

Morphometric Variations in the Skull of the Red Deer (*Cervus elaphus* L.) in Bulgaria

Georgi Markov

Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2 Gagarin Street, 1113 Sofia, Bulgaria; E-mail: georgimar@gmail.com

Abstract: A population craniometric analysis of the red deer (*Cervus elaphus* L.) from Bulgaria was carried out using 19 linear skull and dental parameters. The analysis included 93 adult individuals (74 males and 19 females), originating from the main hunting economic areas in the country, i.e. Central Stara Planina Mountains, Strandzha-Sakar area, Ludogorie-Dobrudzha region and the Rila-Pirin region. The data confirmed a high degree of sexual dimorphism of the skull. The univariate and multivariate statistical assessment of the population skull features in the Bulgarian red deer, performed separately by gender, demonstrated craniological similarity among the specimens of the same sex. It was more pronounced in females. The estimated similarity of the craniometric features in the males of the four Bulgarian populations to the craniometrically determined subspecies *Cervus elaphus pannoniensis* and *Cervus elaphus hippelaphus* showed that the Bulgarian red deer is craniometrically closer to *C. e. pannoniensis* than to *C. e. hippelaphus*.

Keywords: *Cervus elaphus*, craniometry, sexual dimorphism, Bulgaria, gammataxonomy

Introduction

According to the historic records, the red deer (*Cervus elaphus* L.) is an autochthonous species for the territory of Bulgaria (SPASSOV, POPOV 2007). It was presented permanently in the Bulgarian mammalian fauna throughout the Holocene (SPASSOV, ILIEV 1994, SPASSOV *et al.* 2001).

As in the historical times (SPASSOV, ILIEV 1994), nowadays (MARKOV 1959, DRAGOEV 1978, BOTEV 1985) the environment offers good conditions for the development of the red deer in the present territory of Bulgaria. It is the main hunting species (STENIN 2008, OBREtenov 2010) of great importance in the forest ecosystems in the country.

The variations in the number of the red deer in Bulgaria were caused by anthropogenic influences. In the recent times there were several dramatic changes. The first of them was a marked decline in 1915-1918, when only a few individuals remained in fragmented local populations. In 1932-1933 the red deer occurred only in the Stara Planina and Rila

mountains. A slow recovery took place in the subsequent years and the period of intensive increase in numbers began in the 1950s (1430 red deer inhabited the country in 1952). This process was supported by re-acclimatisation of animals, bred in farms. In the 1970s more than 10 000 red deer were found in five natural and 18 artificial habitats (DRAGOEV 1978). The species numbers reached 28 370 individuals in 1992, and according to the spring census taken in 2012, there were almost 21 000 individuals (AEFA 2012). Nowadays, the red deer distribution in Bulgaria is concentrated mainly in forest biotopes, which cover habitats in the Central and Eastern Stara Planina, Central (Sashtinska) Sredna Gora, Rila-Rhodope and Strandzha-Sakar mountain areas, Kraishte-Osogovo and Ludogorie-Dobrudzha regions.

The relatively well-known genetic diversity of the red deer in Bulgaria has revealed: (i) high population genetic diversity of species in the country (HARTL *et al.* 1993b, CHASSOVNIKAROVA *et al.* 1993, MARKOV

1998); (ii) very high levels of polymorphism and heterozygosity as compared to other European populations (HARTL *et al.* 1993a, HARTL *et al.* 1995); and (iii) presence of at least two different genetic lines of the red deer in Bulgaria (MARKOV *et al.* 2012). In contrast to this, the craniometric features of the population of the red deer in habitats with diverse ecological conditions are still not well known.

Considering the lack of knowledge on craniometric characteristics of the red deer in the main populations in Bulgaria and general biological relevance of the analysis of the mammalian skull to resolve several issues in their microevolution and adaptations, the aims of the present study were as follow: (i) to analyse the extent of sexual dimorphism in skull features; (ii) to review the population morphometric variations in the skull of the red deer from mountainous and lowland habitats; and (iii) to assess the craniometric similarity of the red deer from the main populations in Bulgaria to the eastern European red deer (*Cervus elaphus hippelaphus*) and the south-eastern European red deer (*Cervus elaphus pannoniensis*).

