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Morphological patterns of the European bison (*Bison bonasus*) skull

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This study aimed to investigate the effects of environmental factors, sexual selection, and genetic variation on skull morphology by examining the skull structure of the European bison, a species at risk of extinction, and comparing it to other bovid species. The skull of the *European bison* was significantly bigger than that of other species of the tribe *Bovini*, and the results revealed considerable morphological differences in skull shape compared to other *Bovini* samples. The bison skull exhibited a broader shape in the frontal region and a more laterally oriented cornual process. The frontal bone also significantly influenced skull shape variation within the *European bison* subspecies. Also, the findings indicated that cornual processes size significantly affected skull shape. Male *Bison* had larger skulls and more developed cornual ridges, and these morphological changes in the frontal region are thought to have also influenced the nuchal region, maxillary region, and facial bones. Furthermore, the differences in skull size observed between the Lowland-Pszczyna and Lowland-Białowieża lines, which share a closer origin than the Lowland-Carpathian lines, may reflect environmental and genetic adaptations over time. This research provides a reference point for future studies on the ecological and evolutionary factors influencing bison skull variations, with significant implications for the conservation of this species.

Keywords *Bovidae*, *Bovini*, Cornual process, Frontal bone, Geometric morphometrics, Skull morphology

The *European bison* (*Bison bonasus*), known as the wisent, is Europe's largest mammal. Following World War I, the species faced extinction in the wild, with the last individual being killed in the Białowieża Forest in 1919[1]. However, the species survived in a few Zoos and enclosures across Europe. Thanks to an international breeding program initiated in the 1920s and 1930s, the population began to recover, starting with re-establishing a herd in Białowieża. Since then, multiple free-ranging populations have been created, with numbers now reaching tens of thousands. In historical times, there were two subspecies of bison. The Lowland bison (*Bison bonasus bonasus*) occupied the forest areas of central and eastern Europe, and the *Caucasian bison* (*Bison bonasus caucasicus*) was found in the Caucasus Mountains. The *Pszczyna bison* herd, brought to Silesia in 1865 from the Białowieża the European bison population has identified two genetically distinct lines: the *Lowland-Carpathian* line and the *Lowland-Białowieża*line[2,3]. In the autumn of 1865, four Lowland European bison were transported from Białowieża to Murcki, and a year later, they were released into a large enclosure in the Pszczyna Forest. This population, having diverged genetically over time, is now recognized as the Lowland-Pszczyna line[4]. As a representative of the European megafauna, the European bison serves as an umbrella species, playing a crucial role in its ecosystem. The species is listed as Near Threatened (NT) on the IUCN Red List[5], underscoring the urgent need for its conservation.

The skull and its morphological features play a key role in the systematics and phylogeny of ruminants[6]. Variations in skull shape and its structures reflect the potential to respond to a wide range of selective pressures[7]. Horns, present in both male and female bovids, are a solid sexual differentiating feature[8]. The size of the cornual processes in growing individuals positively correlates with skull size and body mass, as more giant skulls and heavier body masses provide greater structural support for the development of larger cornual processes. The skull of bison of both sexes shows intensive development until the age of 5, after which the growth rate slows down significantly, likely indicating the completion of major cranial structural formation and the transition to a more stable adult morphology[9].

As one of Europe's last surviving megafauna species, the *European bison*is critical in maintaining biodiversity and promoting ecosystem health. Studying its biology, morphology, and genetics allows conservationists to gain

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deeper insights into its survival strategies and evolutionary adaptation processes[10,11]. By examining its anatomical structures, notably features like skull shape and size variations, and investigating genetic diversity within and between populations, scientists can better understand how this species has adapted to its environment over time[12,13].

This study aims to investigate the evolutionary adaptations and morphological diversity of the *European bison*skull. This complex and phylogenetic structure is crucial in understanding developmental processes in evolutionary change[14,15]. The research assumes that the skull morphology of the *European bison* has been shaped by long-term inbreeding and genetic factors due to the species' isolation in protected habitats like the Białowieża Forest. In these isolated populations, restricted gene flow is expected to drive morphological diversity. The first part of the study focuses on situating the *European bison* within the broader tribe *Bovini* by comparing its skull morphology with other species within the family. This comparative analysis will provide an evolutionary framework, offering insights into shared ancestral traits and divergent ecological adaptations that have influenced skull morphology across the *Bovini* lineage. Through this, the study will assess how evolutionary pressures and ecological niches have shaped both the commonalities and unique traits found in the *European bison*'s skull. In the second part of the study, the focus shifts to intraspecific variation within the European bison population, exploring morphological differences between sexes and subspecies. A key hypothesis is that male bison will exhibit morphological differences from females because their highly developed cornual processes are known to impose developmental pressures on the skull.

Results

Skull and cornual process size

Figure 1 illustrates the distribution of skull sizes for all samples used in the study, while Table 1 provides pairwise group comparisons. The skull of the *European bison* was notably larger compared to other members of the *Bovini* family ($p \leq 0.01$). The *European bison* skulls displayed a relatively homogeneous distribution, similar to that observed in the *Bos* samples. In contrast, members of the *Bubalus* genus are characterized by the smallest skulls, with substantial variability in size. This variation may be attributed to the more significant number of subspecies within the *Bubalus* group, which included four distinct subspecies, compared to only two in the *Bison* and *Bos groups* ($p \leq 0.01$). This broader taxonomic diversity likely contributed to the observed size variability. The size difference across the *Bovini* family was statistically significant when assessing the entire dataset ($p \leq 0.01$).

Within the *European bison* samples, skull size comparisons revealed that the *Lowland-Białowieża* line had significantly larger skulls than the other two ($p \leq 0.05$). In contrast, the *Lowland-Pszczyna* and *Lowland-Carpathian* lines exhibited similar skull sizes, with no statistically significant differences between them. Comparisons of cornual process size yielded results consistent with those for skull size. In both cases, sex emerged as a distinguishing factor, with males exhibiting larger skulls and more prominent cornual processes than females ($p \leq 0.001$).

