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## Short Note

## Morphology, chemical composition, mechanical properties and structure in antler of Sardinian red deer (Cervus elaphus corsicanus)

Jamil CAPPELLI<sup>1,2,\*</sup>, Alberto Stanislao Atzori<sup>3</sup>, Francisco Ceacero<sup>4</sup>, Tomas Landete-Castillejos<sup>1,2,5</sup>, Antonello Cannas<sup>3</sup>, Laureano GALLEGO<sup>1</sup>, Andrés José García Díaz<sup>1,2,5</sup>

<sup>1</sup>Departamento de Ciencia y Tecnología Agroforestal y Genética, ETSIAM, Universidad de Castilla-La Mancha (UCLM), 02071 Albacete, Spain

<sup>2</sup>Sección de Recursos Cinegéticos y Ganaderos, Instituto de Desarrollo Regional (IDR), Universidad de Castilla-La Mancha (UCLM), 02071 Albacete, Spain

<sup>3</sup>Dipartimento di Agraria,Sezione di Scienze Zootecniche, Università di Sassari, via E. De Nicola 9, 07100 Sassari, Italy <sup>4</sup>Department of Animal Science and Food Processing, Faculty of Tropical AgriSciences. Czech University of Life Sciences Prague, Kamicka 129, 165 21, Prague 6 - Suchdol, Czech

Republic

<sup>5</sup> Instituto de Investigación en Recursos Cinegéticos, IREC (CSIC, UCLM, JCCM), Campus Universitario s/n, 02071 Albacete, Spain

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## Abstract

The deer population present in Sardinia and Corsica represents an endemic subspecies Cervus elaphus corsicanus. We describe for the first time the characteristics of cast antlers of Sardinian red deer from the forest complex Sette Fratelli, south-east part of the island. Moreover, we describe the material mechanical properties, the structural ones, and the mineral profile of antlers from adults, comparing them with the antler characteristics of the subspecies C. e. hispanicus examined exactly with the same methodology. Sixty-one deer casted antlers were collected and classified as belonging to adults (35) or sub-adults (26). A first part of the study described the common features of the antlers of sub-species C. e. corsicanus through the analysis of morphology in all deer antlers. Subsequently, a more detailed study used 12 adult deer antlers for a destructive analysis. Statistical comparisons were conducted using ANOVAs between characteristics of the two age subgroups, and using Pearson's correlation coefficients between the antlers morphological variables. In general, morphological antler measures had greater values in adults than in sub-adults. In comparison with Iberian deer, Sardinian adult antlers have a more simple structure with lower values in morphological features, mechanical properties and structural characteristics.

The deer population present in Sardinia and Corsica represents an endemic subspecies Cervus elaphus corsicanus (Pitra et al., 2004). At present, there are approximately 4270 animals in Sardinia but the subspecies is classified as "endangered" by the IUCN (IUCN, 2004). The Sardinian deer survived naturally only in a few mountain areas: one of this is the Sette Fratelli Mountain (Casula et al., 2013). This subspecies is slightly smaller and more slender than continental red deer. Its antlers differ from the European subspecies by weight, shape and by the number of tines, which is limited to 4-5, against the ordinary 8-10 tines of an adult deer in Central-Europe (Caboni et al., 2006). In the distal part of the beam tines are placed on the same plane. The bez or second tine (from base) can be found only in few trophies of this subspecies (Beccu, 1989). Studies on antler characteristics of Iberian deer (Estevez et al., 2008; Landete-Castillejos et al., 2007a) prove that morphological variables, composition and mechanical properties can be used to diagnose diet and management problems of a deer population. Thus, we describe for the first time the characteristics of cast antlers of Sardinian red deer from the forest complex Sette Fratelli, to provide reference values for further studies which may help the conservation of deer. The aims of this study are: to describe the antler morphometry, the mechanical properties, the structure and the mineral profile of antlers from adults and subadults of this subspecies, and to compare them with the well studied C. e. hispanicus.

