (Rohlf, 2010), allowing to characterize the morphospace of the compared astragali.

Subsequent principal component analyses (PCA, i.e. the relative warp analysis in geometric morphometrics) were performed on the Procrustes shape coordinates in order to summarize morphometric variation and mean shapes of each aspect in the reduced dimensional space of the original dataset obtained within the sample at the population level. One metric of overall dissimilarity among groups that defines the distances in Kendall's shape space as an absolute magnitude of the shape deviation, was computed and considered. Procrustes distance (D_{Pr}) was employed for the quantification of mean shape differences among the analyzed populations (computed as the square root of the sum of squared distances between the corresponding landmarks of two superimposed shapes at centroid size, see Bookstein, 1991). Centroid size, defined

as the squared root of the sum of squared distances of a configuration of landmarks from their centroid ("gravity center") (Bookstein, 1991), was used as a size proxy, thus a measure of relative dimensions of the specimens. In order to explore possible size reflection in the observed morphological variability, we performed ordinary least-squares regressions analyses of $\log_{10}$-transformed centroid sizes onto the first three principal components for each margin. Furthermore, univariate analysis of variance (ANOVA), coupled with Tukey's Honest Significant Difference (HSD) multi-comparison tests after the Tukey-Kramer modification for unequal sample sizes, has been used to identify sources of significant difference in size (centroid size) and shape (PC scores) between samples.

The analysis of the size and shape variables and visualization of mean shape differences were carried out in Paleontological Statistics (PAST) (Hammer et al., 2001), IBM Statistical Package for Social Sciences (SPSS) 20 and MorphoJ (Klingenberg, 2011).

Institutional abbreviations and involved samples − **AUTH**: Museum of Geology and Paleontology, School of Geology, Aristotle University of Thessaloniki, Greece (Apollonia, **APL**); **HEP**: Senckenberg Center for Human Evolution and Paleoenvironment, Archaeozoology collection, University of Tübingen (Bialowieza forest *Bison bonasus*, **EMB**); **IPHES**: Catalan Institute of Human Paleoecology and Social Evolution, Museum of Prehistory and Palaeontology of Orce
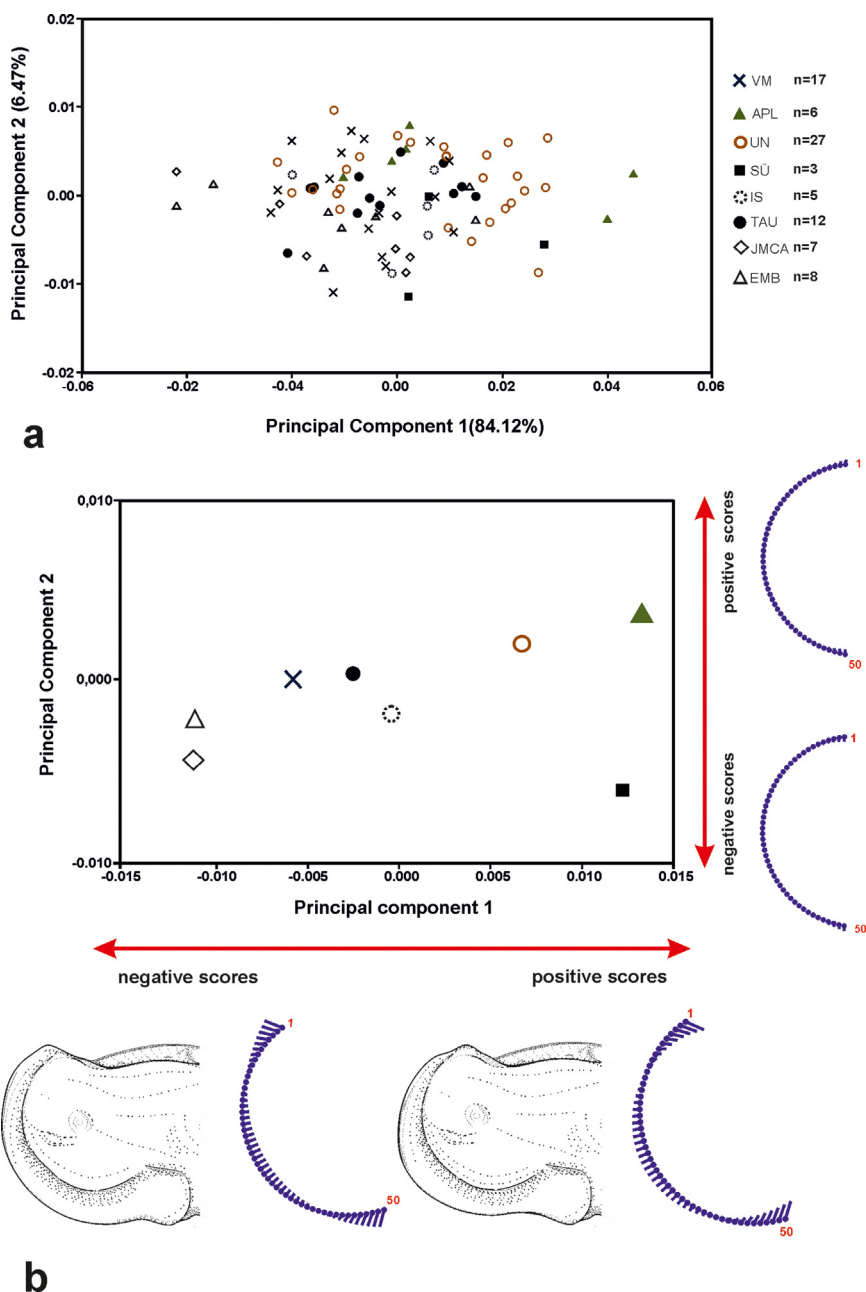
Fig. 3. Results of the principal component analysis for the 50 LMs shape coordinates on the proximal trochlear margin of the astragalus (**a**) and the visualization of the observed mean shape differences ("lolipop" graphs) among the groups (*n* = 85) along the two first principal components (**b**), which account for nearly 91% of the original variance in terms of landmarks positions.