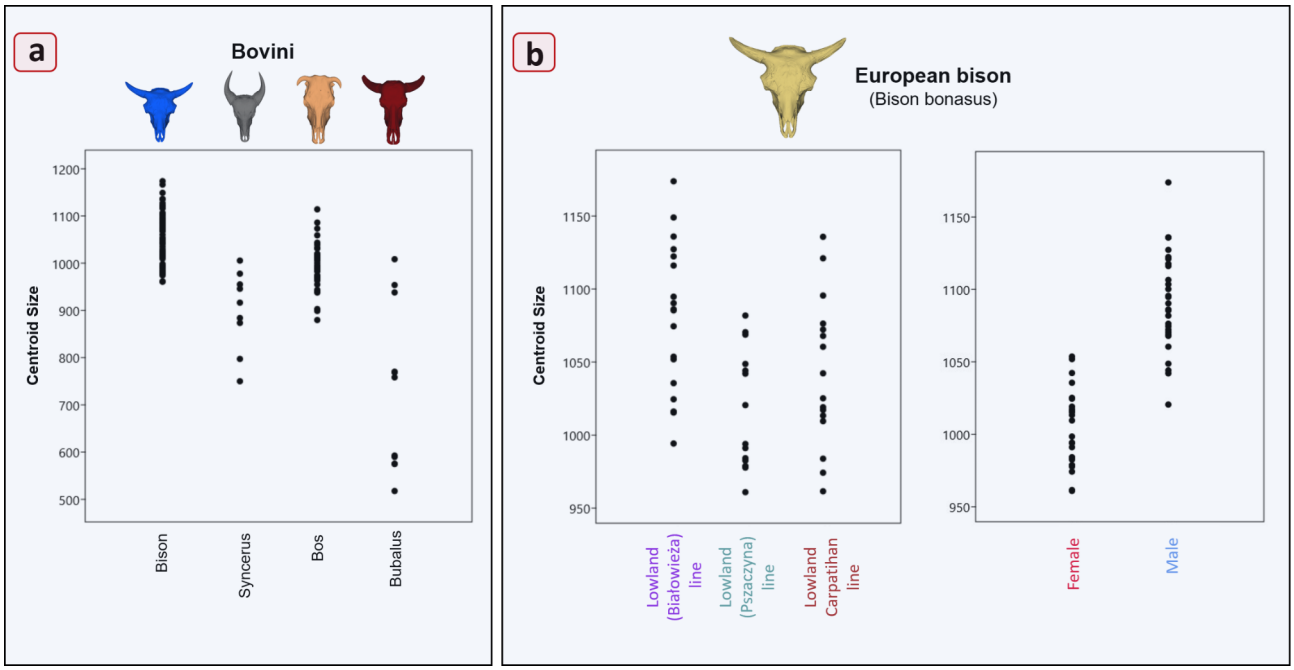

Fig. 1. Jitter Plot of centroid size between the groups. (**a**) Centroid sizes for tribe *Bovini*, (**b**) Centroid sizes for 3 different lines of *European bison*.

Group	Measurement	Pair	Difference	Lower CI	Upper CI	*p*-value
Bovini	Skull size (CS)	*Bison-Syncerus*	151.4489	83.4597	219.4382	$p \leq 0.001$
		Bison -Bos	56.8076	18.2346	95.3807	$p \leq 0.01$
		Bison -Bubalus	320.4568	258.1099	382.8037	$p \leq 0.001$
		Syncerus - Bos	94.6413	24.2511	165.0315	$p \leq 0.01$
		Syncerus - Bubalus	169.0079	83.2522	254.7636	$p \leq 0.001$
		Bos - Bubalus	263.6492	198.6924	328.6059	$p \leq 0.001$
European bison (Line)	Skull size (CS)	*Białowieża - Pszczyna*	64.3619	27.4367	101.287	$p \leq 0.001$
		Białowieża - Carpathian	37.0776	0.8916	73.2636	$p \leq 0.05$
		Pszczyna - Carpathian	27.2843	−13.6453	68.2139	p: 0. 111
		Female Male	88.5086	72.2288	104.7884	$p \leq 0.001$
	Cornual process Size (CS)	*Białowieża - Pszczyna*	64.3619	27.4367	101.287	$p \leq 0.001$
		Białowieża - Carpathian	37.0776	0.8916	73.2636	$p \leq 0.05$
		Pszczyna - Carpathian	27.2843	−13.6453	68.2139	p: 0. 282
		Female Male	88.5086	72.2288	104.7884	$p \leq 0.001$

Table 1. Results of ANOVA.