The forest complex Sette Fratelli is located in southeastern Sardinia (39°14' N, 9°30' E, 210-500 m of altitude). Sixty-one antlers, grown

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and casted in the same year, were collected inside the forest natural area. Antlers were assigned as belonging to adults (35) and sub-adults (26), according to the presence of the following features: the brow tine, the central tine and a bifurcation in the distal part of the main beam (Beccu, 1989). The antlers collected showed no sign of diagenetic al-



Figure 1 - Sampling technique for adult deer antlers: the antler bone was cut at various level along the vertical axis, in each sampling position were obtained a complete transverse cross-section (to study the structural characteristics) and a cylinder from which two cortical bone bars were extracted to study the mechanical properties and the mineral profile.

<sup>\*</sup>Corresponding author

Email address: jamil.cappelli@uclm.es (Jamil CAPPELLI)

teration, no weathering (grey color) and no cracks. In order to describe their morphological characteristics, the following measurements were recorded: total curve length, first tine length, second tine length, central tine length, burr perimeter, first half perimeter (between first tine and central tine), second half perimeter (between central tine and the crown), weight, and scoring (CIC, 1960). Thereafter, 12 antlers of adult individuals were selected in order to obtain antlers that represent the characteristics of the adult antler, following these criteria: the greatest average total length of the main beam and the greatest number of tips. Since they cannot be assigned to a particular male, and thus we are not certain which left and right antlers constitute a pair, only left antlers were used in this part of the study. Samples were collected from four positions along the main antler beam (Fig. 1). From each position, a 1 cm slice (complete transverse cross-section) and a cylinder about 5-6 cm (to prepare bars from the cortical wall) were obtained (Landete-Castillejos et al., 2010). Intrinsic mechanical properties (Cappelli et al., 2015) were measured in two ways: bending tests were carried out in a Zwick/Roell 500N machine and the following mechanical properties were measured: Young's Modulus of elasticity E, Bending strength BS, and the Work to peak force W; impact tests were carried out in a CEAST-IMPACTOR II machine (CEAST S.p.A., Pianezza, Italy), in order to obtain the impact energy absorption (U). Mineral content (Ca, Mg, K, P, B, Co, Cu, Fe, Na, Mn, S, Se, Si, Sr, Zn) was measured from cortical bone samples through spectrophotometry (Landete-Castillejos et al., 2007b). Transverse cross sections were used to measure the average cortical thickness and to calculate the ratio between cortical and total area, cortical/total ratio. Density of cortical bone was calculated dividing the weight by the volume of the bone bars fragments. Another fragment was used to measure ash content after burning in a muffle furnace (HTC 1400, Carbolite, UK). Statistical analyses were performed using MINITAB 16 and SPSS version 19 (SPSS Inc., Chicago, IL, USA); comparisons were conducted using ANOVAs between characteristics of the two age subgroups, and also using Pearson's correlation coefficients to describe the relationship between the antler morphological variables. Descriptive statistics were also applied to the morphological, structural and chemical characteristics of a selected adult deer antler (N=12) for each sampling position along the antler main beam.

The morphometric characterization of the antlers of adult and subadult *C. e. corsicanus* is shown in Fig. 2. Generally, adult deer antlers were heavier  $(0.52\pm0.03 \text{ VS } 0.34\pm0.03, p<0.001)$  and with higher scoring  $(66.7\pm1.8 \text{ VS } 52.4\pm2.2, p<0.001)$ . Pearson's correlation analysis within each age class (Tab. 1) showed that in subadults the strength of association between the morphological variables was high and strongly significant. Afterwards, 12 adult deer antlers were selected to characterize the structural characteristics, mechanical properties and mineral profile. The means of each studied position (Tab. 2) showed that distal positions (3 and 4) had lowest values with a gradually decreasing trend form the base to the antler's tip.