Fig. 3. Résultats de l'analyse en composantes principales pour les 50 LMs coordonnées sur l'extrémité trochléaire proximale de l'astragale (**a**) et visualisation des différences moyennes de forme observées (graphiques « lolipop ») parmi les groupes (*n* = 85), selon les deux premières composantes principales (**b**), 91 % de la variance initiale en termes de position des *landmarks*.

in Granada, Spain (Venta Micena, **VM**); **IQW**: Senckenberg Research Station of Quaternary Palaeontology, Weimar, Germany (Untermassfeld, **UN**; Taubach, **TAU**; Süssenborn, **SÜ**); **NHM**: Natural History Museum of London (Joint Mitnor Cave Assemblage Zone sites, **JMCA**); **UNIFE**: Department of Biology and Evolution, University of Ferrara, Italy (Isernia La Pineta, **IS**).

3. Results

In terms of shape, the anatomical descriptions are restricted to the features that represent roughly the general morphological spectra of the astragali across different localities. The PC analyses of the selected characters showed an overall slight distinction between bison
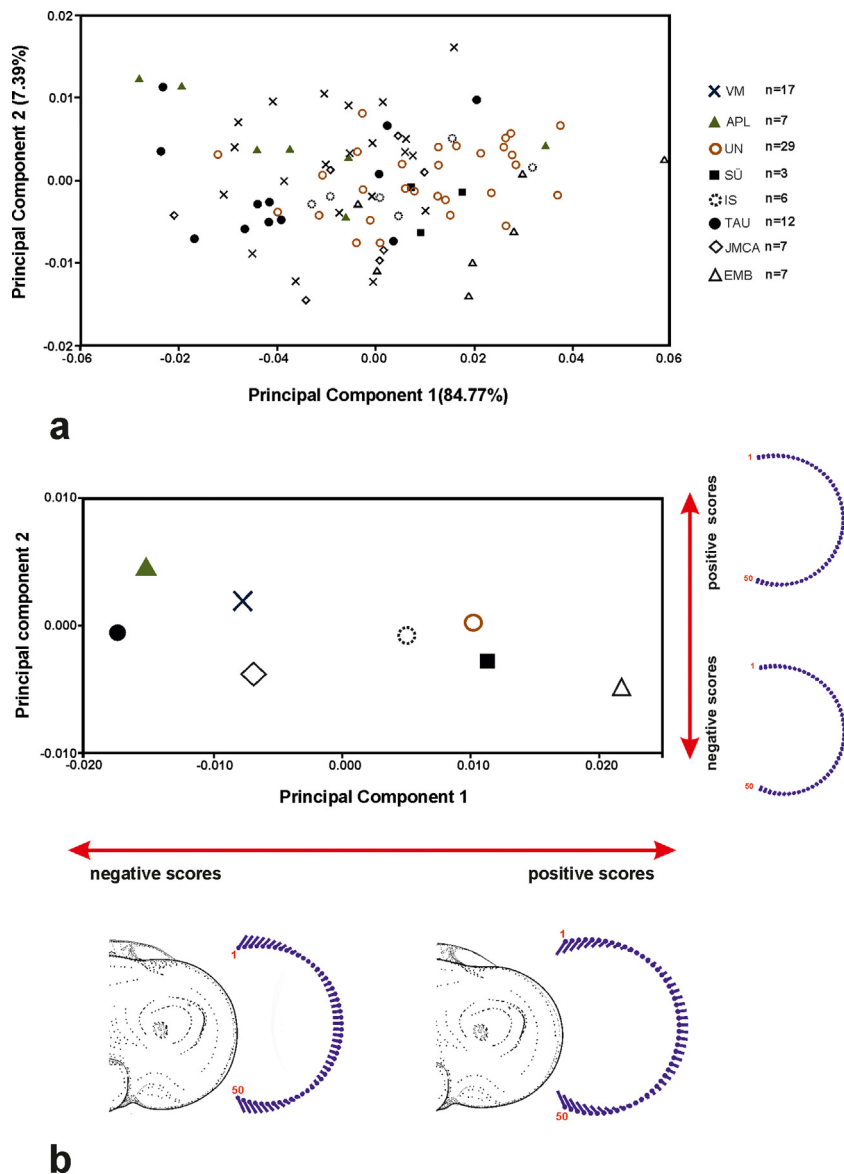
Fig. 4. Results of the principal component analysis for the 50 LMs shape coordinates on the distal trochlear margin of the astragalus (**a**) and the visualization of the observed mean shape differences ("lolipop" graphs) among the groups (*n* = 88) along the two first principal components (**b**), which explain nearly 92% of the original variance in terms of landmarks positions.

Fig. 4. Résultats de l'analyse en composantes principales pour les 50 LMs coordonnées sur l'extrémité trochléaire distale de l'astragale (**a**) et visualisation des différences moyennes de forme observées (graphiques « lolipop ») parmi les groupes (*n* = 88), selon les deux premières composantes principales (**b**), ce qui explique approximativement 92 % de la variance originale en termes de position des *landmarks*.

originating from different time frames and geographical regions, including both early and later forms. This separation is characterized by an intergroup overlapping; resulting significantly in the compactness of the clusters and therefore it was necessary to average observations for each group (Figs. 3b and 4b). The two first principal components (PC1 and PC2) convey a significant amount of the variance expressed by the 50 variables generated from the examined Cartesian coordinates, together explaining over 90% of the total variance for the proximal and distal articulation margins, respectively (Figs. 3 and 4). Given the considerable amount of variation accounted by the

first axis in both analyses, in terms of landmarks positions, it is reasonable to consider that shape differences among the studied taxa are primarily explained by this component.

In the analysis of the proximal astragalar facet in the upper ankle joint, the first component, summarizing approximately 84% of the variation in the sample (Fig. 3a), is defined by the movement of landmarks positioned on the most proximo-anterior and the most distoposterior extremities, respectively and also those outlining the central area of this radius (Fig. 3b). A significant range of variation is evident along this axis regarding the mean

Table 1

Procrustes distances (D_{Pr}) observed by pairwise comparisons among the studied bison populations regarding the mean shape differences of the proximal (above the diagonal) and the distal (below the diagonal) medial astragalar margins.

