Morphological insights from Ecology and Evolution: Mandibular plasticity and phenotypic variation in Cervini from different populations and contrasted habitats

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Abstract:
Understanding how genetic variations and environmental constraints influence morphological differences is crucial to interpreting phenotypic diversity. The mandible is a key structure widely used to make inferences about feeding ecology and habitat conditions in early ungulate growth. However, most studies focus on interspecific comparisons, and few address mandibular plasticity and variations at inter- and intrapopulation scales. In this study, we used a 2D geometric-morphometric approach to analyse how these factors shape the mandibles of two Cervini species characterised by different contexts, populations and belonging to distinct lineages: the red deer (*Cervus elaphus*) and the rusa deer (*Rusa timorensis*).
For the European red deer, we found clear variations in mandibular morphology along a north-south transect from Norway to southern Spain. These variations are related to the need for different biomechanical forces to masticate vegetal resources with distinct physical properties, under contrasting climates. The mandibles of red deer in southern Europe are more robust, probably due to the higher proportion of highly fibrous, tough herbaceous monocotyledons in their diet. In contrast, northern populations have slender mandibles, reflecting the consumption of a softer diet. The American population of red deer from Argentina shows a different trend, both in size and shape.
A recent study showed that New Caledonian rusa deer constituted a single genetic population. Here, we identified significant intrapopulation differences between contrasting local habitats. Gouaro-Déva rusa deer are significantly smaller, which we interpret as the consequence of greater stress, impacting the growth and ontogenic development of these animals. Deer from tropical forests have a more robust mandible than in grassy areas, aligning with the biomechanical needs of their respective diets.
Morphological variations in deer jaws at intraspecific and interspecific scales provide insights into the ecology and environment of these animals, and offer potential for future (paleo)ecological research using geometric morphometric approaches.

Keywords: geometric morphometrics, environmental constraints, ecological plasticity, Cervini, (eco-)Phenotype.

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Cervini, (eco-)Phenotype, environmental constraints, ecological plasticity, geometric morphometrics

Introduction

Understanding the relationship and interactions between environmental factors and historical contingencies is a classic question in evolutionary biology. It is essential for elucidating how populations interact and respond to their habitats and the underlying processes behind phenotypic diversity. As stated by Seilacher (1970), morphology is shaped by a combination of genetic inheritance, structural constraints and environmental adaptations. This framework provides a solid theoretical basis for studying how these three factors interact.

Mandible morphology is a key functional trait closely related to animal diet. Its highly plastic tissue is constantly remodelled by the functional stresses it undergoes (Currey, 2003; Wolff, 1892). Among the factors influencing mandibular morphology, the mechanical forces acting during mastication in response to the hardness, toughness or size of ingested particles play a significant role (Clauss et al., 2008; Fletcher et al., 2010; Janis, 1995; Paschetta et al., 2010; Varela and Fariña, 2015; Wang et al., 2022). After a change in diet, a few months can be enough to detect significant differences in shape (Neaux et al., 2022). As a result, the morphology of the mandible provides valuable ecological information, particularly on diet (De Felice et al., 2020; Neaux et al., 2022, 2021; Spencer, 1995). However, most studies investigating the relationship between mandible morphology and feeding ecology focus on interspecific comparisons, emphasizing the significant impact of phylogenetic affinities compared with that of adaptation to available resources in the habitat (Fletcher et al., 2010; Pérez-Barbería and Gordon, 1999; Raia et al., 2010; Zhou et al., 2019). There are comparatively few studies addressing these issues at both inter- and intrapopulation scales (Kangas et al., 2017; Ozaki et al., 2007), although several ungulate species provide good candidates, with their wide geographical distribution and well-known ecological plasticity.

In this study, based on a 2D geometric morphometric approach, we aim to contribute to filling this gap by investigating how genetic variants and environmental constraints affect the mandibular bone morphology of the red deer *Cervus elaphus* and the rusa deer *Rusa timorensis*,

two *Cervini* species characterised by different contexts and belonging to distinct lineages (Mackiewicz et al., 2022).

Cervus elaphus dispersed in Europe during the middle Pleistocene (Croitor, 2018). The current genetic structure of *Cervus elaphus* is divided into three main genetic lineages (western European, eastern European, Mediterranean ; Niedziałkowska et al., 2011; Skog et al., 2009; Zachos et al., 2016), resulting from the contraction of the distribution range of the species into refugial areas during the last glacial maximum (Hewitt, 1999; Jones et al., 2020; Meiri et al., 2013; Taberlet et al., 1998). Red deer populations originating from the Iberian glacial refuge were the primary source of postglacial recolonisation and subspecific radiation in north-western Europe (Carranza et al., 2016; Meiri et al., 2013). Northern Norway and Southern Spain represent the extreme north and south of the European *Cervus elaphus* native distribution along a clear bioclimatic gradient (Lovari et al., 2018). The species has also been introduced beyond its native range, where it has often established successful and sometimes invasive populations, for example, in Australia (Kelly et al., 2023), New Zealand (Latham and Nugent, 2017) or Argentina (Novillo and Ojeda, 2008). This reflects its remarkable ecological plasticity (Azorit et al., 2012; Bugalho and Milne, 2003; Gebert and Verheyden-Tixier, 2001; Geist, 1998). The wide geographical distribution demonstrates its broad climatic tolerance and its ecological plasticity. The well-documented genetic of *Cervus elaphus* makes it an excellent species for studying the influence of genetic and environmental factors on mandibular bone morphology at an interpopulation scale.

Rusa timorensis (Blainville, 1822) is endemic to the Indonesian islands of Bali and Java, where it is considered vulnerable by the IUCN Red List (Hedges et al., 2008). There, it occupies a diversity of habitats, from forests to grasslands. Its diet is adaptable, with a preference for herbaceous monocotyledons (Hedges et al., 2008). In 1870, twelve rusa deer individuals were introduced to New Caledonia, which now hosts the largest population of the species in the world (Savouré-Soubelet et al., 2021). New Caledonia is a biodiversity hotspot (Mittermeier et al., 2011; Myers

et al., 2000) where the rusa deer is an exotic invasive species (Barrau and Devambez, 1957; Gargominy et al., 1996). It is regarded by local institutions as a "natural disaster", because of the major threat it poses to both agriculture and ecosystems. In New Caledonia, herbaceous monocotyledons represent the major part of the diet of this cervid as soon is available. However, they are characterised by great dietary plasticity and are able to turn to alternative food resources when this preferred resource is lacking (Berlioz et al., 2020, unpublished CNRS report). This plasticity enables it to occupy a wide range of habitats. A recent study (Frantz et al., 2024) revealed a unique, genetically homogeneous population throughout New Caledonia, while empiric observations of local Neo-Caledonian hunters suggest animal size variations based on geographic origin on the Grande Terre. This context offers a unique opportunity to explore the mandibular bone morphological variation within a population in response to different environments while breaking free from genetic influence.

This study aims to determine the relative importance of both genetic and environmental factors in shaping *Cervini* mandibular bone morphology at the intraspecific scale. For *Cervus elaphus*, we expect that an interaction between these two factors can explain the observed morphological patterns, while *Rusa timorensis* is anticipated to respond to the characteristics of local habitats. This study will also help better interpret mandibular morphological changes in future paleoecological studies.

Material and **methods**

Material

These individuals belong to 8 populations of extant cervid species (7 populations of *Cervus elaphus* and one of *Rusa timorensis*), with different genetic origins and contrasting habitats (Fig. 1). For most specimens, the sex and date of death are known, and the habitat is well-characterized in all cases. To limit ontogenetic allometry's impact on shape variations, we studied 315 hemi-mandibles of *Cervini*, focusing on adults with tooth eruption and dental wear

compatible with an age of more than 5-6 years old, when the mandibular growth is normally completed (Azorit et al., 2003).

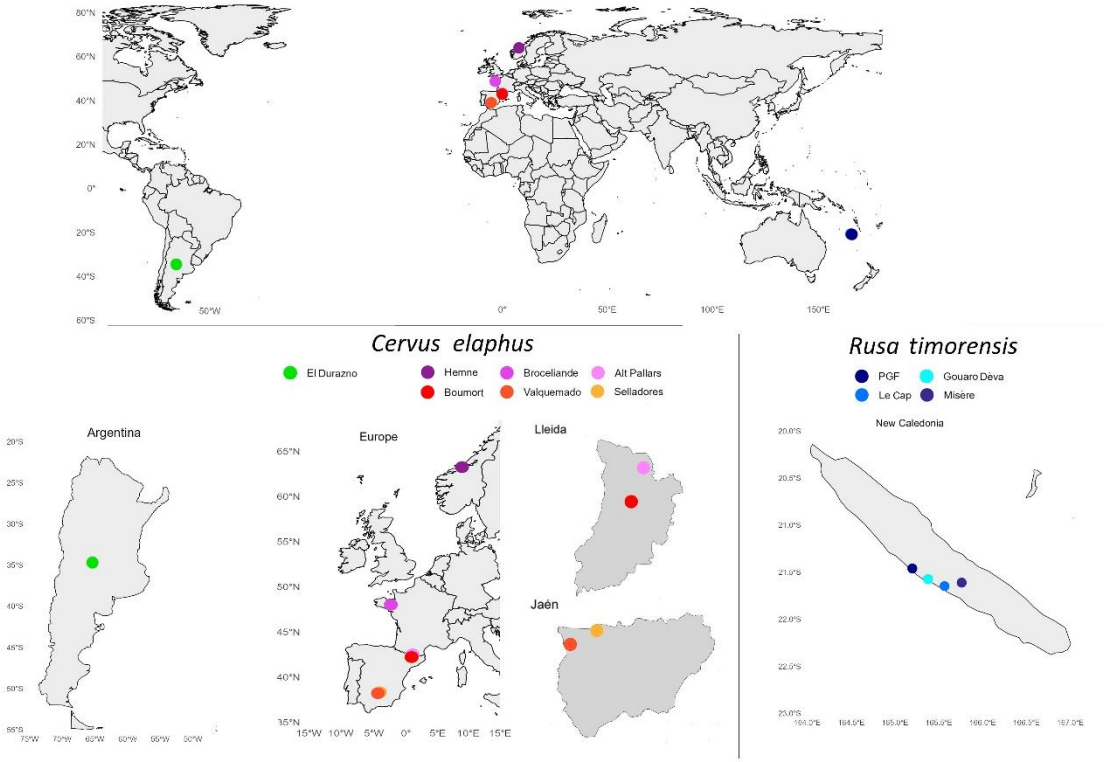

Figure 1: Location of the eight Cervini populations included in the study. ***Cervus elaphus***: El Durazno, Hemne, Broceliande, Alt Pallars, Boumort, Selladores, Valquemado. The focus on the Lleida region gives a better view of the Alt Pallar and Boumort populations. The focus on Jaen area gives a better view of the Valquemado and Selladores populations. ***Rusa timorensis***: New Caledonia (Gouaro Déva, Le Cap, Misère, Parc des Grandes Fougères (PGF)).

Red deer material

The red deer population of **El Durazno** (N= 21; Fig. 1) originates from a hunting ground located 20 km north of Buena Esperanza in the Province of San Luis, Argentina (-34.5459; -65.2982) in the Pampa Ecoregion (Table 1). The area is a grassy steppe, primarily covered with tussock

bushes. It typically includes small groves of chañar trees (*Geoffroea decorticans*). In some places, isolated caldén trees (*Prosopis caldenia*) are also present (Anderson et al., 1970).