**Figure 2** – Descriptive statistics of antler morphological characteristics of *Cervus elaphus corsicanus*. Mean for antlers from subadult and adult deer (N=61). Probability calculated using ANOVA; levels *p*<0.01 and *p*<0.001 are indicated by \*\* and \*\*\*, respectively. Second half perimeter cannot be measured in antlers of subadult animals.

These findings show that antlers from adults have more tines, greater weight and longer total length. The comparison with *C. e. italicus* (Zachos et al., 2014; Mattioli and Ferretti, 2014) shows that Sardinian red deer has a quite similar number of tines per antler  $(2.11\pm0.16 \text{ in}$  Sardinian deer VS  $2.9\pm0.9$  for *C. e. italicus*), but a lower average weight (0.52 kg in Sardinian red deer VS 0.9 kg in *C. e. italicus*). Furthermore, in the studied Sardinian subpopulation only a 20% of antlers have three-tined antlers; whereas the *C. e. italicus* recorded a 41.5%. This can be explained by the fact that antler size is strongly affected by ecological factors during their growth (Brown, 1990). The Sardinian deer antler morphology matches the maintenance phenotype model described by Geist (1998), according to which the phenotype plasticity is pushed to a model of efficiency.

Recent studies show that the composition of the antlers can tell us great information that may be useful to know the status of a wildlife population (Landete-Castillejos et al., 2013); moreover, the monitoring of morphometrics could help to assess how individuals react to conservation measures (Mattioli and Ferretti, 2014). Generally, the mechanical and structural properties of Sardinian antler bone show a decrease in the observed values. This is coherent with the hypothesis that the decreasing trend shows the depletion of those minerals in the body (Landete-Castillejos et al., 2007a, 2010), thus reflecting the physiological exhaustion to grow the antlers. Comparing antler characteristics of C. e. corsicanus and C. e. hispanicus, the values in most traits are lower for the first subspecies. The average antler weight, first tine length, burr perimeter, and total length are lower by a 65%, 45%, 30% and a 26% respectively (data for C. e. hispanicus in Fierro et al., 2002). This could be due to its more slender body size. Animals with a smaller skeleton could invest less mineral bone resources for the growth of their antlers. Various relationships indicate that body morphometry characteristics were related to antler measurements (Ceacero, 2016). Regard-

Table 1 – Pearson's correlation coefficients between measured morphological variables of subadult and adult deer antlers of *Cervus elaphus corsicanus*. Probability at levels p<0.01 and p<0.001 are indicated, respectively, by \*\* and \*\*\*.

Variables	Total length	First tine length	Central tine length	Burr perimeter	First half perimeter
Subadult deer					
First tine length	0.833***				
Central tine length	0.813***	0.751***			
Burr perimeter	0.749***	0.732***	0.663***		
First half perimeter	0.782***	0.636**	0.784***	0.811***	
Antler weight	0.929***	0.887***	0.870***	0.816***	0.775***
Adult deer					
First tine length	0.625**				
Central tine length	0.639***	0.440**			
Burr perimeter	0.753***	0.537***	0.451**		
First half perimeter	0.640***	0.319	0.667***	0.567***	
Second half perimeter	-0.521	0.043	-0.798**	0.421	0.326
Antler weight	0.761***	0.545***	0.775***	0.666***	0.791***

Table 2 – Means ( $\pm$ S.E.) of the four studied positions for structural characteristics, mechanical properties and mineral profile of the adult deer of *Cervus elaphus corsicanus* (selectedantlers, N=12). The last column shows mean ( $\pm$ S.E.) differences between the basal position (Position 1) and the distal position (Position 4) in percentage. ANOVA analyses with Tukey testshow (in superscripts) differences among the positions.