Tableau 1

Distances Procuste (D_{Pr}) observées par comparaison des populations de bisons étudiées au regard des différences moyennes de forme des extrémités astragalaires médiales proximales (au-dessus de la diagonale) et distales (en dessous de la diagonale).

|	VM	APL	UN	IS	SÜ	MIS5e	TAU	EMB
VM	–	0.0195	0.0127	0.0078	0.0192	0.0074	0.0041	0.0068
APL	0.0082	–	0.0071	0.0148	0.0111	0.0255	0.0164	0.0249
UN	0.0182	0.0259	–	0.0089	0.0104	0.0188	0.0097	0.0184
IS	0.0218	0.0213	0.0061	–	0.0144	0.0117	0.0059	0.0112
SÜ	0.0300	0.0287	0.0090	0.0092	–	0.0237	0.0161	0.0242
MIS5e	0.0070	0.0127	0.0179	0.0129	0.0194	–	0.0106	0.0039
TAU	0.0103	0.0062	0.0279	0.0229	0.0303	0.0119	–	0.0100
EMB	0.0306	0.0386	0.0135	0.0184	0.0151	0.0290	0.0398	–
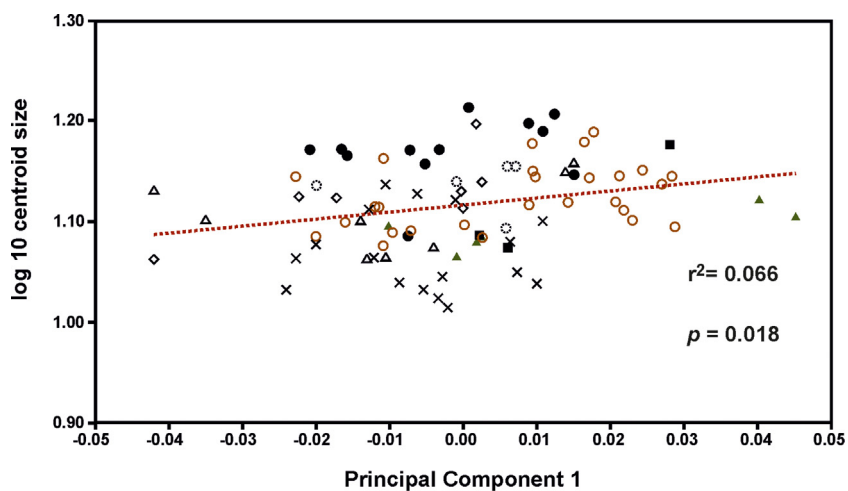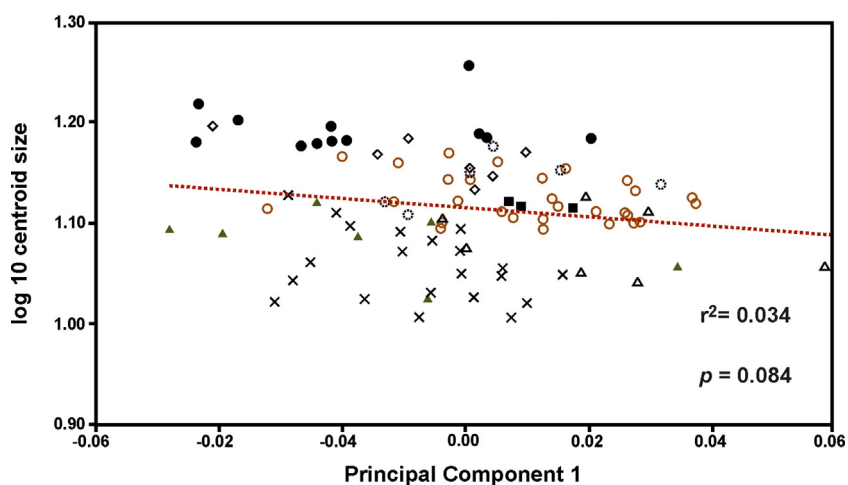
Fig. 5. Regression analyses of $\log_{10}$-centroid size and the first principal component for the (**a**) proximal and (**b**) distal astragalar facets.

Fig. 5. Droite de régression du premier axe de l'analyse (PC1) en fonction de la $\log_{10}$-taille centroïde des facettes proximales (**a**) et distales (**b**) de l'astragale.