Table 1: Information on the habitats of cervid populations. *Information on biomes is taken from https://ecoregions.appspot.com/. Description of the local vegetation for the populations living in geographical Europe is taken from Ozenda and Borel, 2000. For El Durazno and the Neo-Caledonian localities, the description is based on field observations.*

	Group	Biome	Local vegetation description
Cervus elaphus	Alt Pallars	Temperate Broadleaf & Mixed Forests	High mountain: subalpine, alpine and nival vegetation belts
	Boumort	Temperate Broadleaf & Mixed Forests	Lower and mid-mountain: collinean and montane vegetation belts
	Broceliande (Paimpont)	Temperate Broadleaf & Mixed Forests	Hyperhygrophilous Armorican sector
	El Durazno	Temperate Grasslands, Savannas & Shrublands	Semiarid Pampas
	Hemne	Boreal Forests/Taiga	Orocaledonian vegetation unit (mountain tundra)
	Selladores	Mediterranean Forests, Woodlands & Scrub	Siliceous, with round-leaved oak
	Valquemado	Mediterranean Forests, Woodlands & Scrub	Calcicolous, with round-leaved oak
Rusa timorensis	Gouaro Déva	Tropical & Subtropical Dry Broadleaf Forests	Sclerophyllous & meso-sclerophyll forests, extensive grasslands, Niaoulis savannah
	Le Cap	Tropical & Subtropical Dry Broadleaf Forests	Niaoulis savannah & agricultural grasslands
	Misère	Tropical & Subtropical Dry Broadleaf Forests	Mesophyll forest
	Parc des Grandes Fougères	Tropical & Subtropical Moist Broadleaf Forests	Dense tropical rainforest

Mean annual precipitation is 510 mm, about 80% of which falls between October and April, with low water availability during winter (Berton and Echeverría, 1999). The mean annual temperature is 17°C, with a strong continental effect (43°C in summer and 15°C in winter; Anderson, 1979). Although there is no data on the diet of *Cervus elaphus* in the southern portion of the province of San Luis; there is data in the neighbour province of Pampa, where this deer has 39.5% grasses in its annual diet (Pordomingo and Rucci, 2000). Specimens included in this study were hunted between 2022 and 2023. The El Durazno population was introduced to Argentina in 1904 (Novillo and Ojeda, 2008). Specimens came from various populations across Eastern Europe, specifically Germany, Austria, and the Carpathian Mountains. Deer from Eastern Europe represent a unique genetic lineage (Jaksic et al., 2002; Skog et al., 2009). In Argentina, red deer are considered an invasive exotic species (Merino et al., 2009)

The western European populations of *Cervus elaphus* included in the present study all belong to the Western European genetic lineage (Skog et al., 2009). This lineage is further structured (Niedziałkowska et al., 2011; Zachos et al., 2016) with Iberian deer (*Cervus elaphus hispanicus*) forming a genetically distinct group from the other western European populations among the Western European lineage (Carranza et al., 2016).

The red deer population from the municipality of **Hemne** (N = 35; Fig. 1; Table 1) comes from the southwest of Trøndelag county, in Mid-Norway (63.2885, 9.2157). The region is characterized by areas with open coniferous forest under an oceanic climate in a low-altitude coastal fjord landscape (Albon and Langvatn, 1992; Aune, 1973). The tree cover mainly consists of Scots pine (*Pinus sylvestris)* and *Betula* spp., with juniper (*Juniperus communis*) and ericaceous species (*Calluna*, *Empetrum*, *Vaccinium myrtillus*) making up the shrub layer. The herbaceous layer includes monocotyledonous and dicotyledonous patches of anthropogenic origin (Aune, 1973). Although there is no stomach content data for these animals, the red deer population of Åstfjorden, located in a similar environment 18 km away, consumes 55.5% graminoids, 29.9% herbs, and 14.6% shrubs. In this area, snow cover prevents access to the herbaceous layer for 4 to 5 months a year (Mysterud, 2000). Hemne red deer were hunted between September and December 2014. Norwegian red deer belongs to the sub-species *Cervus elaphus atlanticus* and differs genetically from the French and Spanish red deer populations (Zachos et al., 2016).

The French population from **Paimpont** (N = 28; 48.0723, -2.1640, Fig. 1, Table 1) originates from a small remaining portion of the **Broceliande Forest**, covering an area of 1200 hectares. It comprises dry moorland and deciduous woodland, under a temperate climate with oceanic influences (MNHN Natura 2000, 2017). The Broceliande population has strong genetic affinities with the Domaine National de Chambord (France) population, which has been regularly used as a source population to replenish French deer populations (Dellicour et al., 2011; Klein, 1990). The deer included in this study were hunted between September 2005 and January 2006. In the 20th century, red deer abundance drastically decreased in the Iberian Peninsula. They were only found in the southwestern area, especially in Sierra Morena. Many deer that recolonised other Spanish hunting areas in the 20th century came from these mountains (Arenzana et al., 1965). **Alt Pallars** red deer (N = 16; 42.5452, 1.2789, Fig. 1, Table 1) come from a National Game Reserve of Alt Pallars, a high mountain game reserve covering an area of 81,772 ha in the Spanish Axial Pyrenees. Climate is Atlantic and subalpine, with wet and temperate summers and very

cold winters and spring with significant snow cover (Gort-Esteve et al., 2023; Pérez-González et al., 2023). The sub-alpine belt is characterised by fir (*Abies alba*) and mountain pine (*Pinus uncinate*). The montane belt is characterized by Scots pine (*P. sylvestris*) and extensive deciduous forests composed of sessile oak (*Quercus petraea*), hazel (*Corylus avellana*), birch (*Betula pendula*), aspen (*Populus tremula*) or ash (*Fraxinus excelsior*). The montane landscape also includes rocky outcrops and Pyrenean broom (*Genista balansae*) and grasslands are abundant. Human activity (managed meadows and a few agricultural fields) is significant in the valley bottoms (Gort-Esteve et al., 2023). The red deer from Alt Pallars were hunted between 2015 and 2019, mainly during the hunting season between September and February. The population of the National Game **Reserve of Boumort** (13,097 ha), located in the Pre-Pyrenees (N = 18; 42.2313, 1.1235, Fig. 1, Table 1), is also located in the Spanish Pyrenees. Compared to Alt Pallars, this region is characterized by lower mountains, and an overall milder climate with hot and dry summers and cold winters with less snow accumulation. Most rainfall occurs in spring and autumn (Gort-Esteve et al., 2023; Pérez-González et al., 2023). Subalpine grasslands can be found, but the dominant vegetation consists of pine forests with an undergrowth of bearberry (*Arctostaphylos uvaursi*) and juniper. Additionally, there are Scots pine and Pyrenean pine (*P. nigra subsp. salzmanii*) forests. At lower elevations, there is a mixture of holm oaks (*Q. ilex*) forest and extensive areas of thermophilic bushes. The red deer from Boumort were hunted mainly between September and February, from 2013 to 2018. Alt Pallars red deer consume 49.1% of graminoids, whereas Boumort deer only consume 32.9% (Gort-Esteve et al., 2023). A genetic study including these two Pyrenean red deer populations has recently been conducted (Pérez-González et al., 2023). Despite being geographically close, they belong to genetically different clusters: Alt Pallars deer show more genetic similarities with *Cervus elaphus* from the south of France while the red deer of Boumort are genetically closer to the Iberian red deer (*Cervus elaphus hispanicus*).

Deer sampled from Southern Spain come from the two independent mountains **Selladores-Contadero** (N=31; 38.3497, -3.8428) and **Valquemado** (N=36; 38.2287, -4.1624, Fig. 1, Table 1), both located in Sierra Morena Oriental, in the Natural Park of Sierra de Andújar, Jaén. The deer studied from Valquemado were hunted in 1994-95 and the deer from Selladores-Contadero in the 2018-2019 hunting season, from October to February. These areas are characterised by a Mediterranean ecosystem with mild winters, precipitation in spring and autumn, very little rainfall and high summer temperatures. In both localities, the Mediterranean pasture habitat predominates is the Dehesa of holm oak and grassland vegetation, and some areas of reforestation pine forests, and mastic trees (Marañón, 1991). In this area, the red deer annual diet is mainly composed of herbaceous monocotyledons (57.1%; Azorit et al., 2012), with a peak in spring. Browses constitute an important food resource at the end of winter and of summer, while fruits are consumed more in autumn and winter. In these regions, the genetic identity corresponds to the Iberian deer subspecies (*Cervus elaphus hispanicus*).

El Durazno material is currently stored in the mastozoological collection of the Bioresearch Center of the National University of the Northwest of the Province of Buenos Aires, Argentina. Hemne and Paimpont specimens are stored at the Palévoprim lab (UMR6272, CNRS & University of Poitiers, France). Alt Pallars, Boumort, Valquemado, and Selladores-Contadero specimens belong to the Vertebrate Laboratory collection of RNM-175 Research Group, housed at the University of Jaén, Spain.

Rusa deer material

The unique population of New Caledonian rusa deer (Frantz et al., 2024) lives in a wide range of habitats, from man-managed plains to pristine dry forests, within various microclimates and varying densities of herbivores (Fig. 1). There, its natural disaster status justifies almost unlimited hunting 365 days a year. Between 2008 and 2022, a “jawbone reward” has been introduced to encourage the local residents to shoot them. This study centres on animals sampled throughout the year in 2018-2019 from four contrasting local New Caledonian habitats.

To date, there have been no studies of stomach content in these animals. The first sample comes from a cattle-breeding area in the surroundings of the municipality of **Poya** (N=54, 165.1972, -21.4605), on the west coast of New Caledonia, between Poya Bay and the Goulvain cape. In this coastal region, the vegetation consists mainly of niaoulis savannah, composed of trees and shrubs scattered throughout a dense herbaceous layer typical of the western coast of New Caledonia, and agricultural grasslands. This area is particularly arid. The second habitat is the **Grandes Fougères Provincial Park** (N=26, 165.7598, -21.6106), which straddles the municipalities of Farino, Moindou, and Sarramea. This dense tropical rainforest, spanning 4,500 hectares, is home to many endemic vegetal and animal species. Deer have caused significant damage in the area, especially to woody vegetation. However, deer pressure on the vegetation has slightly decreased since the implementation of a more intensive hunting program. A third group of animals (N=38) was hunted in the **Gouaro Déva Provincial Park** (Bourail; 165.3769, -21.5735). This protected natural area of 7,800 hectares includes 1,700 hectares of sclerophyllous forest, making it the largest dry forest area in New Caledonia. The park also has extensive grasslands, niaoulis savannah and meso-sclerophyll formations. Since 2008, an intense and effective daily hunting effort has significantly improved the vegetation growth in the park, which was previously severely affected by the high deer density. The last sample consists of 12 specimens from a mountainous area called **"Vallée Misère",** located between Moindou and Nessadiou. This forest, characterised by mesophyll vegetation, is seldom hunted due to access challenges, which is only possible through private properties. Due to the very high density of deer, the undergrowth of this forest is literally devastated, laid bare by the over-consumption of the herbaceous layer and any regrowth of woody plants, as well as by trampling.

All rusa deer specimens included in this study are part of a larger collection of biological material from New-Caledonian rusa deer belonging to the University of Poitiers (CVCU) and housed at the Palévoprim laboratory (UMR7262, CNRS & University, Poitiers, France).

Methods

We applied 2D geometric morphometric method, a powerful and fully quantitative approach to capture interspecific, interpopulation, and intrapopulation shape variations (Hammer and Harper, 2008; Lawing and Polly, 2010; Zelditch et al., 2012). To do so, each specimen was positioned perfectly horizontally by placing it on its lingual surface on a support made of ground coffee, following the protocol described in Azorit et al. (2020).

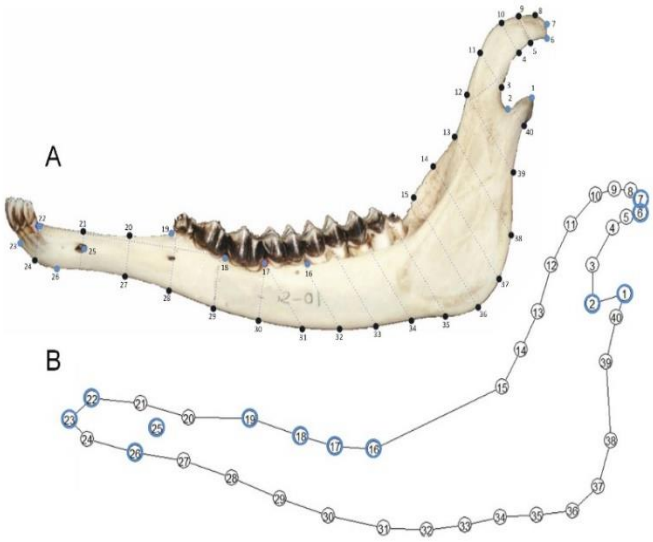

Figure 2: Landmarks (blue) and semi-landmarks (black) used to capture the mandibular bone morphology through a 2D geometric morphometric analysis on the buccal side of the left hemimandible. A: mandible of a red deer specimen from the Selladores-Contadero population showing the linear scheme by Makefan8 program to digitize the semilandmarks. B: Consensus shape for *Cervus elaphus*. The anatomical structures and regions corresponding to numbers 1-40 are described in Table 2.