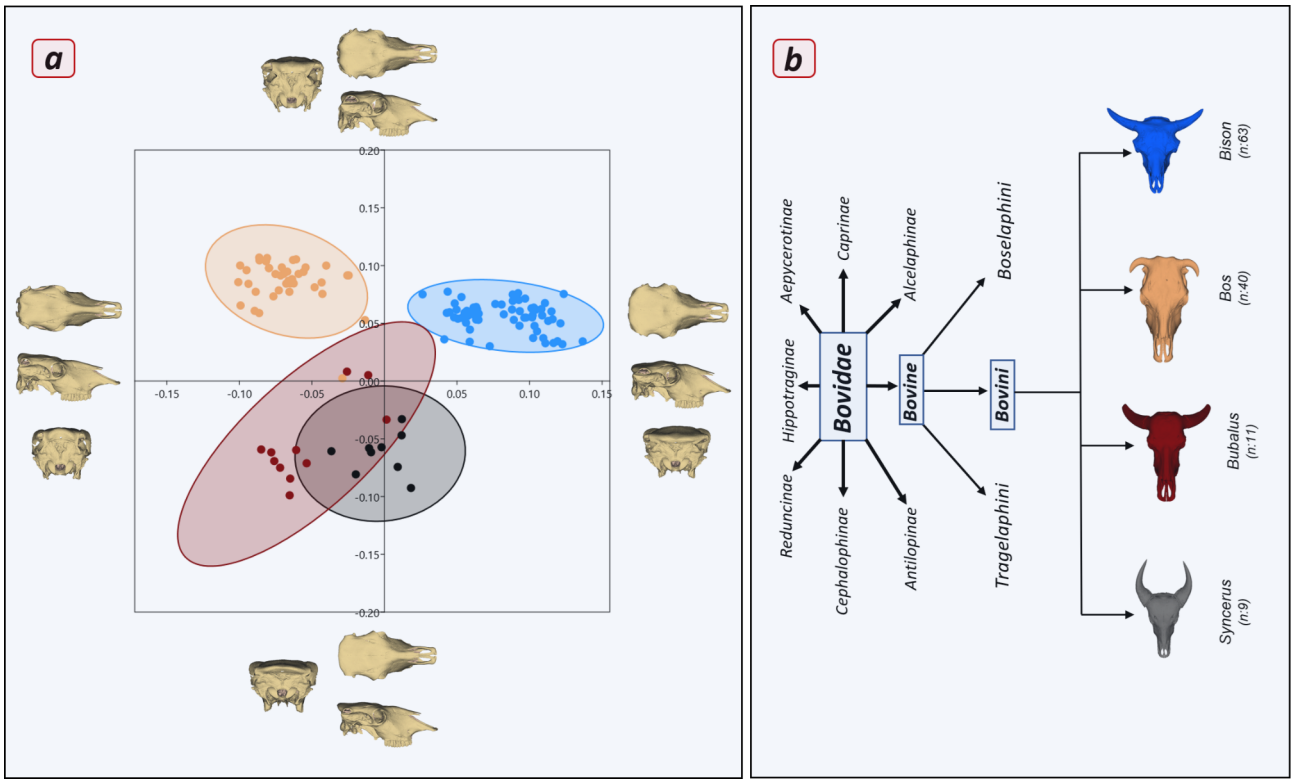

Fig. 2. (**a**) Results of the PCA (Ellipses represent 95% confidence intervals around the means); (**b**) Zoological classification of the *European bison* in the family *Bovidae*[47].

Skull shape variations

The distribution of tribe *Bovini* according to the PCA results is presented in Fig. 2A. In contrast, Fig. 2B illustrates the sample sizes (n numbers), species names used in the study, and a species tree tracing the evolutionary lineage from the Bovidae family to the species analyzed. The analysis revealed (PC1 and PC2) that the *Bison* skull shape displayed distinct morphological variation compared to other members of *Bovini*. All *Bison* samples exhibited positive PC1 values, whereas all others, except five *Syncerus* specimens, showed negative PC1 values. This indicates that even the PC1 values alone were statistically sufficient to distinguish the *Bison* skull from other species ($p \leq 0.01$). Regarding PC2 results, *Bison* showed positive PC2 values similar to *Bos*'s, further distinguishing the *Bison* skull from the *Bubalus* and *Syncerus* genera.

The positive PC1 value, which accounted for the most significant shape variation, was associated with a broader skull, especially in the frontal region, and a more laterally oriented cornual process. In contrast, negative

PC1 values indicated a narrower, more elongated skull, with the cornual process positioned closer to the nuchal region and a narrower frontal region. Positive PC2 values also indicated a larger frontal region, while negative PC2 values were linked to a significantly more robust cornual process base, contributing further to species differentiation.

The principal component analysis (PCA) results for European bison skulls within their group are depicted in Fig. 3A. The skull shape variations among groups with different genetic characteristics were similar, suggesting that these variations in *European bison* are more individualistic than strongly linked to genetic lineage. However, sex had a clear and significant impact on skull shape. The PC1 and PC2 results revealed a complete separation between male and female specimens, indicating that sex was a crucial determinant in skull morphology.

The specific shape changes associated with negative and positive values of the first two principal components are illustrated in Fig. 3B. A negative PC1 value was associated with a narrower, elongated skull, whereas a positive PC1 value represented a broader skull, particularly the frontal squama. Additionally, in the positive PC1 configuration, the nuchal plane was significantly more extensive, and the facial part of the skull exhibited lateral expansion. One of the most striking morphological changes observed with a positive PC1 value was the raised middle part of the frontal squama, creating a lump-like prominence. In contrast, a negative PC1 value corresponded to a flatter frontal bone roof.

Regarding PC2, a positive value corresponded to a more oval skull. Furthermore, the cornual processes were thicker in specimens with positive PC2 values than those with negative PC2 values. A broader nuchal plane was another prominent feature associated with positive PC2 values, further contributing to significant shape variations across the skulls.

Inter- and intragroup shape differences

The Procrustes ANOVA revealed morphological differences among the predefined groups (Bison, Syncerus, Bos, and Bubalus) ($P<0.05$). Pairwise comparisons showed that the most notable distinctions were between Bison and Syncerus and between Syncerus and Bos. In contrast, no significant differences were observed between Bison and Bos, or between Bos and Bubalus.

According to the result of Procrustes ANOVA, the comparison between Białowieża and Carpathian lines showed a significant effect ($F=41.605$, $p<0.001$), with a substantial proportion of the variation in shape attributed to group membership ($R^2 = 0.50983$). In contrast, the Białowieża and Pszczyna comparison revealed no significant difference ($F=0.4691$, $p=0.492$), suggesting that the morphological variation between these two lines is minimal ($R^2 = 0.01188$). On the other hand, the Pszczyna and Carpathian comparison showed a highly significant morphological divergence ($F=72.005$, $p<0.001$), with a large proportion of shape variation accounted for by the group ($R^2 = 0.71288$).