shape differences of this aspect (Fig. 3a, b), as shown by the D_{Pr} values (Table 1). The astragalus of the Apollonia bison and *Bison schoetensacki* population from Süssenborn (high positive values along PC1) are characterized by a proximodistal (superior-inferior) compression and an anteroposterior deepening of the articular surface for the distal tibia. This morphological feature, also describes, to a lesser degree, the astragalus of the other Epivillafranchian form, *Bison menneri* from Untermassfeld. The mean configuration found in the astragali of the British *Bison priscus* along that of *Bison bonasus* seem to segregate them from the aforementioned general pattern, exhibiting a wider proximal joint surface (low PC1 negative values). A rather intermediate morphological condition could possibly been depicted by the astragali of the Taubach priscoid bison, *Bison schoetensacki* from Isernia La Pineta and, to some extent, the Venta Micena bison. PC2 represents only minor variation (accounting for 6.5%, Fig. 3a), reflected primarily in the extremities of the proximal articular surface (Fig. 3b). The astragali of the Apollonia bison fall mostly within the morphospace defined by the positive values, whereas the British *Bison priscus* and the *Bison schoetensacki* from Süssenborn tend towards the opposite direction of this component.

A different pattern is apparent in the analysis of the distal trochlear margin on the medial side of the astragalus (Fig. 4a, b, Table 1). Regarding the morphological traits exposed, it appears that sample overlapping is again detectable rather than distinctive morphotypes, as visualized along the PC1 axis (summarizing ~85% of total variation) and indicated by the mean shape differences expressed in D_{Pr} values. The position of the modern wisent corresponds to forms that score mostly positively on the first component. The relative displacement of landmarks (Fig. 4b) reflects a more obvious anteroposterior compression and the proximodistal expansion of the trochlear margin along the transverse tarsal joint. Furthermore, one group, consisting of *B. menneri* (Untermassfeld) and the middle Pleistocene *B. schotensacki* (Isernia La pineta, Süssenborn) is distributed towards the positive end of

PC1. The priscoid bison from Taubach and the Apollonia bison tend to be placed on the other edge of this morphological spectrum (PC1 negative scores), displaying a relatively anteroposteriorly broader and shortened distal articular radii. The early Pleistocene astragali of Venta Micena bison and the British priscoid form seem to resemble, in a lesser extent, the above anatomy in their distal astragalar facet. Similarly, the second component does not seem particularly diagnostic as the majority of the studied populations falls close to the origin of PC2, possibly with the exception of the Apollonia bison (positive scores), the British priscoid bison and the European wisent (negative scores). The slight morphological variation along PC2 (explaining ~7.4%, Fig. 4a) reflects the anteroposterior relative displacement of landmarks, mainly on the posterior edge of the distal astragalar facet (Fig. 4b).

Size-shape relationship is given in the linear regressions of PC1 with centroid size for both margins (Fig. 5, plots of the rest PCs not shown here). In the studied material, shape was not found to be a function of size given that there is no significant correlation between centroid size and the first component and that the comparison of the studied populations revealed clear size differences (Table 2). A set of analysis of variance (ANOVA, pairwise comparison tests among the samples of different localities) was conducted on the first component scores derived by the PCA on the Procrustes coordinates (Table 3). The observed shape variation of the two astragalar facets along PC1 is supported by significant ANOVA results ($P < 0.05$). Regarding the proximal margin, significant intergroup differences are not evident based on the Tukey-HSD pairwise comparison, however due to the conservative character of the test; differences could be suggestively detected between the Apollonia bison in relation to *B. bonasus* (Table 3). On the other hand, multiple comparisons succeed to distinguish the distal margin shape of several populations. More particularly, the Tukey-HSD test identifies differences between *B. menneri* and the other Early Pleistocene bison from Venta Micena and Apollonia and the *B. priscus* from

Table 2
(a) Analysis of Variance (ANOVA) on centroid size differences and **(b)** results of pairwise comparison (Tukey-HSD) tests among bisons of different Pleistocene localities for the proximal (above the diagonal) and the distal (below the diagonal) medial astragalar margins, respectively (bold values indicate significant differences at $P < 0.05$ confidence level). SS: sum of squares; MS: mean square; *F*: F statistical value; *P*: significance of *F*.