Variables	Position 1	Position 2	Position 3	Position 4	Difference base/tip (%)
Cortical/total ratio	$0.54\ \pm 0.02\ ^{\text{A}}$	$0.37\ \pm 0.01\ ^{\rm C}$	$0.43 \ \pm 0.02 \ ^{B}$	$0.42 \ \pm 0.03 \ ^{BC}$	$-21.04 \pm 4.26$
Average cortical thickness (mm)	$4.48 \ \pm 0.16 \ ^{\text{A}}$	$3.27\ \pm 0.09\ ^{B}$	$3.39\ \pm 0.14\ ^{B}$	$3.27\ \pm 0.16\ ^{B}$	$-26.54 \pm 3.36$
Density of cortical bone (g/mL)	$1.81 \ \pm 0.02 \ ^{\rm A}$	$1.77\ \pm 0.02\ ^{\rm A}$	$1.60\ \pm 0.04\ ^{B}$	$1.50 \ \pm 0.05 \ ^{\rm C}$	$-17.63 \pm 2.38$
Young's modulus of elasticity (E),(GPa)	$14.72 \pm 0.41$ <sup>A</sup>	$13.93\ \pm 0.34\ ^{\rm A}$	$12.22\ \pm 0.52\ ^{B}$	$10.74\ \pm 0.57\ ^{\rm C}$	$26.53 \pm 3.57$
Bending Strength (BS), (MPa)	$275.74\ \pm 16.38^{A}$	$271.92\ \pm 5.00\ ^{\rm A}$	$252.39\ \pm 8.19\ ^{AB}$	$225.66\ \pm 13.37^B$	$-22.26 \pm 5.02$
Work to peak force (W), (kJm <sup>-2</sup> )	$41.35 \ \pm 2.37 \ ^{\text{A}}$	$39.03\ \pm 1.60\ ^{A}$	$35.66 \pm 2.17$ <sup>B</sup>	$32.07\ \pm 3.05\ ^{B}$	$-22.68 \pm 7.20$
Impact work (U), (kJm <sup>-2</sup> )	$22.63 \pm 1.16$	$21.64 \pm 1.18$	$22.28 \pm 0.94$	$20.87 \pm 1.10$	$-6.10 \pm 5.44$
Ashes (%)	$59.09 \pm 0.31$	$58.94 \pm 0.52$	$58.75 \pm 0.48$	$59.33 \pm 0.52$	$0.40 \pm 0.59$
Ca (wt%)	$21.17 \pm 0.36$	$20.99 \pm 0.18$	$20.57 \pm 0.26$	$20.63 \pm 0.27$	$-2.40 \pm 1.24$
Mg (wt%)	$0.446 \pm 0.007^{\rm A}$	$0.447 \pm 0.006^{\rm A}$	$0.436 \pm 0.008^{AB}$	$0.430 \pm 0.007^{B}$	$-3.64 \pm 1.17$
Na (wt%)	$0.555\pm0.009$	$0.556 \pm 0.008$	$0.535\pm0.010$	$0.534 \pm 0.018$	$-3.30 \pm 4.28$
P (wt%)	$9.90 \hspace{0.2cm} \pm \hspace{2cm} 0.18$	$9.94 \pm 0.09$	$9.72 \pm 0.14$	$9.62 \pm 0.11$	$-2.63 \pm 1.25$
B (ppm)	$4.04 \pm 0.12$	$4.10 \pm 0.13$	$4.11 \ \pm 0.14$	$4.05 \pm 0.13$	$0.13 \pm 0.93$
Co (ppm)	$0.036 \pm 0.010$	$0.058\pm0.007$	$0.047\pm0.010$	$0.034\pm0.008$	$-15.09 \pm 29.04$
Cu (ppm)	$0.76\ \pm 0.05$	$0.72\ \pm 0.04$	$0.85 \ \pm 0.04$	$0.87\ \pm 0.06$	$-17.25 \pm 7.41$
Fe (ppm)	$5.12 \hspace{0.2cm} \pm 1.95$	$12.49 \pm 5.14$	$10.22 \ \pm 1.26$	$9.38 \pm 2.23$	$45.41 \pm 12.55$
K (ppm)	$360.48 \pm 7.72 \ ^{A}$	$341.16\ \pm 5.67\ ^{AB}$	$319.05 \ \pm 7.07 \ ^{BC}$	$304.03\ \pm 11.00^{C}$	$-15.18 \pm 3.72$
Mn (ppm)	$0.47\ \pm 0.05$	$0.42 \hspace{0.2cm} \pm \hspace{0.2cm} 0.03 \hspace{0.2cm}$	$0.56 \hspace{0.2cm} \pm \hspace{0.2cm} 0.06 \hspace{0.2cm}$	$0.61 \pm 0.11$	$-3.78 \pm 6.45$
S (ppm)	$1279.49\ \pm 17.61^{B}$	$1279.76\ \pm 18.22^B$	$1376.97\ \pm 18.24^{A}$	$1382.27\ \pm 12.45^{A}$	$8.26 \pm 1.77$
Se (ppm)	$1.07\ \pm 0.10$	$0.82 \pm 0.15$	$1.16 \ \pm 0.12$	$1.23\ \pm 0.11$	$15.58 \pm 11.73$
Si (ppm)	$28.73\ \pm 3.84\ ^{\rm C}$	$29.73 \hspace{0.2cm} \pm 4.04 \hspace{0.2cm} ^{BC}$	$40.25\ \pm 5.15\ ^{A}$	$38.90\ \pm 3.83\ ^{AB}$	$25.54 \pm 10.34$
Sr (ppm)	$240.28 \pm 7.22$	$243.11 \pm 7.47$	$237.71 \pm 8.26$	$236.59 \pm 8.55$	$-1.57 \pm 1.69$
Zn (ppm)	$68.73\ \pm 2.78\ ^{A}$	$67.57 \ \pm 2.66 \ ^{AB}$	$66.71\ \pm 2.35\ ^{AB}$	$65.97\ \pm 2.11\ ^{B}$	$-3.51 \pm 1.58$