Size and shape

Figure 4 illustrates the interactions between age, cornual process size, skull size, and the two first principal components. The analysis first revealed that age had no significant effect on skull size. Also, age did not appear to affect skull shape in the *European bison* significantly. However, there was a statistically significant relationship

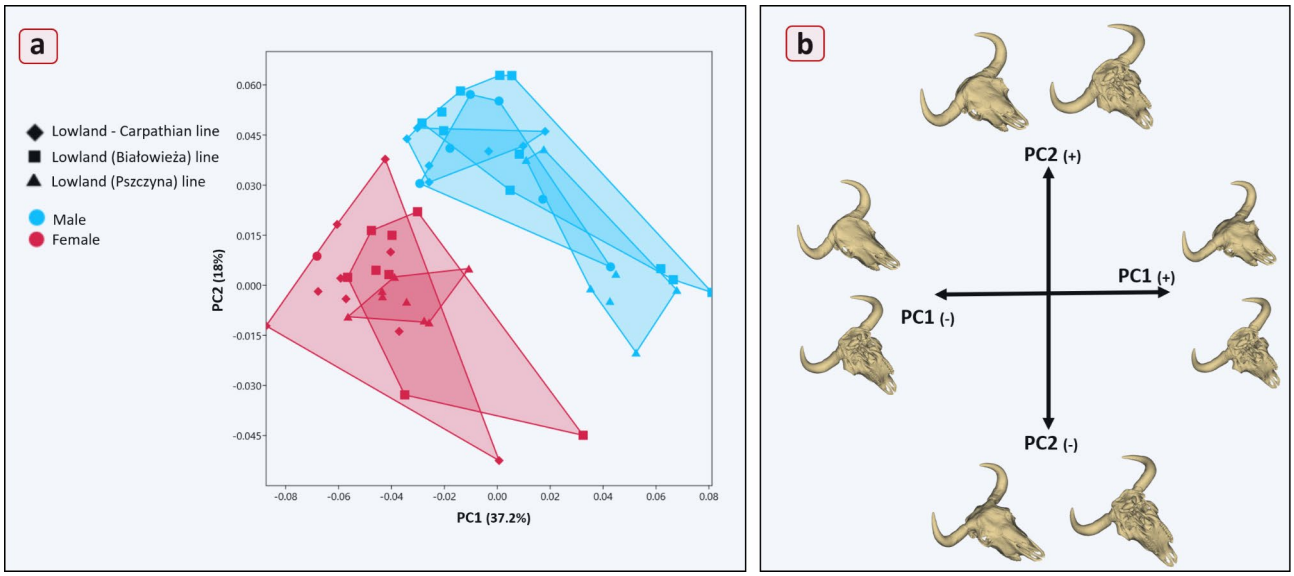

Fig. 3. The first two principal components of the analysis of European bison' skulls. (**a**) Scatterplots of the first two principal components from the skull of three distinct populations: *Lowland-Carpathian* line, *Lowland-Białowieża* line, and *Lowland-Pszczyna* line (4 male sample lines are unknown); (**b**) Negative and positive shape variations for the first two principal components. PCA results of the first two axes account for 55.2% of the total variance. Shape changes are shown by 3D warping.

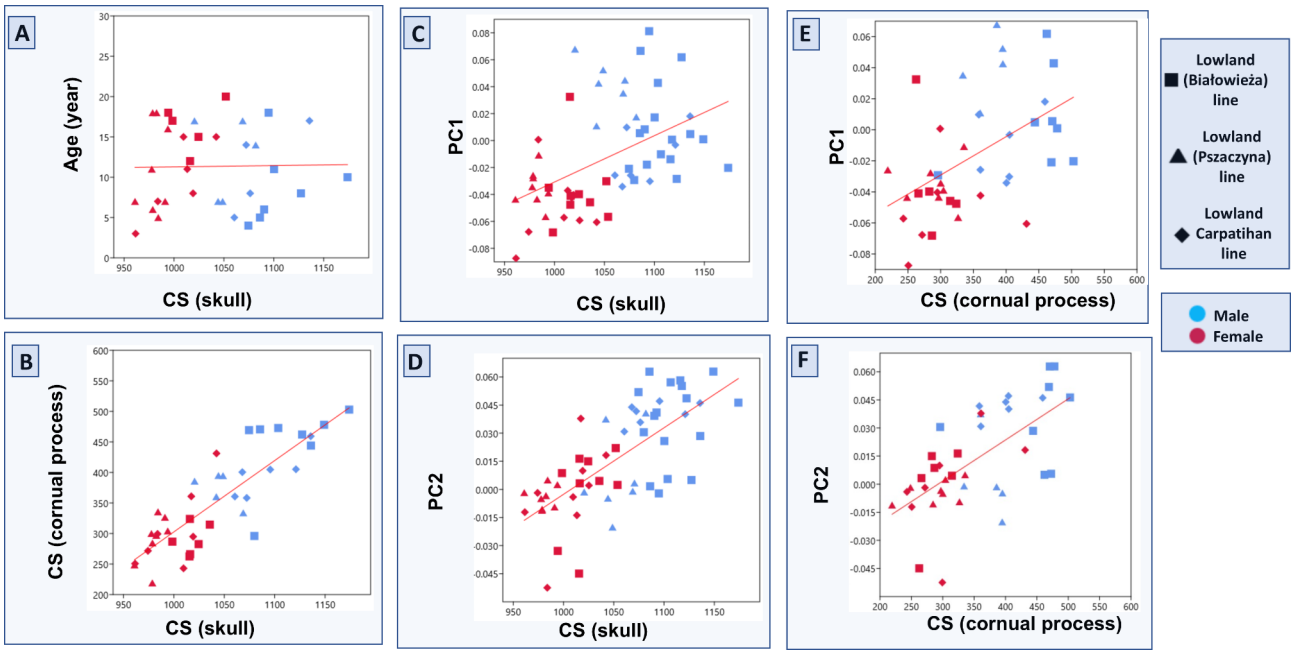

Fig. 4. Relationships between age, skull size, cornual process size, and the first two principal components.

between skull size and cornual process size (R^2: 0.72, F: 47.67, $p < 0.001$) and their corresponding PC1 (R^2: 0.56, F: 62.58, $p < 0.001$) values, which explains the most substantial shape variation in these measurements.