Tableau 2
(a) Analyse de variance (Anova) des différences de tailles centroïdes et **(b)** résultats des tests de comparaison (Turkey-HSD) par paire de bison de différentes localités du Pléistocène pour le bord astragalaire médial proximal (au-dessus de la diagonale) et distal (en dessous de la diagonale) (Les valeurs de *p* en gras indiquent des différences significatives, < 0,05). SS : somme des carrés ; MS : moyenne des carrés ; *F* : valeur de F ; *p* : significativité de *F*.

a		SS		MS		F		p
Proximal margin		76.808		10.973		9.903		**0.000**
Distal margin		173.965		24.852		37.355		**0.000**

b	VM	APL	UN	IS	SÜ	JMCA	TAU	EMB
VM	–	0.907	**0.000**	**0.011**	0.526	**0.012**	**0.000**	0.280
APL	0.527	–	0.418	0.448	0.988	0.595	**0.000**	0.995
UN	**0.000**	**0.015**	–	0.999	0.999	0.1000	**0.003**	0.873
IS	**0.000**	**0.005**	0.826	–	0.988	1.000	0.436	0.816
SÜ	**0.025**	0.635	1.000	0.898	–	0.999	0.139	1.000
JMCA	**0.000**	**0.000**	**0.006**	0.679	0.135	–	0.110	0.931
TAU	**0.000**	**0.000**	**0.000**	**0.001**	**0.000**	0.147	–	**0.001**
EMB	0.565	1.000	**0.012**	**0.005**	0.611	**0.000**	**0.000**	–

Table 3
(a) Analysis of Variance (ANOVA) on PC1 (RW1) scores and **(b)** results of pairwise comparison (Tukey-HSD) tests among bisons of different Pleistocene localities for the proximal (above the diagonal) and the distal (below the diagonal) medial astragalar margins, respectively (*P*-values < 0.05 indicate significant differences, in bold). SS: (between groups) sum of squares; MS: mean square; *F*: F statistical value; *P*: significance of *F*.
Tableau 3
(a) Analyse de variance (Anova) de PC1 (RW1) et **(b)** résultats des tests de comparaisons (Turkey-HSD) par paire de bison de différentes localités du Pléistocène pour le bord astragalaire médial proximal (au-dessus de la diagonale) et distal (en dessous de la diagonale). (Les valeurs de *p* en gras indiquent des différences significatives, <0,05.) SS : somme des carrés ; MS : moyenne des carrés ; *F* : valeur statistique *F* ; *p* : significativité de *F*.

a		SS		MS		*F*		*p*
Proximal margin		0.005		0.001		3.056		**0.007**
Distal margin		0.014		0.002		6.175		**<0.001**

b	VM	APL	UN	IS	SÜ	JMCA	TAU	EMB
VM	–	0.190	0.179	0.997	0.599	0.994	0.999	0.991
APL	0.980	–	0.984	0.838	1.000	0.111	0.494	0.087
UN	**0.016**	**0.024**	–	0.982	0.999	0.140	0.701	0.096
IS	0.776	0.464	0.998	–	0.954	0.937	1.000	0.923
SÜ	0.670	0.396	1.000	1.000	–	0.385	0.834	0.354
JMCA	1.000	0.988	0.323	0.930	0.822	–	0.936	1.000
TAU	0.798	1.000	**<0.001**	0.196	0.210	0.910	–	0.915
EMB	**0.007**	**0.005**	0.786	0.699	0.989	0.067	**<0.001**	–

Taubach as well. Furthermore, significant differences are observed between the three aforementioned bison populations and the European wisent (Table 3).

4. Discussion and conclusions

Regarding the geometric patterns revealed on the medial side of the astragalus, several shape trends are mainly recognized based on the combined morphological traits of each examined anatomical region (Fig. 6). Furthermore, a significant overlapping of the majority of the examined bison populations is apparent; however mean shape changes along PC1 do not seem to follow size variability and possibly are associated with an open-close habitat gradient. A moderate anteroposterior shortening and proximodistal deepening of the proximal and distal trochlear radii, respectively, is roughly represented by the astragali of the Epivillafranchian bison form *B. menneri* and the *B. schoetensacki* representative of the middle Pleistocene from Süssenborn (Fig. 6). Part of the studied extinct and extant *Bison* material was assigned exclusively to the same part of the exposed morphological spectra. More specifically, the early Pleistocene bison from Apollonia seems to be placed alongside the former group, according to the shape of the proximal trochlear margin, but resembles the priscoid bison from Taubach based on the extensive broadening of the distal margin (Fig. 6). On the other hand, the reverse configuration, in relation to the one described for the Apollonia bison, is uncovered in the modern wisent, the relatively wide and shallow proximal astragalar trochlea of which also corresponds to the morphology of the astragalus of the British Last Interglacial *B. priscus* population (Fig. 6). Concerning the shape of the two astragalar margins on the rest of the material, a rather intermediate morphological pattern can be distinguished for the *B. schoetensacki* population from Isernia La Pineta. The astragali of the Venta Micena primitive form are characterized by a fairly wide and shallow proximal trochlea, whereas a more prominent anteroposterior broadening can be exposed in the distal part of the bone (Fig. 6).