All photos were taken with a Canon 7D digital camera, and a professional photographic table equipped with a graduated arm and lighting, allowing the camera to be perpendicular to the specimen. Each photo was taken at 50 cm distance with the same objective and settings, minimising image distortion and parallax error. We focused on the left hemimandible, but when unsuitable, we photographed the right hemimandibles and flipped it horizontally to obtain the jaw buccal surface. Adapting the existing protocol developed for similar mandibular studies (Azorit et al., 2020), we digitized 40 landmarks and semilandmarks on the mandible to capture

its shape using TPSDig2 (Rohlf, 2021). Since the homology is provided by the geometry of the mandibular structures or by biological characteristics, unique and easy to identify in all the individuals studied, the reference points 1, 2, 6, 7 and the group of 16-19, 22, 23, 25 and 26 are considered type II and I landmarks, respectively (Fig. 2, Table 2). To obtain a description of the mandibular curved edges, we used the Makefan8 tool of the Integrated Morphometric Software 8 Package (IMP 8) (Sheets, 2014). It is a graphical tool for selecting points to digitize semilandmarks along a curve and it is based on the creation of a scheme of equidistant lines between two landmarks. Figure 2 shows the linear reference scheme created to digitize the semilandmarks along the curves in a standardized manner across all specimens. Then, the semilandmarks were slid, to the position minimizing the squared Procrustes distance between the form of the hemimandible of a given specimen and the consensus reference form from all specimens, using gmShiny (v. 0.1.4) (Baken et al., 2021; Zelditch et al., 2012).

Table 2: Description of landmarks and semilandmarks used for capturing the mandibular shape for geometric morphometric analyses.

Landmarks	Names and Description
1	Tip of the condylar process
2	Ventral margin of the mandibular sigmoid notch
6	Ventrocaudal margin of the coronoid process
7	Tip of the coronoid process
16	Posterior alveolar margin of *M1*
17	Anterior alveolar margin of *M1*
18	Posterior alveolar margin of *P3*
19	Anterior alveolar margin of *P2*
22	Posterior alveolar margin of *C*
23	Ventral margin of the *I1*
25	Mental foramen
26	Posterior margin of the mandibular symphysis
Semilandmarks	**Description traits**
3 to 5	Concavity of the posterior border of the coronoid apophysis
8 to 15	Convexity of the anterior edge of the coronoid process
20 to 21	In the mandibular diastema, at the level of the Semilandmark 27 (20) and of the Landmark 25 (21)
24	Rostro-ventral margin of the horizontal ramus of the mandible, at the level of the posterior alveolar margin of C
27 to 38	Convexity of the ventral margin of the horizontal ramus of the mandible to the posterior margin of the angular process
38 to 40	Concavity of the caudal edge of the mandible ramus

Geometric morphometric analyses were performed with the R package Geomorph v. 4.0 (Adams et al., 2024; Baken et al., 2021). We obtained the centroid size (i. e. the square root of the sum of the squared distances of a set of landmarks from their centroid) for each specimen.

Statistical analyses

We first explored the differences in centroid size between groups using analyses of variance (ANOVAs) for each of the three datasets (for *Cervus elaphus* plus *Rusa timorensis*; for all *Cervus elaphus*; and for all *Rusa timorensis;* Annex 1), followed by Duncan's post-hoc tests where appropriate. We also did a generalized Procrustes analysis on the raw landmark coordinates for all specimens of each of the three datasets to remove differences in size, translation, and rotation. We then explored the structure of the variance using a Principal Component Analysis (PCA) on each of these datasets. The ANOVAs on the PC scores representing more than 80% of the variance were performed to detect between-group (species; populations; habitats)

differences. Where differences were detected, post hoc Duncan's tests were conducted to identify between which groups these differences were located. For axes showing significant differences, we describe the morphological variations along each PCA axis.

Then, with Canonical Variate Analyses (CVA) maximising the between-group differences, we focused on the morphological variations enabling us to differentiate between populations (dataset *Cervus elaphus*) and habitats (dataset *Rusa timorensis*). We also performed ANOVAs on canonical variates, followed by Duncan post-hoc tests where appropriate. We describe the morphological differences along each axis where significant differences exist between groups. Together, PCA and CVA provide a comprehensive understanding of the data structure and group differences. Finally, for the *C. elaphus* populations for which these data were available, we looked at the correlation between mandible shape variations and two explanatory variables: the percentage of herbaceous monocotyledons consumed annually and the latitude.

Procrustes regressions and Partial Least Square (PLS) analyses enabled us to investigate the relative influence of the animals' size (allometry/isometry) and population (for *Cervus elaphus*) or habitat (for *Rusa timorensis*) on the mandibular morphology of the deer.

Results

Interspecific differences:

There is a significant difference of centroid size between the two species ($p < 2.2e-16$; Table S1, Annex 1), with *Cervus elaphus* being significantly larger. The first four principal components (PC1-4) of the PCA represent over 80% of the variance (Annex 1). ANOVAs on the PC scores show that species statistically differ on PC1 ($p < 2e-16$; Table S1), PC3 ($p = 7.12e-15$; Table S1) and PC4 ($p = 0.0141$; Table S1).

Cervus elaphus occupies the lower values on PC1 (45.3%; Fig. 3), with thinner and more slender mandibles compared to *Rusa timorensis*, which has higher values on PC1. The angle made by the ramus and the corpus on the internal side of the mandible is more perpendicular in *Rusa*

timorensis and more open in *Cervus elaphus*. The angular process (anatomical region 34 to 39 in Fig. 2) of *Rusa timorensis* is more voluminous, rounded and robust. Its coronoid process is thicker, less elongated posteriorly and less pointy. The posterior margin of the mandibular symphysis is more concave in *Cervus elaphus*, and straighter and oriented upwards in *Rusa timorensis*. On PC3 (10.2%; Annex 1), *Cervus elaphus* occupies the higher values. Shape deformation reflects a more pronounced concavity in the anterior margin of the mandibular

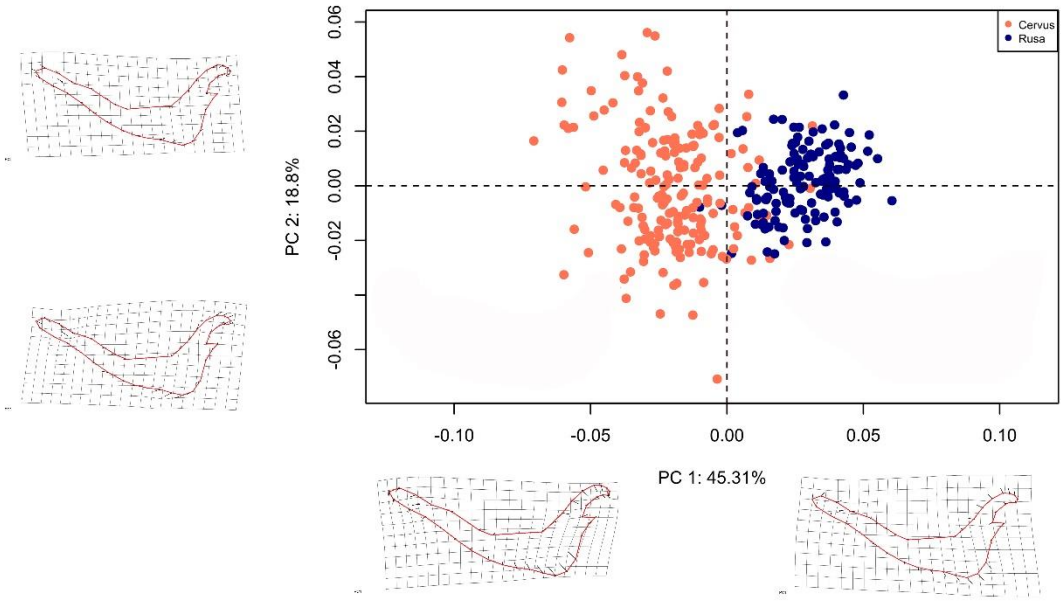

Figure 3: Biplot representing the two first principal components of the PCA exploring interspecific mandibular shape variations between Cervus elaphus and Rusa timorensis. Shape deformations are shown along PC1 and 2 for each species (C. elaphus: coral; R. timorensis: navy blue). The consensus shape for all Cervini is shown in grey to aid interpretation.

angle, at the level of the vascular incisure, for *Cervus elaphus* compared to *Rusa timorensis*. On PC4 (5.8%; Annex 1), interspecific differences are less obvious and seem to be due to a few outlying individuals.

Interpopulation differences: between *Cervus elaphus* populations

There are significant differences in centroid size ($p < 2.2e-16$; Table S1) among the seven *Cervus elaphus* populations considered. Based on Duncan's post-hoc tests (Table S2; Annex 1), the El Durazno deer is significantly larger than the European populations, whereas Boumort deer

constitute the smaller population. The second smallest *Cervus elaphus* are those of Valquemado population. It significantly differs from the larger deer found in Alt Pallars and Broceliande. Hemne and Selladores-Contadero fall between Valquemado and the group of Alt Pallars and Broceliande, and do not statistically differ in size from either of them.

PC1-5 of the PCA exploring the shape deformation among these populations represent more than 80% of the variance (Annex 1). ANOVAs on PC scores indicate significant differences between populations on each of these PC ($p < 0.05$ in all cases; Table S1). Interpopulation differences are identified based on Duncan's post-hoc tests (Table S2). On PC1 (percentage of explained variation: 37.3%; Fig. 4, A and B), the El Durazno population of *Cervus elaphus* stands out as having the most negative values. The Boumort population has the highest values and is statistically different (Fig. 4 B; Table S1 and S2) from all the other populations. Alt Pallars, Hemne and Broceliande populations differ significantly from the first two, with high to medium values on PC1. Finally, Selladores-Contadero and Valquemado are similar to each other, with high values on PC1, and do not differ significantly from Alt Pallars and Boumort, respectively (Table S2; Annex 1). Morphologically, specimens in the low PC1 range have a more elongated diastema than populations with higher values. These specimens also have a lower vertical ramus and a less pronounced mandibular sigmoid notch. Their corpus is straighter and less convex in its ventral margin. The posterior margin of the mandibular symphysis is more concave in these

individuals with low PC1 values. Alt Pallars deer occupy the lower values of PC2 (25.9%; Fig. 4 A; Table S2; Annex 1), statistically differing from Selladores-Contadero and Valquemado, which are in the higher range. Hemne falls between these two extremes and is statistically different from

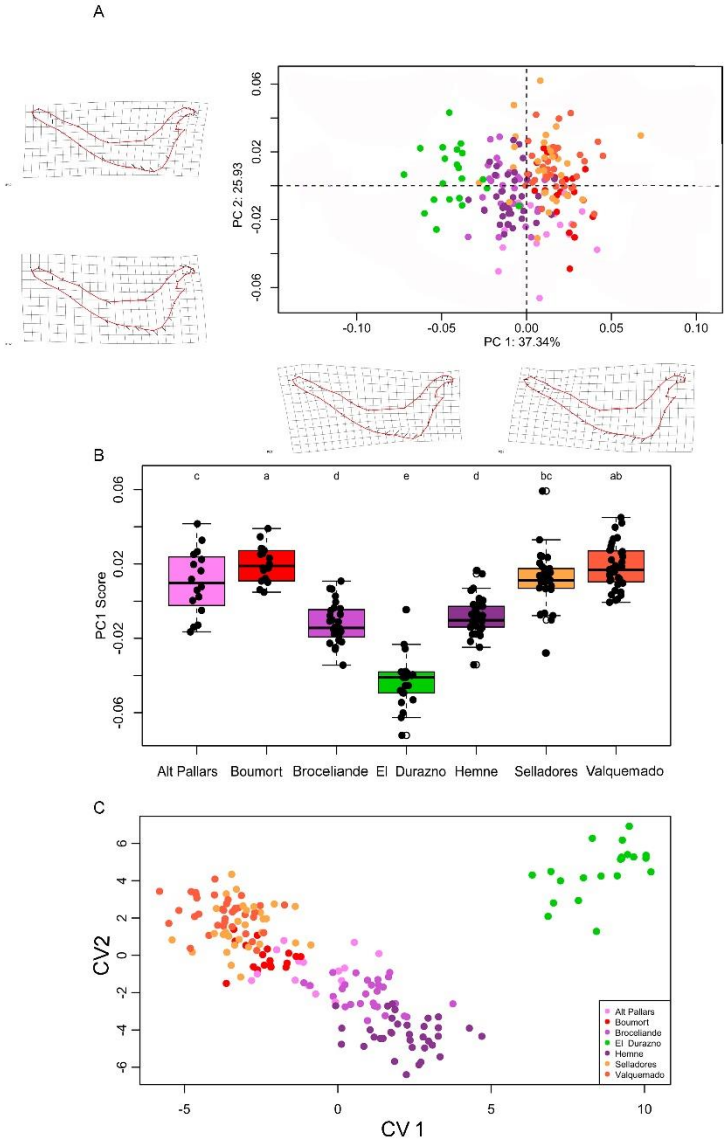

Figure 4: A: Biplot representing the two first principal components of the PCA (63.3% of the variance) exploring Cervus elaphus *interpopulation mandibular shape variations. Shape deformations are shown along PC1 and 2 for each population (Alt Pallar: pink; Boumort: red; Broceliande: mauve; El Durazno: green; Hemne: purple; Selladores: dark yellow; Valquemado: orange). The consensus shape for all C. elaphus is in grey to aid interpretation. B: Focus on the variation on PC1. Letters (a-e) represent interpopulation differences based on Duncan post hoc test. C: Biplot representing the two first canonical variates of the CVA.*

both. Boumort, Broceliande and El Durazno populations have values between Hemne and the two populations of Southern Spain, with El Durazno being closer to the populations in Southern Spain and Boumort and Broceliande being closer to Hemne. These statistical differences are reflected in deformations along PC2 (Fig. 4 A; Annex 1). In individuals with negative values of PC2, the ramus and corpus of the mandible appear more robust, with a more pronounced and rounder angular process. The angle between the ramus and the corpus of the mandible is perpendicular for specimens with lower PC2 values and becomes more obtuse in those with higher values. Additionally, in specimens with lower PC2 values, the ramus of the mandible is higher, and the corpus of the mandible is more elongated and directed upward.