As anticipated, the cornual process size was positively correlated with skull size ($p \leq 0.01$), indicating that larger skulls generally had larger cornual processes. This suggests that the development of the cornual process is proportionate to overall skull growth. Furthermore, skull size statistically significantly impacted PC1 and PC2 ($p \leq 0.001$), indicating that skull shape changes are closely linked to skull size. As skull size increased, the specimens exhibited more prominent positive PC1 and PC2 features. Regarding morphology, more giant skulls tended to have wider transverse dimensions (as reflected by positive PC1 values) and displayed more oval or rounded shapes (as indicated by positive PC2 values).

Discussion

The findings of this study provide new insights into the morphological relationships between skull size, cornual process size, and shape variation in the tribe *Bovini*, with a particular focus on the *European bison*. One of the most striking results is the significant correlation between skull size and cornual process size, which was an expected outcome. This finding aligns with previous studies on bovids, confirming that larger skulls support larger, more developed cornual processes. The lack of a significant relationship between age and both skull and cornual process size suggests that, beyond a certain point in development, growth in these traits is largely independent of age. Sexual dimorphism is more dominant in determining these morphological features, as evidenced by the clear separation of males and females. Additionally, the effect of size on shape variation, particularly as captured by the principal components, highlights the importance of allometry in skull morphology. Larger skulls tend to have wider transverse dimensions and more rounded shapes, indicating that size contributes significantly to the overall shape, independent of other factors. These results provide a more detailed understanding of the morphological variation within the tribe *Bovini*, particularly in the context of the *European bison*. Future research could expand on these findings by exploring the genetic and ecological factors that drive this variation and its implications for species classification and conservation efforts.

The skull of the *European bison* is significantly larger than that of other members of the tribe *Bovini*. This size difference indicates a robust skeletal structure that can support the physical demands of the bison's environment, including its role as a large herbivore in various habitats across Europe. The considerable morphological differences in skull shape compared to other *Bovini* can be attributed to evolutionary adaptations shaped by diet, habitat, and social behavior. The *European bison*exhibits a broader skull, distinguishing it from other species. This may provide advantages in feeding, enabling the bison to graze more efficiently on various vegetation[16,17]. The broader skull also supports larger masticatory muscles, facilitating the processing of rigid plant material, a significant aspect of the bison's diet. In addition to its skull shape, the cornual processes of the *European bison* differentiate it from *Bubalus* (water buffalo) and *Syncerus* (*African buffalo*). The base of the cornual process in *Bubalus* and *Syncerus*is more massive, suggesting a stronger emphasis on horn growth for purposes such as dominance displays, territorial defense, or mating competitions[18]. In contrast, the cornual processes of the *European bison* are observed to be separated from the frontal squama and have a thinner base. This anatomical difference may indicate that, while cornual processes play a role in bison social dynamics, their morphology has evolved to serve different functions compared to the more robust horns of *Bubalus* and *Syncerus*. The shape

of the bison's cornual process could also reflect adaptations to the environments in which these species live. Bison inhabit open grasslands and forest edges, where their primary defensive strategies may not rely heavily on weapons[19]. Instead, their social structures and behaviors—such as forming herds—may play a more crucial role in protection against predators. As a result, the evolutionary pressures on bison may have led to the development of skull morphology that prioritizes other survival strategies over the robust cornual processes seen in their relatives. The significant morphological differences among the tribe *Bovini* highlight the diverse adaptations that have evolved in response to environmental pressures, feeding strategies, and social dynamics. Understanding these morphological traits provides valuable insights into the evolutionary history of the *European bison* and its relatives and their ecological roles in their respective habitats.

Solounias[20]identified distinct patterns in premaxillary morphology that correspond to dietary habits, with narrower, pointed premaxillae typically associated with browsers and flatter, wider premaxillae characteristic of grazers. This morphological differentiation is crucial for herbivores, as it directly influences their ability to select forage, chew efficiently, and maximize their intake of nutrients. Tennant[21]further emphasized that snout shape plays a significant role in determining foraging efficiency, as a pointed snout allows herbivores to be more selective. In contrast, a blunter snout enables a more generalized, high-volume cropping of vegetation. In the present study, the European bison's premaxilla was blunt and broad, corresponding with the species' classification as a strict grazer[22,23]. Ruminants, such as *European bison*, primarily feed on grass, which requires a more generalized cropping mechanism to consume large quantities of low-quality forage. The premaxillary morphology observed in the bison reinforces this ecological adaptation. Compared to other *Bovini* species in the study, the bison displayed more pronounced grazer characteristics, as reflected in its premaxilla shape. This finding is significant because it not only reinforces the link between skull morphology and dietary adaptation but also suggests that within the tribe *Bovini*, the *European bison* may be more specialized for grazing than some of its relatives. For instance, in another tribe of Bovini, more pointed rostral shapes may indicate a greater tendency toward mixed feeding or browsing. In contrast, the bison's blunt and broad premaxilla supports its adaptation to environments where high-volume, low-selectivity feeding is essential for survival, such as grasslands.

The frontal bone was observed to be one of the most significant contributors to skull shape variation in the *European bison*. This prominence of the frontal bone in shaping skull morphology aligns with its critical role in housing and supporting the horn structures (cornual processes), which are essential for species-specific behaviors such as combat, defense, and social hierarchy in the family *Bovidae*. The variation in the frontal bone is particularly notable in terms of width and thickness, as seen in the positive PC1 values, where the skull was laterally expanded, especially in the frontal region. This could be linked to sexual dimorphism, as males tend to have more extensive and more developed horn structures, necessitating a more robust frontal bone to accommodate the increased mechanical stress imposed by horn growth and use during physical interactions[24–26]. The larger size of the frontal bone in males contributes to the overall sexual differentiation in skull morphology between males and females. This difference is a critical distinguishing factor, as males typically possess larger and more developed weapons, while females exhibit smaller or less pronounced horns. Furthermore, this morphological variation of the frontal bone is not isolated; it also has cascading effects on other skull regions. For instance, the nuchal region, which supports the neck muscles, becomes more developed in male *European bison*to counterbalance the added weight and size of the weapons. The increased development in the caudal part of the frontal bone may also influence the shape of adjacent bones, such as the parietal and temporal bones, contributing to differences in the overall cranial structure[27,28]. Moreover, the alteration in the frontal bone also has implications for the facial bones. As the frontal bone expands and morphologically changes to accommodate the weapons, it exerts pressure on the maxilla and nasal bones, possibly leading to secondary adjustments in the facial skeleton.