It is questionable how the spatial and temporal phenotypic diversity could be ascribed to locomotor adaptations and linked to discrete habitats. In the study of DeGusta and Vrba (2003), astragali of forest and heavy cover taxa appeared to be anteroposteriorly compressed, whereas light and open cover taxa show anteroposteriorly expanded astragali. Barr (2014, 2015) claimed that open-country bovids have relatively short astragali in comparison to closed-country bovids, contributing in an increased range of angular excursion. Furthermore, according to these and other authors (e.g., Hildebrand and Goslow, 2001; Plummer et al., 2008, 2015) the measured distal trochlear radius appears to distinguish successfully the open and closed habitat dwellers, as it tends to be greater in open-country bovids in comparison to closed-country forms. Their work has shown that, apart from distal limb relative elongation, increased stride cycles can be achieved via this morphological arrangement, as it hypothetically provides a wider rotational arc and, thus, a correspondingly increased motion range and overall limb velocity.

These assumptions could be also applied on the current findings. Consequently, even within the general restricted locomotor repertoire of bison, it is possible to segregate more and less cursorial forms. In this sense, the shape of the distal astragalar trochlea of Venta Micena and Apollonia primitive bison and the two priscoid bison of the Last Interglacial could be related with an increased medial depth of the tarsal articulation (Figs. 4 and 6), an adaption associated with rapid locomotion and operating in relatively open environments. This grouping is fairly consistent with that reported in a previous study by Maniakas and Kostopoulos (2017), where according mainly to the dental mesowear signature and the relative metapodial shape, the late Villafranchian/Epivillafranchian bison from Apollonia has been found to be suited to overall open and dry habitats with well-developed grassy cover. The rather increased anteroposterior distal astragalar mobility of the Venta Micena bison could be even compatible with the presence of a partially wooded open savanna, as inferred by biogeochemical and ecomorphological data (see Mendoza et al., 2005, Palmqvist et al., 2008).
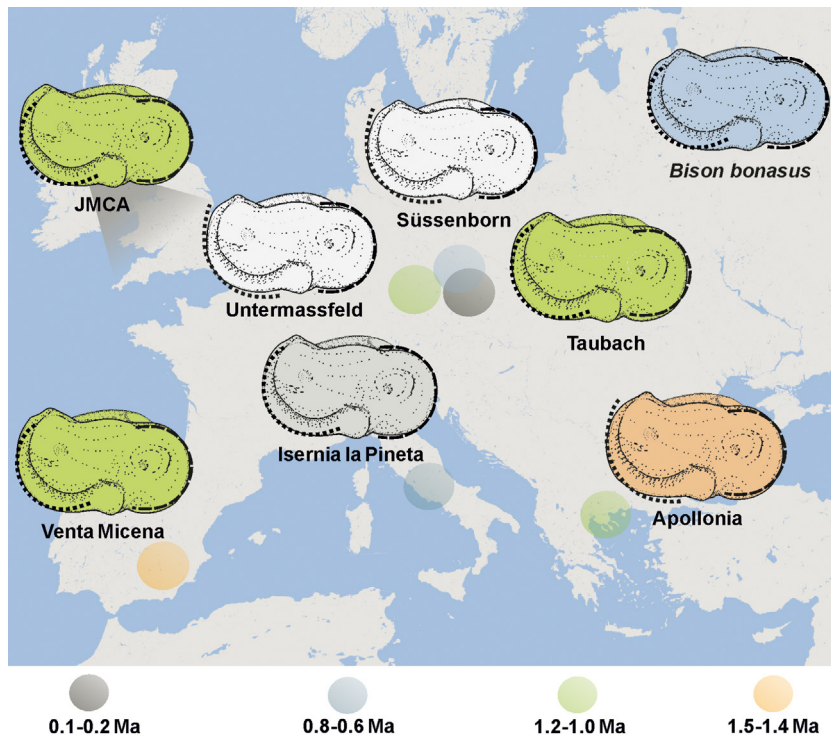
Fig. 6. Schematic drawing of the average observed patterns regarding the shape variation of the astragalar medial trochlear oultlines among the taxa mentioned in the text.
Fig. 6. Schéma de la moyenne des arrangements observés, concernant la variation de forme des contours trochléaires médiaux de l'astragale, parmi les taxons mentionnés dans le texte.