ing structure, the average cortical thickness values for Sardinian deer were much lower than populations of Iberian Red deer: -36% (data for C. e. hispanicus in Landete-Castillejos et al., 2010). The same happens for the mechanical properties: E of Sardinian deer is -17.8% than that of Iberian deer ( $12.89\pm0.33$  VS  $15.69\pm0.32$  in C. e. hispanicus) whereas W is slightly lower (-3%, 38±1.5 in C. e. hispanicus). Also BS and U show lower values  $(306.6\pm6.4 \text{ and } 54.9\pm2.7 \text{ for } C. e. his$ panicus; Landete-Castillejos et al., 2010). The same trend exists for ash content, which showed a -5.2%. The reason may be that the conditions of the natural habitat in which the population is widespread could influence the diet, and a lower availability of resources may end up in antlers of worse quality (Scribner et al., 1989) and its observed differences in mechanical properties. However, it is possible that formal tests for tissue toughness, which were not specifically examined in this study, might be less degraded in our sample when compared to the results of impact and bending tests that we used herein. Additional studies would be required to test this hypothesis because various types of mechanical tests are required to fully assess the antler mechanical properties (Launey et al., 2010; Skedros et al., 2014). Antler minerals play an important role in antler growth, especially for Ca and P (Ceacero, 2016). The comparison between Sardinian and Iberian red deer shows a variable trend: for Ca and P the difference is minimal (-1% and -3%, respectively; Landete-Castillejos et al., 2010), for other minerals the difference is greater (for Na -7.8%, for K -42.7%, and for Co -82.6% in C. e. corsicanus). In conclusion, adult and subadult Sardinian deer showed different morphological characteristics in antlers; this subspecies has a simplified antler structure compared to C. e. hispanicus and to other populations present in Italy (C. e. italicus). This research allows enriching the knowledge of this subspecies through an analysis of the structural characteristics, the chemical content and mechanical properties of antler bone material.

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