The *Lowland-Pszczyna* line, initially genetically characteristic of the *Lowland-Białowieża*line, developed distinct genetic traits after being separated from the main herd in 1865. This divergence is thought to result from a combination of genetic drift and environmental influences over time[4]. Despite the genetic differences, skull shape variation between the two lines remains relatively similar. However, there was a statistically significant difference in skull size, with the *Lowland-Białowieża*line exhibiting larger skulls. This size discrepancy may reflect the influence of environmental factors over the past 150 years. This observation aligns with Bergmann's proposal, suggesting that populations of the same species in colder climates tend to be larger than those in warmer regions, as larger body size aids heat conservation[29]. The *Lowland-Pszczyna* line, located in a slightly warmer environment, may have adapted to this environmental pressure by evolving smaller skulls and overall body size compared to the *Lowland-Białowieża* line. However, while this hypothesis offers an exciting perspective, more extensive data and examples are required to draw definitive conclusions about this adaptation. Nevertheless, these preliminary findings provide a valuable foundation for future studies on environmental and genetic influences on bison morphology.

Numerous studies have examined the allometric properties of the cattle skull, yielding similar findings. The consistent results of these studies are generally attributed to the constraints imposed by a deeply conserved size-shape allometry of the *Bovid*skull, as highlighted by Bibi and Tyler[30]. This conserved allometric relationship has been further suggested to be influenced by the presence and development of horns. Tidière's[31] study underscores that the ontogenetic allometric gradient has likely evolved among species in response to size-related changes in selection pressures, mainly acting on horn length and body mass. Since horn length is often linked to sexual dimorphism, the allometric effects can vary between male and female individuals. For instance, Packard[32], in his allometric study on mountain goats, noted that males exhibited a steep upward trajectory in the allometric equation. At the same time, females followed much shallower paths, thus supporting the hypothesis of sex-related allometric divergence. Similar allometric variation is observed in the skull of the European bison, much like in other bovid species. However, the allometric and shape influence of the cornual process requires special attention in the European bison. In male individuals, the more developed weapons significantly influence skull

morphology by exerting pressure on the frontal bone. This, in turn, affects the facial bones, contributing to the distinct morphological differences between males and females. The well-developed cornual processes in males reinforce their allometric growth, further differentiating skull shape and size in sexually dimorphic patterns[25]. These findings suggest that the evolutionary pressures on cornual process development contribute to overall skull morphology and shape the distinct allometric trajectories between sexes. Understanding this dynamic offers essential insights into how selection pressures, such as size and sexual dimorphism, have shaped the morphological evolution of *European bison* and other *Bovid* species.

Craniofacial elongation is a well-documented aspect of postnatal skull development in mammals, where newborns typically have relatively small faces and rounded heads[33]. As mammals mature, the facial region grows faster than the braincase, resulting in a less spherical head shape and more prominent jaws. However, in the case of *European bison*, age did not significantly impact skull size or shape in this study. This lack of age-related variation can be attributed to the fact that all specimens used were older individuals. One specimen was 4 years old, while the others were all over 5 years of age—an age at which *European bison*are expected to have completed their skeletal development[34,35]. Since craniofacial growth and skull development slow down or cease after full maturity, it is not surprising that age had no measurable effect on the parameters examined in this study. Further studies may comprehensively explore age effects by including younger or juvenile specimens.

In conclusion, this study highlights the frontal bone's and cornual processes' significant role in shaping European bison's skulls, particularly in distinguishing male and female skulls. The morphological variations observed, particularly in the frontal and facial regions, emphasize the impact of horn development on overall skull shape. Additionally, environmental factors may have contributed to size differences between the *Lowland-Białowieża* and *Lowland-Pszczyna* lines, possibly supporting Bergmann's rule. Bergmann's rule suggests that populations of the same species tend to be larger in colder climates than in warmer regions, as a larger body size aids in conserving heat.

This study lays the foundation for future research in several key areas. By providing a detailed analysis of the *European bison*'s skull morphology, mainly focusing on the effects of sexual dimorphism, allometry, and environmental adaptation, it offers a framework for further exploration into the genetic and ecological factors driving these morphological traits. Future studies can build on these findings to investigate the genetic underpinnings of the observed cranial variations, examining how inbreeding, gene flow, and isolation have influenced skull shape and size.

Material method

Samples

A total of 57 *European bison* skulls (31 males and 26 females) of known pedigrees from the Osteological Museum of the Department of Morphological Sciences, Institute of Veterinary Medicine, Warsaw University of Life Sciences (SGGW), were used in this study. These skulls belonged to animals that died between 1950 and 2014, aged 3 to 20. Among the sample, 26 came from the *Lowland-Białowieża* line (9 females, 17 males), 15 from the *Lowland-Pszczyna* line (8 females, 7 males), and 16 from the *Lowland-Carpathian* line (9 females, 7 males).

For the comparative analysis of the European bison skulls, 38 domestic cattle skulls, specifically from Simmental and Holstein breeds, were obtained from the reference collection at Istanbul University-Cerrahpasa (IUC), Faculty of Veterinary Medicine, Department of Anatomy. Additionally, 2 cattle, 9 buffaloes, and 9 Syncerus samples were taken from the study by Bibi and Taylor[25]. In contrast, the remaining 2 buffalo and 4 American bison specimens were provided by the SGGW. Specimens with pathological or fractured skulls were excluded from the study, although skulls with horn or tooth fractures were included, as long as these fractures did not affect the landmarks used. Also, all juvenile skulls were excluded from the study sample. After meeting all criteria, 123 skulls were included in the study. Only adult specimens were used to ensure that the analysis results were not affected by potential changes in skull shape and size caused by growth and development.