The Duncan test reflects fewer differences on PC3 (Annex 1). The Selladores-Contadero population, with higher values, significantly differs from the populations of El Durazno and Boumort, both characterized by lower values. With intermediate values on PC3, the four other populations are not significantly different from Selladores, El Durazno and Boumort.

There is a moderate correlation between the shape of the mandible on PC1 and the latitude (Annex 1: correlation of -0.56 between these two variables, see also the graphic “Correlation between Latitude and PC1”), whereas the correlation is weak between the percentage of graminoids and the latitude (Annex 1: correlation = -0.21, see also the graphic “Correlation between $\%_{graze}$ and PC1”).

ANOVAs performed on the six canonical variables of the Canonical Variate Analysis (CVA) all support significant differences ($p < 2e-16$ for CV1-6; Table S1, Annex 1), further identified via Duncan's post-hoc tests (Table S2). On CV1 (Fig. 4 C), all populations differ statistically. The European populations show a range of low to medium values on CV1, while the Argentina population of El Durazno exhibit the highest value. More specifically, the populations of Selladores-Contadero and Valquemado (Southern Spain) have the lowest values, followed by the Alt Pallars and Boumort populations (Northern Spain). The French population of Broceliande and the Norwegian population of Hemne have medium values. The shape deformations on CV1

mainly concern the length of the corpus of the mandible, the length of its ramus and the shape of the vascular incisure, at the anterior margin of the angular process. Specimens in the low CV1 range are characterised by a shorter corpus and a higher ramus than specimens in the high CV1 range. The vascular incisure is clearly convex in *Cervus elaphus* individuals in the low values of CV1, whereas it is concave in specimens with higher values. All populations differ statistically on CV2 (Fig. 4 C; Tables S1 and S2; Annex 1). The Norwegian population of Hemne is situated in the lower range, followed by Broceliande, Alt Pallars, and Boumort. Selladores-Contadero and Valquemado have intermediate values, while El Durazno occupies the higher range of CV2. Morphological deformations on CV2 are located on the coronoid process, higher and less posteriorly oriented for specimens with lower values. For these individuals, the angular process is also more robust, and the posterior margin of the mandibular symphysis is less concave.

A correlation of 0.85 suggests a strong linear relationship between latitude and CV1 (Annex 1, see also the graphic "Correlation between Latitude and CV1"), whereas a correlation of 0.02 (see also the graphic "Correlation between %graze and CV1" in Annex 1) means there is no relation between the latitude and the percentage of herbaceous monocotyledons.

Inter-habitat and intrapopulation differences: *Rusa timorensis* from contrasting habitats

Based on the analysis of variance (ANOVA), the centroid size of *Rusa timorensis* specimens from the various contrasting habitats differs significantly ($p = 9.61e-05$; Table S1). According to Duncan's post-hoc test, *Rusa timorensis* from Gouaro Déva are significantly smaller than rusa deer from the other three habitats (Table S2; Annex 1).

PC1-8 of the PCA represent over 80% of the variance. Only PC2, 5 and 6 show significant differences between the four habitats (p-values < 0.05 in all cases; Table S1, Annex 1). Based on the Duncan post hoc test (Table S2), we can statistically differentiate between the Gouaro Déva animals, which exhibit the highest values on PC2 (16.2% of the explained variance, Fig. 5 A and B), and the deer from the Parc des Grandes Fougères, which show the lowest values. Deer from

Le Cap and Misère are not statistically different from the others. Morphologically, these differences are found in the condylar process, which goes further back in animals with high PC2 values, and in the mandibular symphysis, which is more upward-oriented in these same

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individuals. In these animals, the angular process is more robust, whereas it is frailer and concave at the level of vascular incisure in animals with low values on PC2.

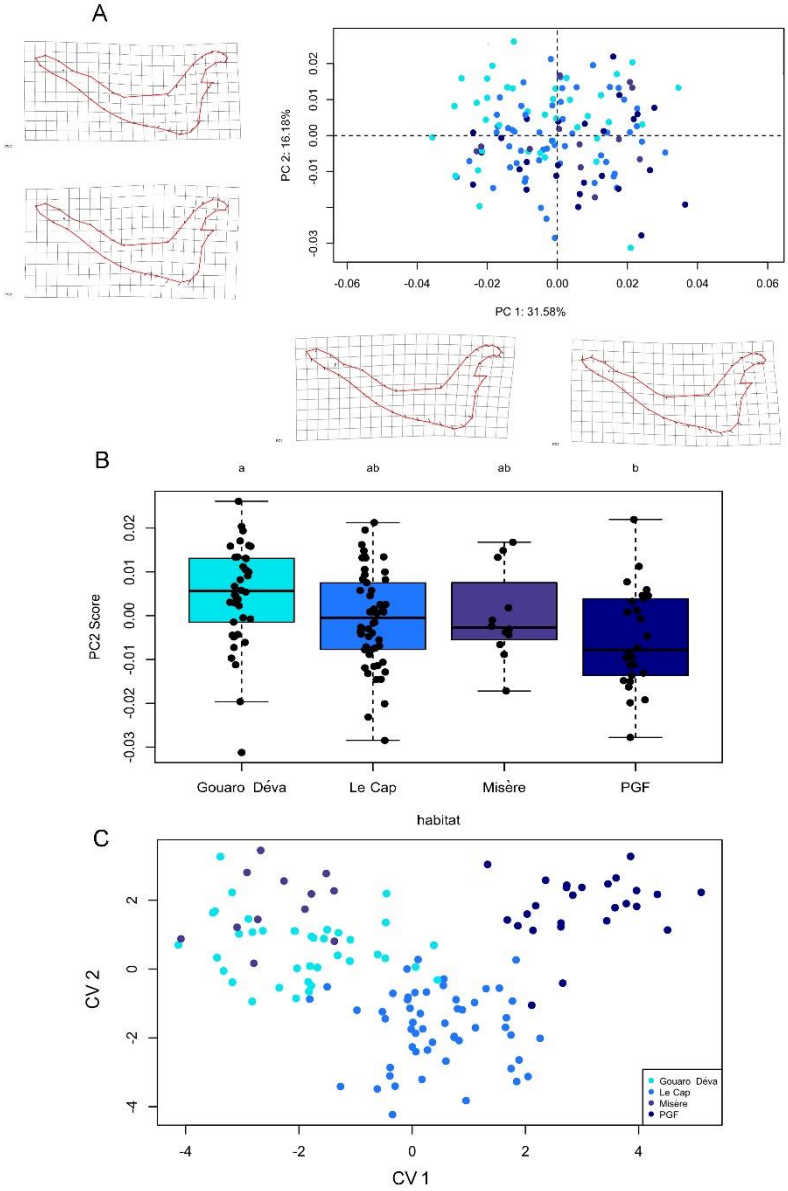

Figure 5: A: Biplot representing the two first principal components of the PCA (47.76% of the variance) exploring Rusa timorensis intrapopulation mandibular shape variations between contrasting habitats. Shape deformations are shown along PC1 and PC2 (Gouaro Déva: Turquoise, Le Cap: royal blue, Misère: plum, Parc des Grandes Fougères (PGF): navy blue). The consensus shape for all R. timorensis is in grey to aid interpretation. B: Focus on the variation on PC2. Letters a and b represent interpopulation differences based on Duncan's post hoc test. C: Biplot representing the two first canonical variates of the CVA.

The fifth principal component represents 5.7% of the variance (Annex 1). Deer from the Parc des Grandes Fougères stand out with high PC5 values, which are statistically different from the low values of Gouaro Déva and Misère deer on this axis. Deer from Le Cap present intermediate values and are not different from the other deer. On PC5, specimens with low values have a well-rounded angular process, unlike specimens with high values, whose angular process protrudes backwards. The rest of the mandible is fairly stable along PC5.

On PC6 (4.4% of the variance; Annex 1), a significant statistical difference exists between the higher values of Gouaro Déva deer and the lower values of Parc des Grandes Fougères deer. Le Cap and Misère deer show intermediate values and are not statistically different from the first two groups. The morphological differences on PC6 are mainly situated in the posterior margin of the ramus of the mandible, which is more robust in animals with low PC6 values. The mandibular symphysis of these specimens is also straighter. On specimens with higher values it is turned slightly downwards.

ANOVAs on the three canonical variables of the Canonical Variate Analysis (CVA; Table S1, Annex 1) all support significant differences ($p < 2e-16$ for CV1-3). Gouaro Déva and Misère deer constitute one group with lower values of CV1 (Fig. 5 C; Annex 1), which is statistically different from Parc des Grandes Fougères deer situated in the higher values of this axis and from Le Cap with intermediates values. The coronoid process of individuals in the high CV1 range is more voluminous. The concavity of the vascular incisure is more pronounced in the lower values of CV1. In addition, the mandibular symphysis of these animals is oriented more upwards than in animals with high values on this axis.

On CV2 (Fig. 5 C; Annex 1), we statistically distinguish Le Cap, with low values, from a group gathering together Parc des Grandes Fougères and Misère, and from Gouaro Déva deer (Table S2). The last three populations present positive values on CV2. The mandibular symphysis is more oriented downwards for individuals with high CV2 values. The vascular incisure of these

animals is slightly more concave, and the coronoid process is slightly lower and projected further back.

Finally, we identify three statistically different groups along CV3. Misère deer are characterized by high values, Le Cap and Parc des Grandes Fougères deer by medium values, and Gouaro Déva deer by lower values. Shape variations along this axis are subtle. The ramus of the mandible appears to be slightly less robust at its anterior margin in animals with low values. The condylar process is projected slightly further back in the same animals.

Allometry

The Procrustes regression (Procrustes distance-based linear model) performed on the first dataset "*Cervus elaphus* plus *Rusa timorensis*" indicates that both "centroid size" and "species" significantly explain shape differences between species (p-values =0.001; Table S3; Annex 1). R^2 indicate that 33.7% of the variance in this dataset is explained by log (Centroid size), and 5.6% by the species (Table S3). But these two variables are not independent (Annex 1: r_pb = -0.878). Within each population, there is a quasi-isometry (Fig. 6).

Regarding *Cervus elaphus* there is a moderate-strong (r-PLS: 0.641; Table S5) and statistically significant (p-value= 0.001; Table S5) correlation between centroid size and mandible shape. The variance explained by size is $R^2 \approx (\text{r-PLS})^2 = 0.428$, indicating that 43% of the variation in shape is due to size, while 57% is explained by other factors (Annex 1). Centroid size explains only half as much of the between-group differences as the population does ($R^2_{population}$ = 0.29; $R^2_{centroid\ size}$ = 0.14; Table S3). For PC1-5, where shape differences were observed between populations, the same observation was made: it is the population that explains most of the variance in the model ($R^2_{Population} > R^2_{Centroid\ size}$ in most cases; Table S4; Annex 1). Moreover, the allometry plot (Fig. 6 A; Annex 1) suggests a quasi-isometry, with the shape of *Cervus elaphus* mandibles not varying significantly when size increases inside each population. Finally, the Partial Least Squares analysis (Table S5), which aims to maximise the covariance between centroid size and

mandibular shape, estimates that the correlation between the centroid size and the mandibular shape is statistically significant (p = 0.001) and moderate to strong (r-PLS: 0.641).