Furthermore, the extensively massive late Pleistocene priscoid forms have been also linked with the dwelling of environments with increased soil moisture as was further indicated in the relative trochlear verticilli splaying (Maniakas and Kostopoulos, 2017). Kahlke (1999) claimed that the bison of the Eemian Interglacial have to be adapted to soft woodland forage in continentally influenced conditions; however, the revealed astragalar morphology of the two *B. priscus* populations links these two forms with a rather advanced cursoriality and open biomes. We can assume that this ecomorphological feature does not necessarily contrast with the vegetational properties suggested for the early and late/latest Pleistocene palaeoenvironments or the need for an increased stability during cursorial locomotion in (constantly or periodically) less solid substrates (see Maniakas and Kostopoulos, 2017). Furthermore, plain dwelling should not be surprising for these two forms given their heavy-built structure. Nevertheless, *B. priscus* was a taxon that originally immigrated the European subcontinent from Asian steppe-like environments following the landscape opening in the later part of early middle Pleistocene (see Kahlke et al., 2011).

In accordance with the aforementioned interpretations, the relatively narrower distal trochlear condyle seems to be functionally related to decreased anterosterior mobility and powerful plantar flexion, i.e., a more closed habitat (Barr, 2014, 2015; Hildebrand and Goslow, 2001; Kappelman, 1988). Under this scope, it is reasonable to attribute the astragalar morphology that characterizes the

group consisting of the Epivillafranchian *B. menneri* and the two middle Pleistocene *B. schoetensacki* representatives (Fig. 5), to the generation of roughly shorter stride cycles, despite their relatively long-legged distal limbs (see Maniakas and Kostopoulos, 2017; Sher, 1997). This would come in some agreement to the reported sparsely wooded grasslands of Süssenborn and Isernia La Pineta (Kahlke et al., 2011; Sala, 1986), as well as to the mixture of open and gallery forested landscapes proposed for Untermassfeld (Kahlke, 2006; Kahlke et al., 2011), especially in comparison to the affiliated southern geographic regions of Apollonia and Venta Micena. This distinction seems to correspond with the assumption of Sher (1997) and Sala (1986) that bison of light construction, like *B. menneri* and *B. schoetensacki*, had to be forest dwellers. On the other hand, their habitat should be expected to differ significantly in vegetational composition in relation to that of the forest-dwelling modern European bison. In order to fit further with the "railed" trochlear condyle configuration (decreased splaying) reported by Maniakas and Kostopoulos (2017) for these forms, solid substrate conditions could be associated with relatively more arid climate conditions and the possible development of mixed sclerophyll woodlands and shrublands.

According to previous studies (Barr, 2014; Plummer et al., 2008, 2015), an analogous tightly interlocking formation mechanism was not supported for the proximal trochlea. Barr (2014) reported no significant differences regarding the proximal radii between open-country bovids

and forest or heavy cover (a term referring to bushlands, woodlands and swampy habitats) dwellers, which presented surprisingly higher values than those found in low cover (referring to light bushlands or tall grass habitats) forms, an inconsistency that was attributed partly to a body size signal. Similarly, confusing results were commented by Plummer et al. (2008, 2015). In the current study, a morphofunctional interpretation for this anatomic region appears also rather problematic, given that the supposed open-country Apollonia bison demonstrates a relatively narrower proximal joint margin in relation to that of the modern wood wisent. These findings perplex the prediction that a larger proximal radius could be associated with an increased range of angular excursion during dorsiflexion (sensu Schaeffer, 1947), indicating advanced cursoriality. Furthermore, the relative magnitude of shape changes between different bison populations was not supported (Table 3b). Following Barr's (2014) allegation, we might also consider the possibility that other factors than habitat have influence on the observed intrageneric shape variation of the trochlear diameter of the tibial articulation, suggesting that further studies are required in order to verify its relation to habitat specific locomotion, particularly at the intra-genus level.

Acknowledgements

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