Specimen digitization and landmarking

A Shining 3D EinScan Pro 2×3D scanner (Shining 3D, Hangzhou, China) was used to create 3D models of the skulls from the SGGW and the reference set from IUC. EXScan Pro (v4.0.0.4), provided by the scanner manufacturer (Shining 3D, Hangzhou, China), was used for 3D model reconstruction. All samples, including those from the study by Bibi and Taylor[30], were saved in ".ply" format. Studies show that the data from the different scanning devices (Artec Spider, Eva, and EinScan Pro 2X) are comparable, with minimal differences in accuracy that did not affect the study's results[36].

Cornual processes and teeth were either missing or damaged in many samples, so these regions were excluded from the landmark-based analysis. A total of 37 anatomical landmarks (Table 2) were selected based on previous studies[32]. These landmarks have been widely used in archaeological studies and are easily replicable by other researchers[37–39]. 3D Slicer software (version 5.2.2) was used for landmarking[35]. Initially, a draft set of landmarks was created, which was then consistently applied to all samples by the same researcher to ensure accuracy in digitization (Supplementary Table 1, Supplementary Table 2). No landmarks were missing from the analysis, and all 37 landmarks were successfully applied to every sample (Fig. 5).

A landmark-based GMM approach was used to analyze cornual process size and determine centroid size (Supplementary Table 2). Traditional metrics, such as length or thickness, often fail to comprehensively understand the volume and dimensions. For instance, measuring the length of a cornual process with a highly curved structure may underestimate its actual length due to its complex geometry. Similarly, thickness measurements at a few select points may not reflect the overall volume or intricate shape. To address these limitations, we employed semilandmarks distributed across the surface of the cornual process to capture its size more effectively. The PseudoLMGenerator module in the 3D Slicer program (version 5.2.2) was utilized. The male *European bison* specimen, "Plotkarz," was selected as the reference model for the initial landmark set. This

Skull	Area	Landmark No	Landmark description
Facial bones	Maxillary area	1,2	Prosthion
		21,24	Postdentale
		22,25	facial tuberosities
		23,26	Premolar
	Nasal area	3,4	Rostral end of nasal bones
		5,6	Naso-incisive notch
		7,8	The most rostral end of the frontal suture
		9	Nasion
	Palate area	29	The rostral point of the hard palate
		30	The end point of the hard palate
	Orbital area	13,15	Entorbitale
		14,16	Ectorbitale
Neurocranial bones	Frontal area	10,11	Supraorbital foramen
		12	Middle point of the supraorbital foramen
	Cornual area	17,19	The rostral point of the cornual process
		18,20	The end point of the cornual process
	Occipital area	31,32	Lower end of the occipital condyles
		33	Opisthion
		34	The external occipital protuberance
		35	Nuchal crest
		36,37	Caudal border of temporal fossa
	Temporal area	27,28	The rostral point of the temporal process

Table 2. Description of landmarks taken on skulls for geometric morphometric analysis.

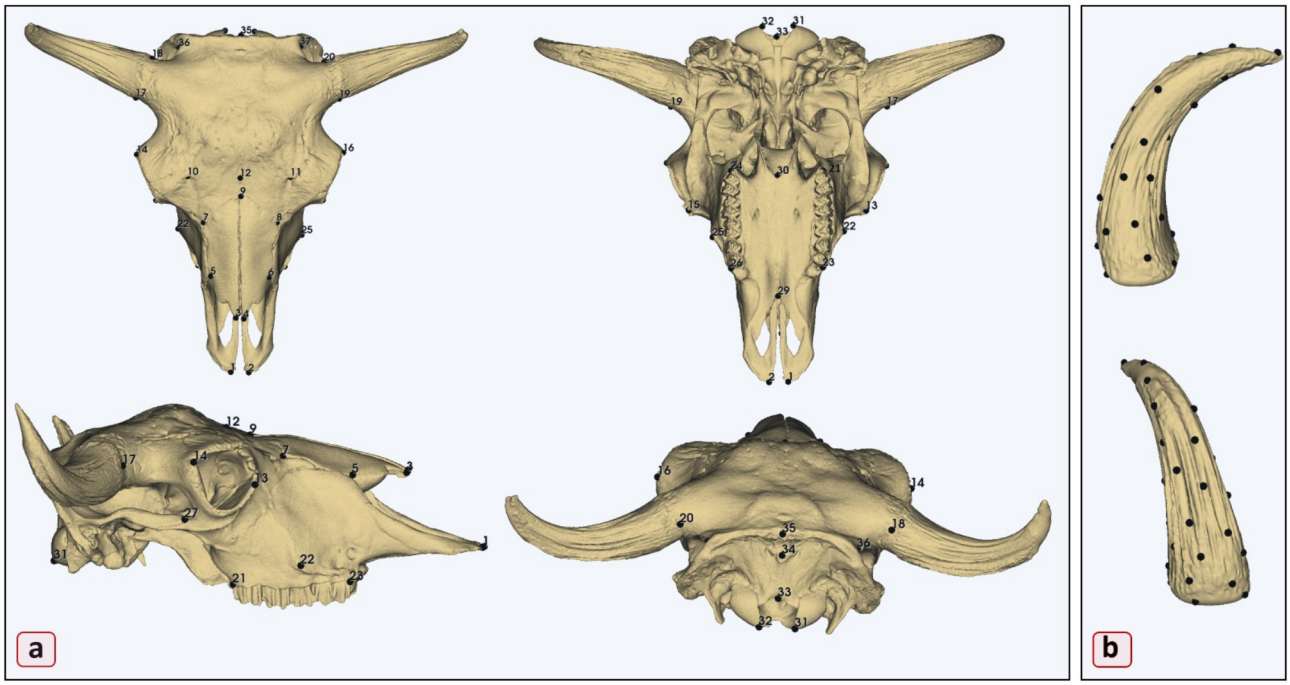

Fig. 5. Landmarks. (**a**) 37 anatomical landmarks on the skull; (**b**) 34 Semilandmarks on the cornual process.