For *Rusa timorensis*, the variance explained by size is $R^2 \approx (r\text{-PLS})^2 = 0.216$, indicating that only 22% of the variation in shape is due to size, while 78% is explained by other factors (Fig. 6 B, Annex 1), such as the habitat to which the individuals belong (p = 0.030; Table S3). Based on R^2, 4.4% of the variance is explained by log (centroid size) and 3.7% by the habitat (Table S3). The

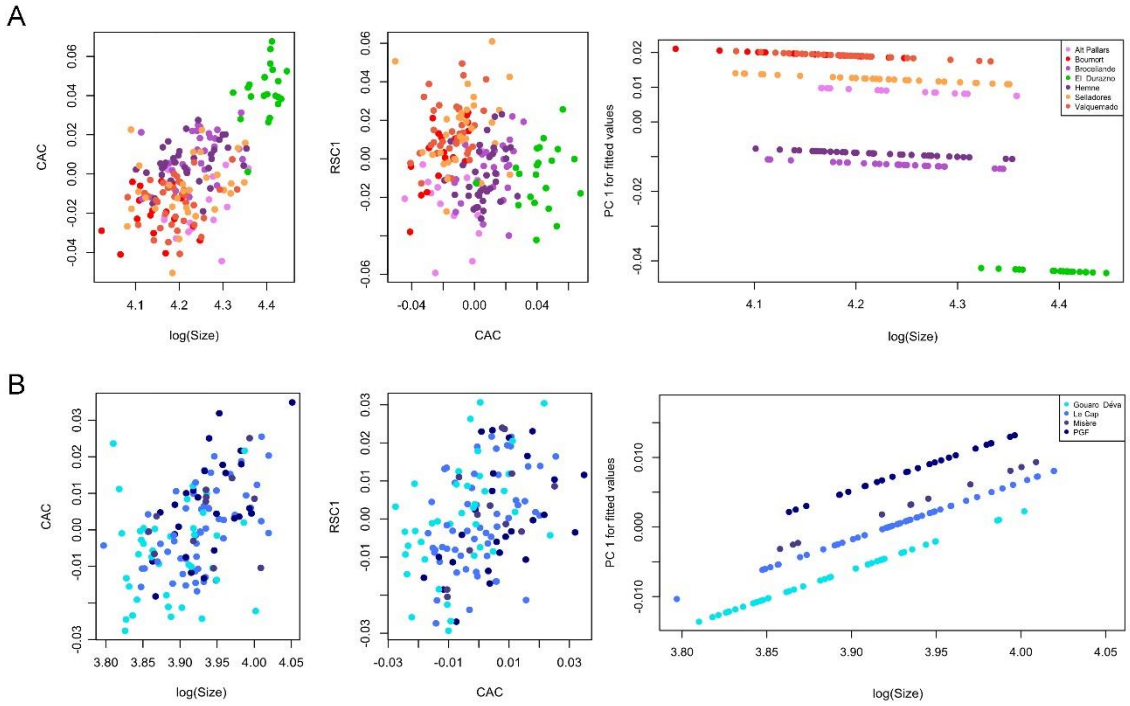

Figure 6: Allometric relationships and morphological variation in Cervus elaphus from different populations (A) and Rusa timorensis from contrasted local habitats (B). Plots illustrate body size and shape variations, both within and among Cervini *populations, highlighting population-specific morphological trends. Log (Size): log-transformed body size; CAC and RSC1 are morphological metrics. CAC: Common Allometric Component; RSC1: Residual Shape Component 1; PC1: Principal Component 1. The rightmost plots provide information regarding allometry or isometry.*

procrustes regressions performed on each axis of the PCA identifying morphological differences between habitats (PC2, PC5, PC6) show that on PC2 and PC6, only centroid size influences variations in mandible shape (p-values of 0.004 and 0.002 respectively; R^2 of 0.07 and 0.09 respectively; Table S4, Annex 1). On PC5, on the other hand, only the habitat is responsible for the morphological variations (p = 0.002; R^2=0.13; Table S4, Annex 1). Finally, the Partial Least Squares analysis estimates that the mandibular bone morphology of *Rusa timorensis* shows a

moderate (r-PLS= 0.465; less than for *Cervus*; Table S5) and statistically significant (p = 0.001; Table S5) correlation between centroid size and mandible shape.

Discussion

Interspecific differences

Shape differences between the two species reflect distinct evolutionary histories (Mackiewicz et al., 2022; Raia et al., 2010). These differences can be related to different biomechanical needs associated with contrasting feeding ecologies for the two species (Clauss et al., 2008). Indeed, *Cervus elaphus*, a generalist ungulate that can adjust its diet from grazing to browsing (Azorit et al., 2012; Bugalho and Milne, 2003; Gębczyńska, 1980) depending on the availability of resources in its habitat (Gebert and Verheyden-Tixier, 2001), has a mandible that is generally more slender and thinner than that of *Rusa timorensis*. The angle made by the ramus and the corpus on the internal side of the mandible is obtuse. The angular process has a concave shape at the level of the vascular incisure. In contrast, *Rusa timorensis* from New Caledonia is highly selective towards herbaceous monocotyledons (Berlioz et al., 2020). The architecture of its mandible is more orthogonal and straighter with a robust angular process corresponding to the insertion zone of the Masseter and Pterygoid muscles (Greaves, 1991; Kangas et al., 2017; Pérez-Barbería and Gordon, 1999). Compared to the red deer mandibles, rusa deer mandibles are better shaped to withstand the larger biomechanical stress and mastication forces expected from a grazer diet (Kangas et al., 2017; Raia et al., 2010).

Differences between *Cervus elaphus* populations

Results showed that the shape variations among red deer are mainly explained by population identity, and to a lesser extent by the size of the mandibles. The quasi-isometry observed and the relative effect of size and population identity on the distribution (Fig. 6; Table S3), means that intrapopulation variations in size have little impact on the morphological variations observed. For this reason, the rest of the discussion focuses on interpopulation differences.

The significant size differences between the seven populations of red deer demonstrate the polymorphic nature of this species (with sizes ranging from 50 kg to 400 kg; Geist, 1998). In Europe, there is a significant size gradient between the morphologically largest populations in Eastern Europe and the smallest in Southern and Western Europe (Lister, 1996). This is often referred to as the Bergmann's rule, which states that larger size provides an advantage under cold climates by reducing thermal loss. Such a trend is also observed at smaller scales, like among Norwegian red deer populations (Langvatn and Albon, 1986). It is now more often associated with the latitudinal clines in the primary productivity and the quality of the plants eaten by the animals. This is because the water, light, and temperature conditions at higher latitudes favour the growth of plants that are more nutritious (richer in proteins and soluble carbohydrates) and more digestible due to their less lignified cell walls (Deinum, 1981; Langvatn and Albon, 1986 and references therein). These characteristics strongly influence animal growth and weight gain (Langvatn and Albon, 1986; McNab, 2010: also referred as the "Resource rule"). However, among the seven red deer populations studied here, we observed no size cline correlated with latitude (Table S3; Annex 1). The study of a larger number of populations along this latitudinal range would probably make it possible to reduce the influence of habitat-specific characteristics (i.e., distance from the coast, topography, altitude, etc.) and thus highlight such a trend.

El Durazno population, which comes from the Eastern European genetic lineage of *Cervus elaphus*, is characterised by larger body sizes. We also found that the largest individuals in our study came from this population. Individuals sampled from populations genetically closer to the Iberian red deer *C. elaphus hispanicus* (Selladores, Valquemado and Boumort) were located at the lower end of the body size continuum. If poor environmental conditions can explain overall interpopulation differences in body size (Azorit et al., 2020), we cannot completely rule out the role of genetics in size variation.

Numerous studies have highlighted the strong association between the shape of the mandible, its biomechanical performances and the physical properties of the diet (Clauss et al., 2008; Fletcher et al., 2010; Pérez-Barbería and Gordon, 1999; Raia et al., 2010; Spencer, 1995; Varela and Fariña, 2015; Wang et al., 2022; Zhou et al., 2019). Here, there is a noticeable morphological gradient in European red deer mandibles along a north-south transect from Southern Spain to Norway, visible both on PC1 and CV1 (Annex 1). The vertical branch and the coronoid process of the southernmost specimens are higher, which increases the biting force of the temporalis muscle (Kangas et al., 2017; Pérez-Barbería and Gordon, 1999). The angle made by the ramus and the corpus of the mandible is more acute and the angular process more developed on its anterior margin, providing a greater insertion area for masticatory muscles such as the masseter and pterygoid (Greaves, 1991; Pérez-Barbería and Gordon, 1999). Overall, the mandible of the southernmost specimens is more robust, allowing for more efficient chewing of hard and tough foods (Clauss et al., 2008). To the north, at the other end of the range, the mandibles are more slender and gracile, indicating that less biting force is needed for mastication of the dominant diet (Fletcher et al., 2010). Kangas et al. (2017) demonstrated a similar mandibular morphological shift along a shorter North-South transect for moose populations in Finland under a boreal climate. These authors associate a less robust mandible for northern moose with a lower percentage of grasses in their diet. In our case, on a broader geographical scale, there is no linear correlation between the percentage of herbaceous monocotyledons consumed and the latitude (Annex 1), meaning that the percentage of herbaceous monocotyledons alone (ranging from 30 to 60% for the populations in this study) is not a sufficient indicator to explain this latitudinal gradient. The picture is indeed much more complex. The transect from Norway to Southern Spain crosses many climatic regions (Dinerstein et al., 2017; Peel et al., 2007) and vegetation units (Ozenda and Borel, 2000). As a result, the taxonomic composition of deer diets varies greatly between populations. In addition, the percentage of graminoids doesn't reflect the overall dietary composition of the animals. Furthermore, annual percentages of bushes and

trees, graminoids, forbs and fruits in the diet do not reflect the important seasonal dietary variations (Azorit et al., 2012; Gort-Esteve et al., 2023; Mysterud, 2000; Pordomingo and Rucci, 2000). These seasonal dietary changes result both from the physiological needs of the animals, notably in terms of nutrients (Verheyden-Tixier et al., 2008), and from behaviours aimed at compensating for restrictions on access to certain resources (linked for example to the snow cover : Albon and Langvatn, 1992; to plant phenology : Azorit et al., 2012; or to the seasonal senescence of certain resources : Bugalho and Milne, 2003). Although not the most important part of the diet, fallback food, which is important when preferred resources are unavailable, is essential at certain times of the year. This is the case, for example, with acorns, which are eaten by males in large quantities in autumn in Southern Spain (Azorit et al., 2012; Berlioz et al., 2017). Such food items may require specific chewing forces to process and, therefore, the development of masticatory muscles. The stems and leaves of some shrubs can be as tough or tougher than some herbaceous monocotyledons (Berlioz, 2017; Merceron et al., 2010). Finally, for the same type of food, the quality and physical composition can vary greatly depending on the season and along a North-South transect (Hebblewhite et al., 2008). Different parts of the plant and plants of different ages also have different physical properties. The example of herbaceous monocotyledons provides a good illustration of this last point. In Southern Europe, summer is a particularly difficult season for ungulates, as severe summer droughts lead to senescence of the herbaceous layer, resulting in scarce food resources, of poor quality, fibrous and abrasive. This resource type requires more force to process. In Northern Europe, on the other hand, while the herbaceous layer is inaccessible to ungulates for several months of the year because of the snow cover (Albon and Langvatn, 1992), when the snow melts, the animals have access to tender young shoots, rich in protein and low in fibre (Hebblewhite et al., 2008; Langvatn and Hanley, 1993), requiring less chewing force. There, herbaceous monocotyledons do not reach fibre compositions as high as those observed in summer in Southern Europe.

Geographical differences in the physical properties of dietary boluses inevitably affects the chewing forces required for comminution. This will consequently affect the development of masticatory muscles and mandibular bone morphology. Although we lack direct measures of the physical properties of the diet, we expect that such factors can be partly responsible for observed the north-south variation in mandibular morphology. To confirm this hypothesis, changes in the physical properties of vegetation resources along a gradient between Norway and Southern Spain would need further investigation.

The Argentinean red deer population from El Durazno is considered separately here as it displays a unique morphology, with a finer mandibular angle and an even more pronounced slender global morphology, that sets it apart from European populations. At El Durazno, the deer have access to a savanna-like herbaceous layer, except in winter when the grass is dry. In winter, these deer mainly feed on fruits and the leaves of trees and bushes (Pordomingo and Rucci, 2000). Both genetics and environmental factors could explain the peculiar aspect of the mandibles of El Durazno deer. To go further and settle this question, exploring the variations in mandibular shape between several populations of Argentinian red deer and representatives of the original gene pool in Central Europe would be interesting.