Material and Methods

The ecological conditions and the food base in the main biotopes of the red deer in Bulgaria are quite different. Generally, the main habitats of the species in the country could be classified as forested habitats

in the mountains and forested localities in the highland fields. Therefore, the description of the complete characteristics of craniological diversity of the red deer in Bulgaria should cover populations, which inhabit both types of biotopes – forested mountainous and forested highland fields. To meet this need, specimens taken from Vitosha Mountain, Central Stara Planina Mountains and from hilly woodlands of the Strandzha-Sakar and Ludogorie-Dobrudzha regions were included in the comparative craniometric analysis (Fig. 1).

The comparative craniometric study included 93 adult individuals (74 males and 19 females) from four populations. The male specimens were taken from two mountainous populations: the Central Stara Planina Mountain population (Population 1; n=29 males) and Vitosha Mountain (Population 4; n=14), as well as from two populations inhabiting hilly landscapes: the Ludogorie-Dobrudzha area (Population 3; n=17) and Strandzha-Sakar area (Population 2; n=14). The females came from two populations: one mountainous (the Central Stara Planina Mountains – Population 1; n=7) and one from forested hills (the Ludogorie-Dobrudzha area – Population 3; n=12).

The analysed sample consisted of specimens kept in the scientific museum collections of public institutes, such as the National Museum of Natural History (Sofia), but the largest part of them belonged to trophy collections of different hunting enterprises in the country (about 85%). The remaining small

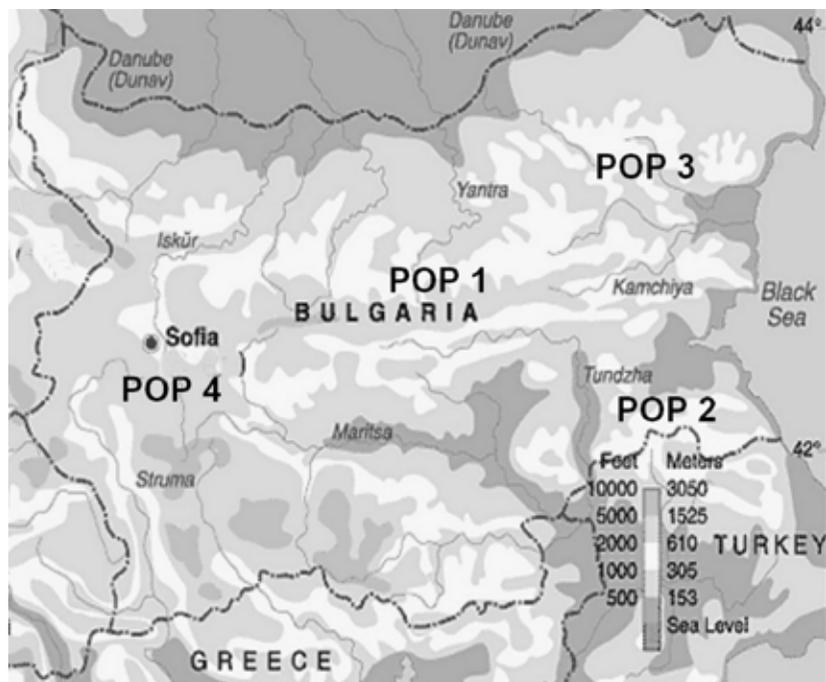


Fig. 1. Geographic location of the Bulgarian red deer (*Cervus elaphus*) populations studied for craniometric diversity: Pop1 – Central Stara Planina Mountains; Pop2 – Strandzha-Sakar region; Pop3 – Ludogorie-Dobrudzha region; Pop4 – Vitosha Mountain