reference sample provided a basis for the configuration of the landmark arrangement. A range tolerance of 10% was applied, which allowed for flexibility in the landmark placement relative to the morphology of the cornual process across different specimens. The template geometry determined the shape and layout of the estimated sampling template, ensuring that the landmarks could adapt to individual shape variations while maintaining consistency. Once the template was created, the landmarks were placed on the surface of the cornual process, ensuring an accurate representation of its morphological features. At this stage, the regularity and distribution

of the sampling points were adjusted to ensure consistent spacing, enhancing the precision and reproducibility of the landmark placement. The final landmark set, comprising 34 semilandmarks, was generated (as shown by black dots in Fig. 5B). An automated process extended the landmark set across the entire dataset. The draft set was applied to all skull specimens using Automatic Landmark Generation via Point Cloud Alignment and Correspondence Analysis (ALPACA)[40]. The ALPACA module within 3D Slicer facilitated this process, allowing for the accurate and efficient application of the Semilandmarks to each specimen.

Geometric morphometrics

GPA and subsequent GMM analyses were performed using the SlicerMorph module within the 3D Slicer software (version 5.2.2)[41–43]. GPA is a widely used method in shape analysis because it standardizes landmark data across specimens, removing non-shape-related variables such as position, orientation, and size. Landmark files for each specimen are provided in Supplementary File.

Landmark configurations from each skull were first translated to the origin, meaning they were shifted in space so that each configuration was centered. Next, the landmarks were scaled to their centroid size, ensuring that all skulls were standardized in size and then optimally rotated. The least-squares criterion was used to minimize the distance between homologous landmarks across all specimens, which allows the skulls to be optimally aligned for further comparison. This process, known as Procrustes superimposition, effectively removes variation due to size, rotation, and translation so the analysis focuses solely on shape differences. After the GPA, all specimens were situated within the same "shape space," centered on a mean shape. This provides a common reference point from which deviations, or shape variations, can be measured.

Morphospace analysis: inter- and intragroup differences

To investigate patterns of shape variation and assess morphological differences among groups, we conducted a Procrustes ANOVA on the aligned landmark configurations[44]. This analysis tested whether predefined groups (*Bovine* and *European bison*groups) exhibited significant differences in their mean shapes. A permutation-based approach with 1000 iterations was used to evaluate statistical significance. Additionally, we performed pairwise Procrustes ANOVA comparisons between group pairs to assess morphological differences further. These pairwise comparisons allowed us to evaluate shape variation's magnitude and statistical significance between specific groups. All analyses were conducted using the Geomorph package (v.4.0.9) in R (RStudio 2024.09.1)[45], which facilitates (GMM) data processing, Procrustes superimposition, and statistical comparisons of landmark-based data.

Cornual process, size, and age

The study aimed to explore the relationship between skull size, age, cornual process size, and shape variations by analyzing the effect of the first two principal components (PC1 and PC2) on these factors. PC1 and PC2 were selected for their ability to explain the most significant changes in skull morphology, making them crucial for understanding the evolutionary and developmental influences on bison skull shape. The skull and cornual process size was calculated using the centroid size (CS), a measure derived from Procrustes shape coordinates that accurately represent size by focusing on the geometric center of mass. The SlicerMorph module within the 3D Slicer software (version 5.2.2) was used to extract the relevant data, including the centroid size values for both the skull and the cornual process. The study leveraged these measurements to assess the impact of age and cornual process size on overall skull morphology and size.

Statistical analysis

An orthogonal projection into a linear tangent space was applied, and a mathematical transformation ensures that subsequent statistical analyses, such as PCA, can be performed on the tangent space coordinates, making the data suitable for multivariate analysis. Two distinct PCA analyses were conducted to explore the shape variation in the skulls. PCA is a powerful statistical tool that reduces the complexity of the dataset by identifying the principal components (PCs), or axes, that account for the most variation in shape among the specimens. This dimensionality reduction helps visualize and quantify the variation across a large dataset while highlighting the key factors driving the differences in skull morphology.

In the first analysis, PCA was performed on the Procrustes shape coordinates for the full dataset of 123 skulls, which included four different subspecies clusters from the broader tribe *Bovini*. This analysis aimed to visualize the distribution of species in a low-dimensional cranial morphospace, which is essentially a mathematical space where each point represents a different skull shape. By examining how the *European bison* skulls clustered or spread out within this morphospace compared to other members of the tribe *Bovini* (such as cattle and buffalo), it was possible to gain insights into the distinctive morphological features of the *European bison*. The second analysis focused exclusively on the *European bison*. It involved PCA on a dataset of 57 skulls to investigate intraspecific variation, particularly across sexes and subspecies within the species.

Size variation was tested using ANOVAs on centroid size data. Following the ANOVA, a Tukey's HSD test was applied as a post hoc analysis to pinpoint specific group differences. Multivariate linear regression was performed to investigate the relationship between age, cornual process size, and skull size. Additionally, the first two principal components (PC1 and PC2), which explain the most significant variation in skull shape, were used for allometric assessment. By examining the relationship between these principal components and centroid size, the study explored how skull shape variations correlate with changes in size (allometry). These analyses were conducted using the PAST statistical program[46].

Data availability

Data is provided within the manuscript or supplementary information files.

Received: 23 October 2024; Accepted: 6 January 2025
Published online: 09 January 2025

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Acknowledgements

This study was funded by the Scientific and Technological Research Council of Turkey (TUBITAK 2219-1059B192301667).The publication was financed by the Science Development Fund of the Warsaw University of Life Sciences - SGGW.

Author contributions

O.G. and T.S. conceived the study. O.G. performed landmark digitization and the statistical analyses. O.G. and T.S. prepared the article. All authors contributed significantly to interpreting the data and results and writing the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-85654-3.

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