Inter-habitat differences: among *Rusa timorensis* population

Together, body size and habitat account for around 8% of the shape variation observed in *Rusa timorensis*. This low percentage is most likely explained by the recent introduction of a limited number of individuals of the species to New Caledonia (Barrau and Devambez, 1957), but nevertheless reflects a biological reality.

At first sight, the comparison of centroid sizes in rusa deer from contrasting habitats yields results that are surprising. Indeed, the rusa deer of the Gouaro Deva Provincial Park, although occupying a habitat rich all year round in herbaceous monocotyledons, the preferred resource of these animals, are characterised by smaller body sizes than the other rusa deer in this study.

Body size differences between ungulate populations from different habitats can be related to the availability, accessibility, and nutritional quality of resources, particularly during animal growth (Azorit et al., 2020; Terada et al., 2012; Toïgo et al., 2006). This can be complicated when access to resources is made difficult, such as in situations of interspecific or intrapopulation competition when animal density is high (Bonenfant et al., 2009; Toïgo et al., 2006). Although there is no official estimate of the population density at Gouaro-Déva, the recovery of vegetation in the area and an improved body shape condition of the deer in recent years testify to the sharp decline in the density of this "natural disaster" since the introduction of the jawbone reward in 2008, designed to protect endemic New-Caledonian flora and fauna from the destruction caused by this invasive deer. An increasing number of studies are examining the impact of human-related disturbances on animal behaviour (Frid and Dill, 2002). Greater animal mobility in response to human disturbance could limit access to resources (Frid and Dill, 2002; Jayakody et al., 2011), thereby impacting animal growth and adult size. At Gouaro-Déva, human recreational activities occur on a daily basis throughout the year (hiking, horse riding, 4x4 excursions, hunting, golf, etc.) and deer mobility is much more important than in other habitats (Berlioz, *Pers. Obs.*). Therefore, we propose that the small size of the rusa deer at Gouaro Déva is the result of higher levels of stress for these animals. Despite the availability of resources, the animals spend more time in constant displacements, and less time foraging. This negative effect of stress has previously been demonstrated in a population of mouflon in Southern Spain (Azorit et al., 2020), where it resulted in poor ontogenic development associated with delayed tooth eruption and a smaller, more gracile adult mandible. As a result, these animals were less able to process hard food. This hypothesis could be tested by conducting an ontogenic study on rusa deer mandibles from the biological collections of the University of Poitiers.

The morphologies of the mandibular bones of rusa deer from the Gouaro-Déva and Grandes Fougères provincial parks clearly differ, partly attributable to the very contrasting vegetation between these two habitats. The Grandes Fougères Provincial Park is indeed 90% dense tropical

rainforest. A high density of deer exploits a few meadows and grassy areas along the tracks and parkings, but the deer also browse intensively on the woody vegetation of the park, causing major damage to ligneous plants (Berlioz, *pers. obs*.). On the other side, while the Gouaro Déva domain includes an important relic of the New Caledonian dry forest, its primary vegetation is large areas of niaouli savannah, characterised by an abundant herbaceous layer rich in monocotyledons.

The angular process of the mandible of the deer from the Grandes Fougères Provincial Park is prominent. This area corresponds to the insertion zone of the masseter muscle on the labial side and the pterygoid muscle on the buccal side (Pérez-Barbería and Gordon, 1999). The coronoid process, where the temporalis muscle is inserted, is longer and wider. The robustness of the mandibles of these deer meets high biomechanical requirements. Indeed, these animals feed on tough vegetal resources like tree fern fronds (Berlioz, *pers. obs*.), requiring substantial biting force to be comminuted. Le Cap and Misère are characterised by more abundant herbaceous layers and more open landscapes compared to the Grandes Fougères Provincial Park, and the morphology of their mandibles shows intermediate values, with slightly greater similarities to the Gouaro Déva deer.

Our results offer a clearer understanding of the relationships between mandible size, variations in mandibular bone morphology, the impact of environmental and genetic factors. By exploring the mandibular morphology of these two present-day cervids living under well-known environmental conditions, this study will provide a benchmark for studying fossil specimens for which environmental and genetic data are generally scarce or unavailable. This study demonstrates the advantages of studying mandibular morphology for palaeoecological and palaeoenvironmental reconstructions.

Conclusion

We have provided evidence supporting the idea that although taxonomy significantly influences variations in mandibular bone morphology between species, this morphology is likely to provide valuable insights into the ecology and environment of species at a finer scale, both within and among populations. We have shown a linear relationship between the robustness of the mandibles of European red deer and the latitude along a North-South aridification gradient. While such a relationship had already been shown for moose (Kangas et al., 2017), it had never been demonstrated for red deer populations on such a large geographical scale. These differences in shape can be linked to the biomechanical forces required to process food, the physical properties of which vary between climatic regions. These interesting results demonstrate the potential of mandibular bone morphology as an eco-indicator to track the responses of ungulates to the current trend of aridification and even desertification in Europe, particularly concerning for the Iberian, Italian and Balkan peninsulas, as well as Eastern Europe, in the context of global climate change (Gao and Giorgi, 2008; Polade et al., 2014).

Since there is only one genetically homogeneous population of rusa deer in New Caledonia (Frantz et al., 2024), this constitutes a unique model for studying the relationship between morphology and environmental factors. This particularity has enabled us to delve deeper into this question and to suggest the impact of other factors on this mandibular shape variation, such as stress induced by human activities. This work also makes a valuable contribution to our knowledge of the New Caledonian rusa deer, an invasive species whose management is essential to protecting this biodiversity hotspot but whose ecology and plasticity are paradoxically still insufficiently documented. It has also enabled us to identify interesting new research perspectives.

Finally, our findings also emphasize the potential of studying well-preserved fossil deer mandibles to contribute to our understanding of palaeoenvironments, their evolution over time,

and the ecological responses of fossil species to environmental changes induced by past climatic oscillations.

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Tables, Figures, Supplementary data:

Tables and SI Tables

Table1: Information on the habitats of cervid populations. Information on biomes is taken from https://ecoregions.appspot.com/. Description of the local vegetation for the populations living in geographical Europe is taken from Ozenda & Borel, 2000. For El Durazno and the Neo-Caledonian localities, the description is based on field observations.

Table 2: *Description of landmarks and semilandmarks used for capturing the mandibular shape for the geometric morphometric analysis*.

Table S1: results of ANOVA analyses (p-values)

Table S2: results of Duncan post-hoc tests

Table S3: results of procrust regressions evaluating how mandible shape vary as a function of size and species (for Cervini), population (for *C. elaphus*) and habitat (for *R. timorensis*).

Table S4: results of procrust regressions (p-values and R^2) for each axis of PCA carrying significant interpopulation and inter-habitat differences.

Table S5: results of Partial Least Squares

Figures

Figure 1: Location of the eight Cervini populations included in the study. *Cervus elaphus*: El Durazno, Hemne, Broceliande, Alt Pallars, Boumort, Selladores, Valquemado. *Rusa timorensis*: New Caledonia (Gouaro Déva, Le Cap, Misère, Parc des Grandes Fougères (PGF)).

Figure 2: Landmarks (blue) and semi-landmarks (black) used to capture the mandibular bone morphology through a 2D geometric morphometric analysis on the buccal side of the left hemimandible. A: mandible of a red deer specimen from the Selladores-Contadero population. B: Consensus shape for *Cervus elaphus*. The anatomical structures and regions corresponding to numbers 1-40 are described in Table 2.

Figure 3: Biplot representing the two first principal components of the PCA exploring interspecific mandibular shape variations between *Cervus elaphus* and *Rusa timorensis*. Shape deformations are shown along PC1 and 2 for each species (*C. elaphus*: coral; *R. timorensis*: navy blue). The consensus shape for all Cervini is shown in grey to aid interpretation.

Figure 4: A: Biplot representing the two first principal components of the PCA (63.3% of the variance) exploring *Cervus elaphus* interpopulation mandibular shape variations. Shape deformations are shown along PC1 and 2 for each population (Alt Pallar: pink; Boumort: red; Broceliande: mauve; El Durazno: green; Hemne: purple; Selladores: dark yellow; Valquemado: orange). The consensus shape for all C. elaphus is in grey to aid interpretation. B: Focus on the variation on PC1. Letters (a-e) represent interpopulation differences based on Duncan post hoc test. C: Biplot representing the two first canonical variates of the CVA.

Figure 5: A: Biplot representing the two first principal components of the PCA (47.76% of the variance) exploring *Rusa timorensis* intrapopulation mandibular shape variations between contrasting habitats. Shape deformations are shown along PC1 and 2 for each population (Gouaro Déva: Turquoise, Le Cap: royal blue, Misère: plum, Parc des Grandes Fougères (PGF): navy blue). The consensus shape for all *R. timorensis* is in grey to aid interpretation. B: Focus on the variation on PC2. Letters a and b represent interpopulation differences based on Duncan's post hoc test. C: Biplot representing the two first canonical variates of the CVA.

Figure 6: Allometric relationships and morphological variation in *Cervus elaphus* from different populations (A) and *Rusa timorensis* from contrasted local habitats (B). Plots illustrate body size and shape variations, both within and among Cervini populations, highlighting population-specific morphological trends. Log(Size): log-transformed body size; CAC and RSC1 are morphological metrics. CAC: Common Allometric Component; RSC1: Residual Shape Component 1; PC1: Principal Component 1. The rightmost plots provide information regarding allometry or isometry.

Supplementary data

Annex 1: R script (R Markdown)

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Table 1: Information on the habitats of cervid populations. Information on biomes is taken from https://ecoregions.appspot.com/. Description of the local vegetation for the populations living in geographical Europe is taken from Ozenda and Borel, 2000. For El Durazno and the Neo-Caledonian localities, the description is based on field observations.

	Group	Biome	Local vegetation description
Cervus elaphus	Alt Pallars	Temperate Broadleaf & Mixed Forests	High mountain: subalpine, alpine and nival vegetation belts
	Boumort	Temperate Broadleaf & Mixed Forests	Lower and mid-mountain: collinean and montane vegetation belts
	Broceliande (Paimpont)	Temperate Broadleaf & Mixed Forests	Hyperhygrophilous Armorican sector
	El Durazno	Temperate Grasslands, Savannas & Shrublands	Semiarid Pampas
	Hemne	Boreal Forests/Taiga	Orocaledonian vegetation unit (mountain tundra)
	Selladores	Mediterranean Forests, Woodlands & Scrub	Siliceous, with round-leaved oak
	Valquemado	Mediterranean Forests, Woodlands & Scrub	Calcicolous, with round-leaved oak
Rusa timorensis	Gouaro Déva	Tropical & Subtropical Dry Broadleaf Forests	Sclerophyllous & meso-sclerophyll forests, extensive grasslands, Niaoulis savannah
	Le Cap	Tropical & Subtropical Dry Broadleaf Forests	Niaoulis savannah & agricultural grasslands
	Misère	Tropical & Subtropical Dry Broadleaf Forests	Mesophyll forest
	Parc des Grandes Fougères	Tropical & Subtropical Moist Broadleaf Forests	Dense tropical rainforest

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Table 2. Labial view of a left deer mandible and description of landmarks and semilandmarks used for capturing the mandibular shape for the geometric morphometric analyses.

Landmarks	**Names and Description**
1	Tip of the condylar process
2	Ventral margin of the mandibular sigmoid notch
6	Ventrocaudal margin of the coronoid process
7	Tip of the coronoid process
16	Posterior alveolar margin of *M1*
17	Anterior alveolar margin of *M1*
18	Posterior alveolar margin of *P3*
19	Anterior alveolar margin of *P2*
22	Posterior alveolar margin of *C*
23	Ventral margin of the *I1*
25	Mental foramen
26	Posterior margin of the mandibular symphysis
Semilandmarks	**Description traits**
3 to 5	Concavity of the posterior border of the coronoid apophysis
8 to 15	Convexity of the anterior edge of the coronoid process
20 to 21	In the mandibular diastema, at the level of the Semilandmark 27 (20) and of the Landmark 25 (21)
24	Rostro-ventral margin of the horizontal ramus of the mandible, at the level of the posterior alveolar margin of C
27 to 38	Convexity of the ventral margin of the horizontal ramus of the mandible to the posterior margin of the angular process
38 to 40	Concavity of the caudal edge of the mandible ramus

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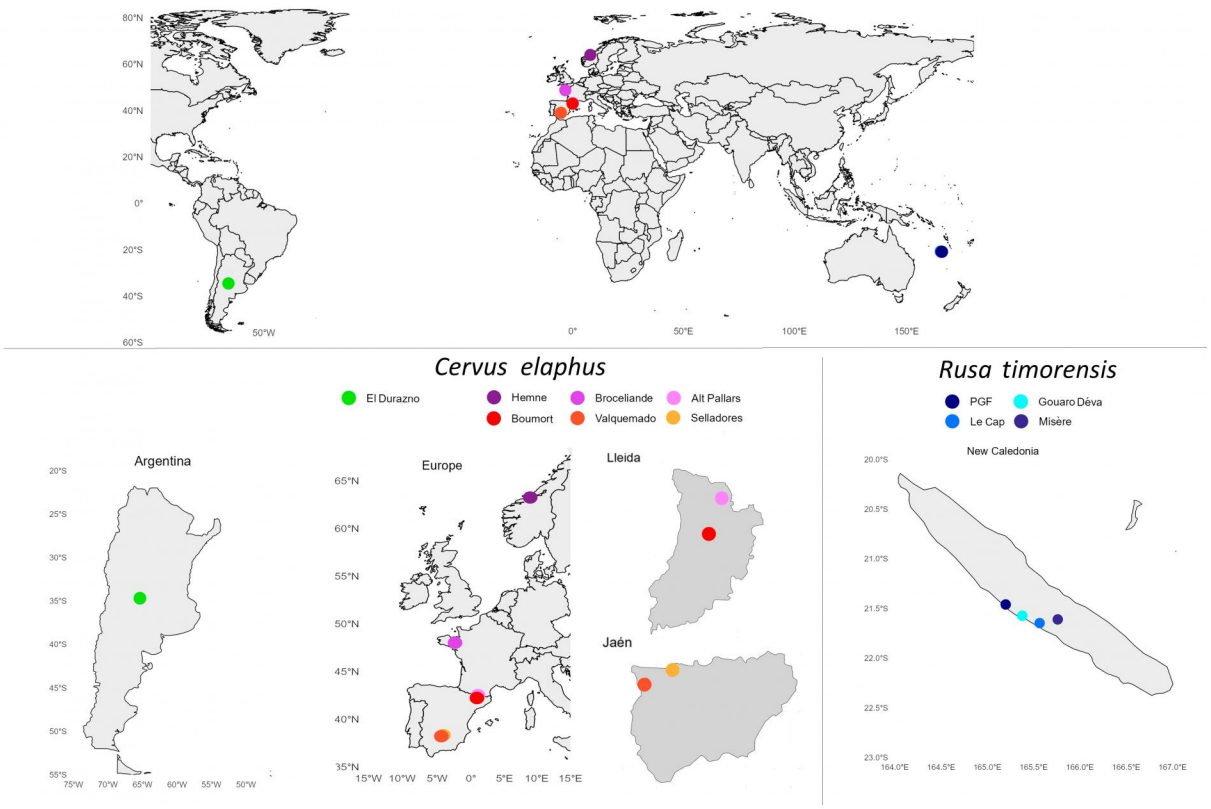

Location of the eight Cervini populations included in the study. Cervus elaphus: El Durazno, Hemne, Broceliande, Alt Pallars, Boumort, Selladores, Valquemado. Rusa timorensis: New Caledonia (Gouaro Déva, Le Cap, Misère, Parc des Grandes Fougères (PGF)).

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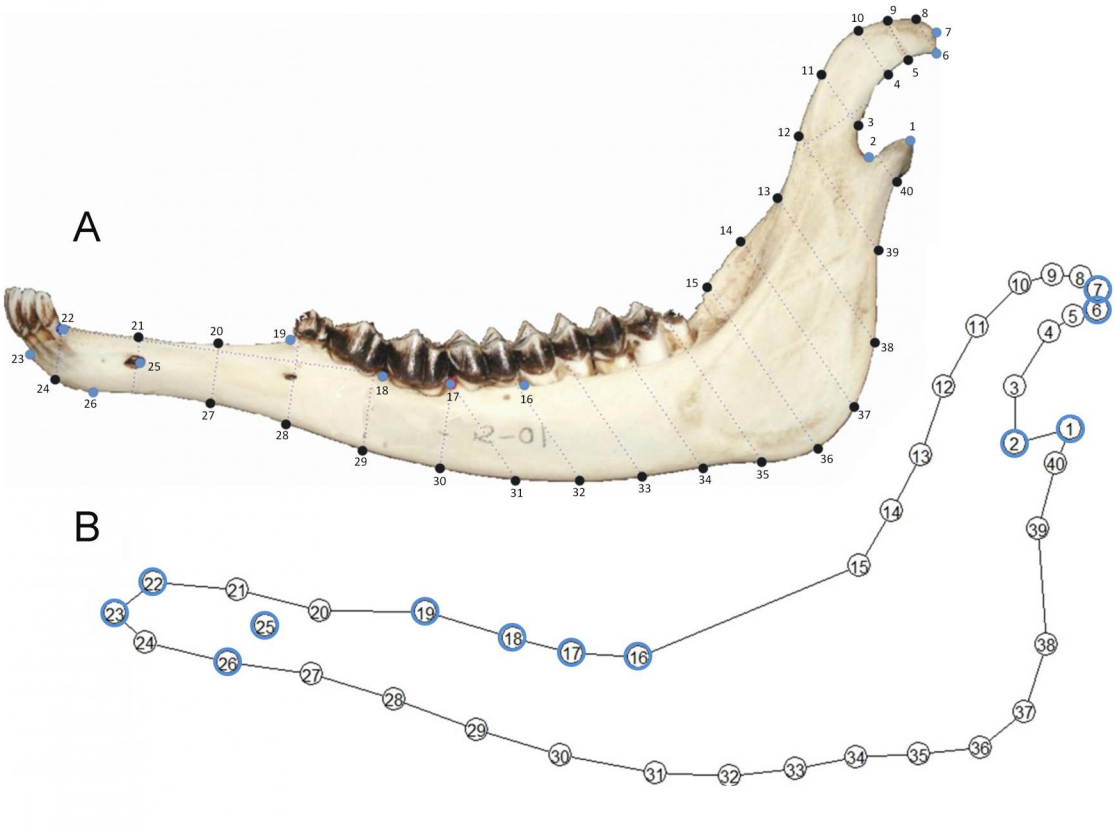

Landmarks (blue) and semi-landmarks (black) used to capture the mandibular bone morphology through a 2D geometric morphometric analysis on the buccal side of the left hemimandible. A: mandible of a red deer specimen from the Selladores-Contadero population. B: Consensus shape for Cervus elaphus. The anatomical structures and regions corresponding to numbers 1-40 are described in Table 2.

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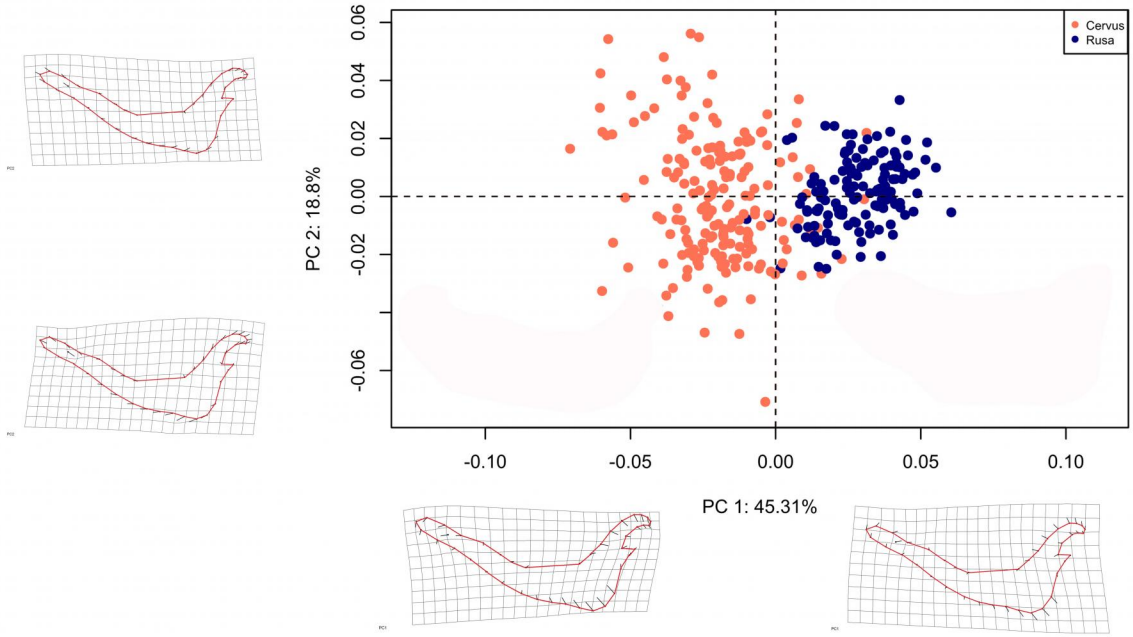

Biplot representing the two first principal components of the PCA exploring interspecific mandibular shape variations between Cervus elaphus and Rusa timorensis. Shape deformations are shown along PC1 and 2 for each species (C. elaphus: coral; R. timorensis: navy blue). The consensus shape for all Cervini is shown in grey to aid interpretation.

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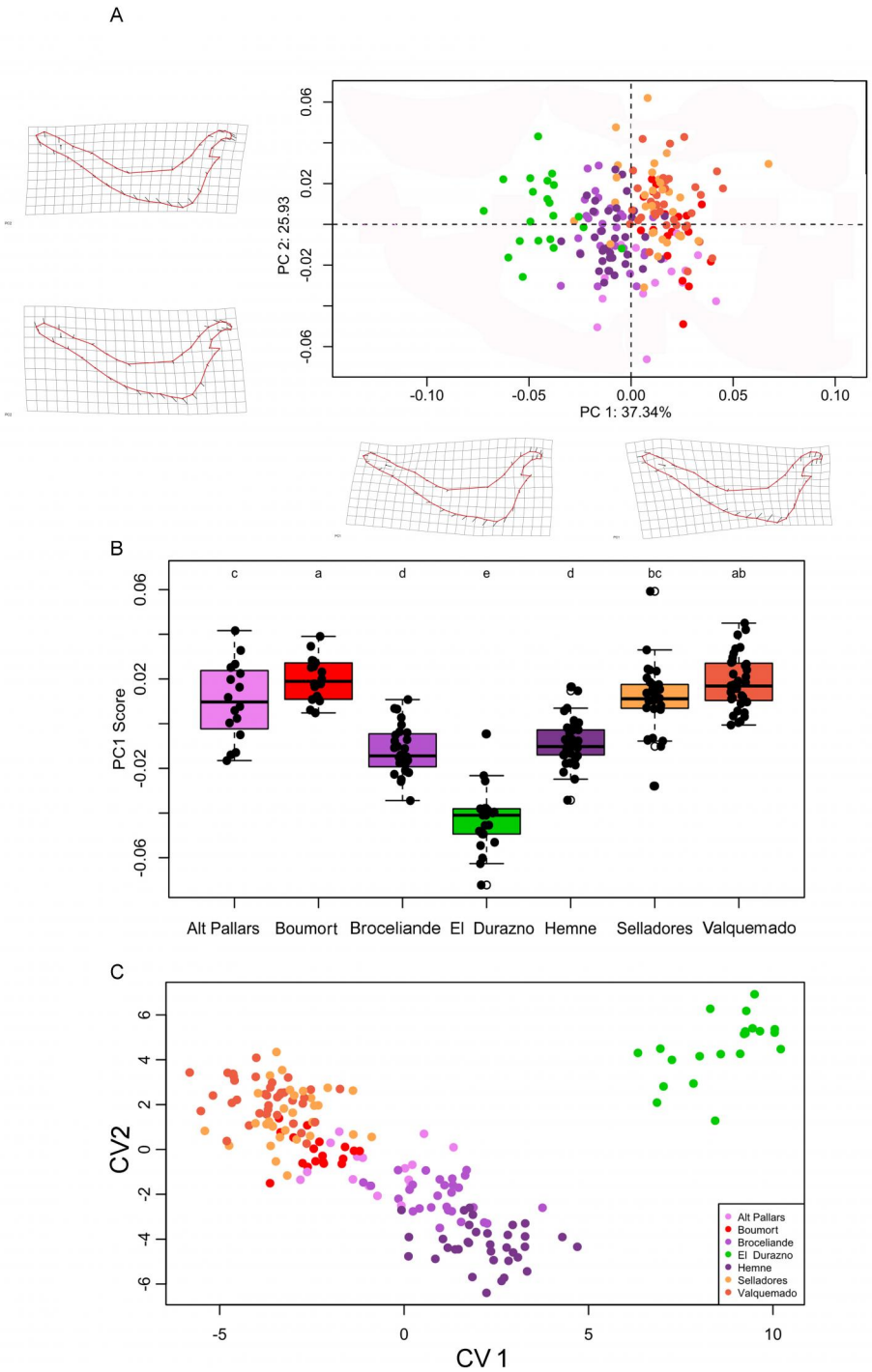

A: Biplot representing the two first principal components of the PCA (63.3% of the variance) exploring Cervus elaphus interpopulation mandibular shape variations. Shape deformations are shown along PC1 and 2 for each population (Alt Pallar: pink; Boumort: red; Broceliande: mauve; El Durazno: green; Hemne: purple; Selladores: dark yellow; Valquemado: orange). The consensus shape for all C. elaphus is in grey to aid interpretation. B: Focus on the variation on PC1. Letters (a-e) represent interpopulation differences based on Duncan post hoc test. C: Biplot representing the two first canonical variates of the CVA.

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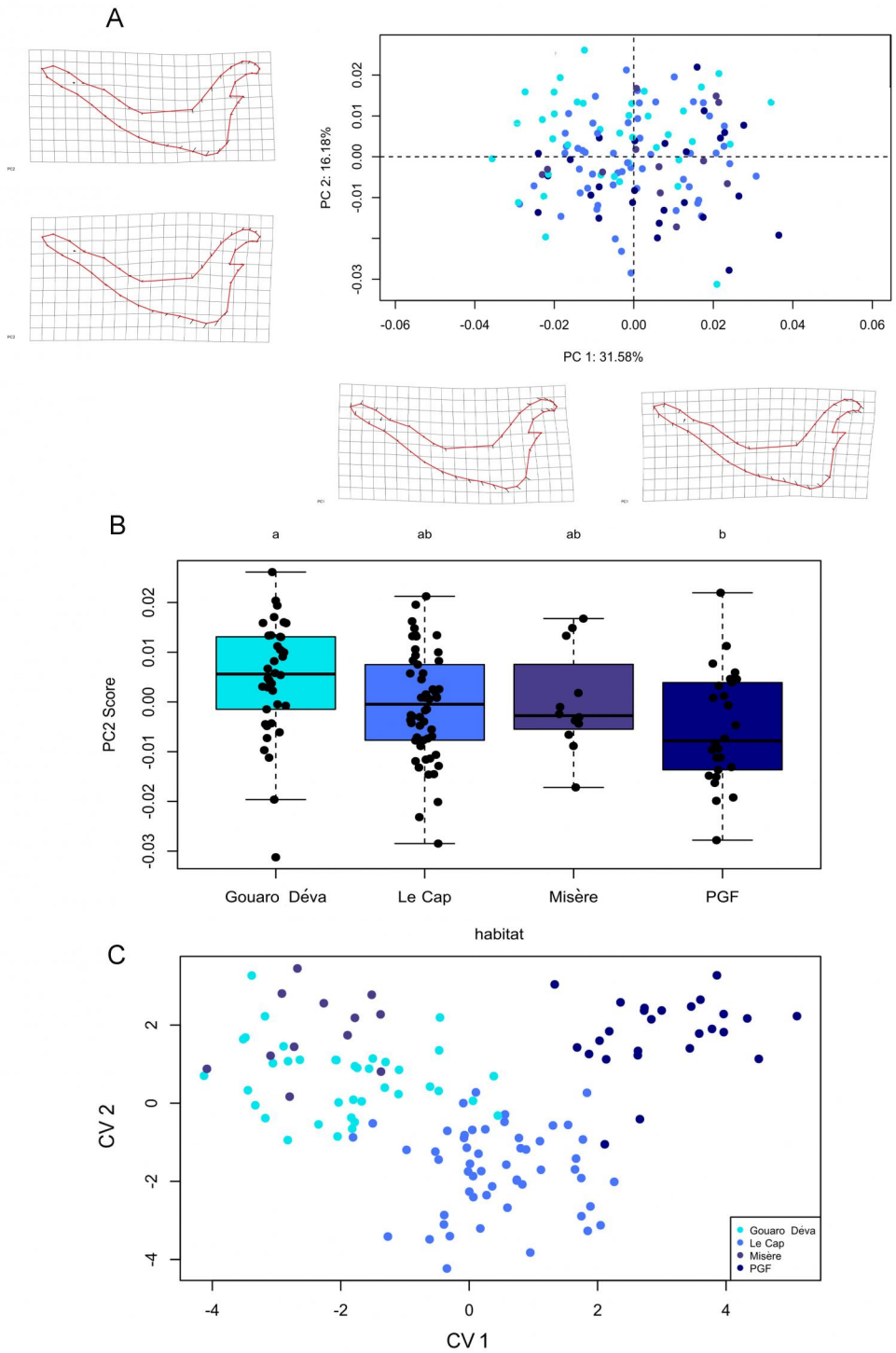

A: Biplot representing the two first principal components of the PCA (47.76% of the variance) exploring Rusa timorensis intrapopulation mandibular shape variations between contrasting habitats. Shape deformations are shown along PC1 and 2 for each population (Gouaro Déva: Turquoise, Le Cap: royal blue, Misère: plum, Parc des Grandes Fougères (PGF): navy blue). The consensus shape for all R. timorensis is in grey to aid interpretation. B: Focus on the variation on PC2. Letters a and b represent interpopulation differences based on Duncan's post hoc test. C: Biplot representing the two first canonical variates of the CVA.

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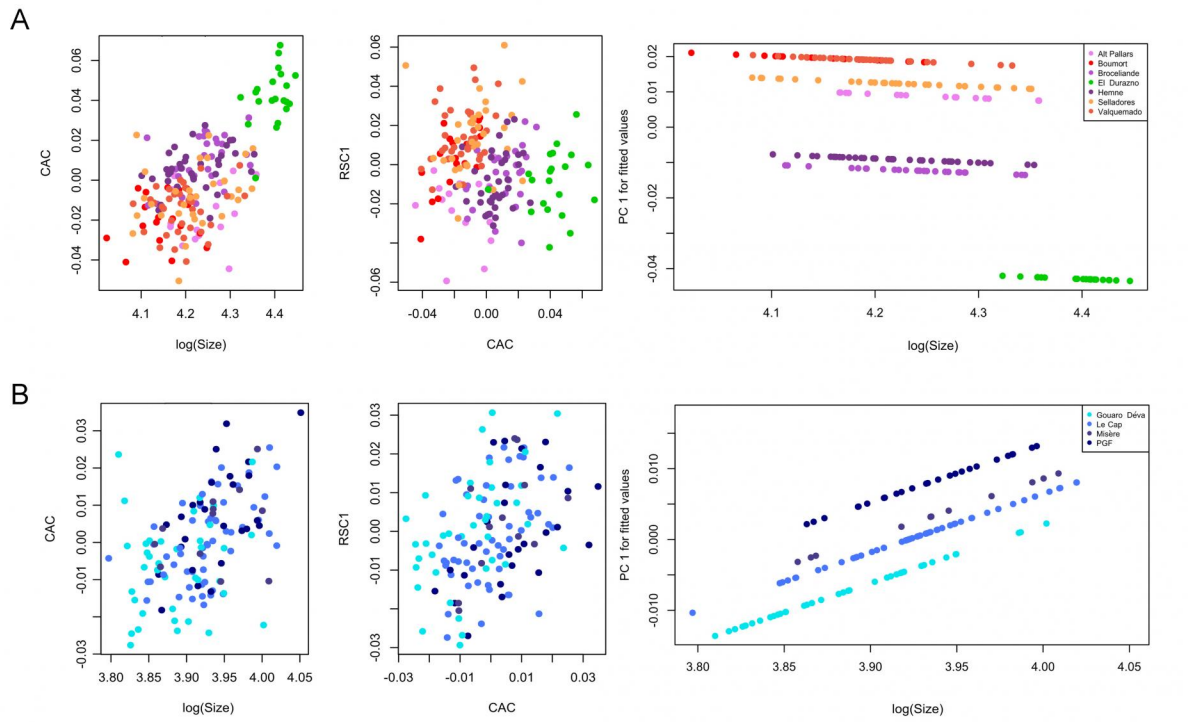

Allometric relationships and morphological variation in Cervus elaphus from different populations (A) and Rusa timorensis from contrasted local habitats (B). Plots illustrate body size and shape variations, both within and among Cervini populations, highlighting population-specific morphological trends. Log(Size): log-transformed body size; CAC and RSC1 are morphological metrics. CAC: Common Allometric Component; RSC1: Residual Shape Component 1; PC1: Principal Component 1. The rightmost plots provide information regarding allometry or isometry.

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Manuscript body

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Tables

Table 1 - Download source file (36.08 kB)
Information on the habitats of cervid populations. Information on biomes is taken from https://ecoregions.appspot.com/. Description of the local vegetation for the populations living in geographical Europe is taken from Ozenda & Borel, 2000. For El Durazno and the Neo-Caledonian localities, the description is based on field observations.

Table 2 - Download source file (15.94 kB)
Description of landmarks and semilandmarks used for capturing the mandibular shape for the geometric morphometric analysis.

Figures

Figure 1 - Download source file (1.06 MB)
Location of the eight Cervini populations included in the study. Cervus elaphus: El Durazno, Hemne, Broceliande, Alt Pallars, Boumort, Selladores, Valquemado. Rusa timorensis: New Caledonia (Gouaro Déva, Le Cap, Misère, Parc des Grandes Fougères (PGF)).

Figure 2 - Download source file (1.21 MB)
Landmarks (blue) and semi-landmarks (black) used to capture the mandibular bone morphology through a 2D geometric morphometric analysis on the buccal side of the left hemimandible. A: mandible of a red deer specimen from the Selladores-Contadero population. B: Consensus shape for Cervus elaphus. The anatomical structures and regions corresponding to numbers 1-40 are described in Table 2.

Figure 3 - Download source file (6.55 MB)
Biplot representing the two first principal components of the PCA exploring interspecific mandibular shape variations between Cervus elaphus and Rusa timorensis. Shape deformations are shown along PC1 and 2 for each species (C. elaphus: coral; R. timorensis: navy blue). The consensus shape for all Cervini is shown in grey to aid interpretation.

Figure 4 - Download source file (3.03 MB)
A: Biplot representing the two first principal components of the PCA (63.3% of the variance) exploring Cervus elaphus interpopulation mandibular shape variations. Shape deformations are shown along PC1 and 2 for each population (Alt Pallar: pink; Boumort: red; Broceliande: mauve; El Durazno: green; Hemne: purple; Selladores: dark yellow; Valquemado: orange). The consensus shape for all C. elaphus is in grey to aid interpretation. B: Focus on the variation on PC1. Letters (a-e) represent interpopulation differences based on Duncan post hoc test. C: Biplot representing the two first canonical variates of the CVA.

Figure 5 - Download source file (2.4 MB)
A: Biplot representing the two first principal components of the PCA (47.76% of the variance) exploring Rusa timorensis intrapopulation mandibular shape variations between contrasting habitats. Shape deformations are shown along PC1 and 2 for each population (Gouaro Déva: Turquoise, Le Cap: royal blue, Misère: plum, Parc des Grandes Fougères (PGF): navy blue). The consensus shape for all R. timorensis is in grey to aid interpretation. B: Focus on the variation on PC2. Letters a and b represent interpopulation differences based on Duncan's post hoc test. C: Biplot representing the two first canonical variates of the CVA.

Figure 6 - Download source file (1.74 MB)
Allometric relationships and morphological variation in Cervus elaphus from different populations (A) and Rusa timorensis from contrasted local habitats (B). Plots illustrate body size and shape variations, both within and among Cervini populations, highlighting population-specific morphological trends. Log(Size): log-transformed body size; CAC and

RSC1 are morphological metrics. CAC: Common Allometric Component; RSC1: Residual Shape Component 1; PC1: Principal Component 1. The rightmost plots provide information regarding allometry or isometry.

Supplementary Online Material

File 1 - Download source file (166 kB)
Table S1: results of ANOVA analyses (p-values)

File 2 - Download source file (52.45 kB)
Table S2: results of Duncan post-hoc tests

File 3 - Download source file (21.84 kB)
Table S3: results of procrust regressions evaluating how mandible shape vary as a function of size and species (for Cervini), population (for C. elaphus) and habitat (for R. timorensis).

File 4 - Download source file (31.17 kB)
Table S4: results of procrust regressions (p-values and R^2) for each axis of PCA carrying significant interpopulation and inter-habitat differences.

File 5 - Download source file (22.38 kB)
Table S5: results of Partial Least Squares

File 6 - Download source file (1.52 MB)
R script (R Markdown)