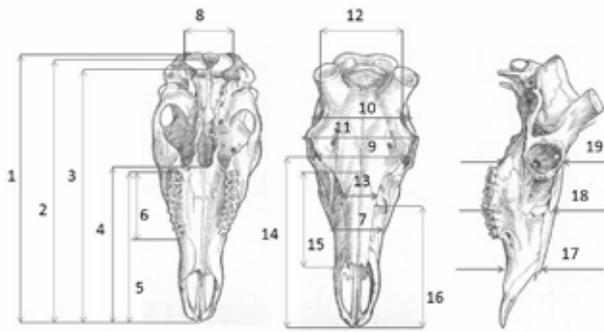


Fig. 2. Measurements of the red deer (*Cervus elaphus*) cranium: V1 – Total length; V2 – Condylbasal length; V3 – Basilar length; V4 – Palatal length; V5 – Frontal length; V6 – Maxillary tooth row length; V7 – Maxillae width; V8 – Condylar breadth; V9 – Orbital breadth; V10 – Cranial breadth; V11 – Smallest orbital breadth; V12 – Braincase breadth; V13 – Nasal breadth; V14 – Rostrum length; V15 – Nasal length; V16 – Length between *p. prosthion* and distal part of the *maxilla*; V17 – Nasal depth I; V18 – Nasal depth II; V19 – Palatal depth; adapted from LOWE, GARDINER (1974) and SOKOLOV, DANILKIN (1981)

part belonged to private collections or was obtained during special field scientific research of the red deer in Bulgaria, which was carried out within the hunting season of this species.

Only adult specimens were analysed craniometrically. The age of each specimen was determined by assessing the state of the tooth system (HABERMEHL 1975).

The craniometric features of the red deer in Bulgaria and the examination of their population degree of similarity and differentiation were based on 19 skull and dental craniometric parameters. The compiled set for measurement of the skull of the red deer (Fig. 2) was developed for the present study using both metric parameters applied by English researchers and recommended for measurement of the British Museum collections (LOWE, GARDINER 1974) as well as metric parameters used by Russian researchers as unified methodology for investigation of deer in Eurasia (SOKOLOV, DANILKIN 1981, SOKOLOV 1992). The measurements were taken with a digital calliper with an accuracy of 0.01 mm.

The initial craniometric parameters were tested for normality using Kolmogorov-Smirnov D-statistics, and for homogeneity of variances using Levene's test. The examination of the craniometric variety of the specimens from geographic populations included the following analysis: (i) calculation of the basic statistics – mean (\bar{X}) and standard deviation (SD) for all studied morphometric characteristics of each investigated group of animals; (ii) checking the significance of differences in mean

values (Breakdown & one-way ANOVA and T-Test for Independent Samples by Groups) of the studied craniometric parameters of the red deer in the investigated units of animals; (iii) characterisation of the multidimensional craniometric variety of the red deer specimens of both sexes using Factor analysis (Extraction method-Principal components; Factor rotation-Varimax normalised); (iii) assessing the craniometric sex dimorphism and differentiation in the Bulgarian red deer, separately for each gender using a Discriminant analysis (Stepwise Discriminant Function Analysis with Model Definition – Forward Stepwise); and (iv) ascertainment of the phenetic relationships among the males from Bulgarian geographic populations and their craniometric similarity to the eastern European red deer (*Cervus elaphus hippelaphus*) and south-eastern European red deer (*Cervus elaphus pannoniensis* = *Cervus elaphus montanus*), by using Cluster Analyses (Joining Tree Clustering), via Single linkage and Euclidean distance matrix.

All calculations were performed using the statistical package STATISTICA 2008 version 8.0 (StatSoft Inc. 2008).

Results

The calculated basic statistical parameters (mean \bar{X} and standard deviation SD) for all investigated morphometric characteristics in both genders of each investigated group of red deer in Bulgaria are presented in Table 1.

The analysis of the statistical significance of the differences between the sizes of the cranium in both genders of the red deer in the combined group of specimens from the four populations showed a significant difference ($p = 0.05$) in 94.74% of the means in all studied craniometric parameters (except the means of V16 differing at $p = 0.299$), and all the means were higher in males.

The Factor analysis revealed two factors explaining the biggest part of the variation in the studied group of the red deer of both sexes – 76.50%. The first factor explained 70.51%, the second – 5.98%. In the first factor the highest values belonged to 15 (78.94%) of the studied parameters (loadings were >0.700), five of which describing the skull lengths (V1 ÷ V3, V14, V15); seven describing the skull widths (V7 ÷ V13); and three – the skull heights (V17 ÷ V19). The highest values (loadings are >0.700) in the second factor belonged to the skull width -V16.

The multivariate analysis of the sexual craniometric differences in the Bulgarian red deer (Stepwise Analysis) resulted in 13 variables combined in sta-

Table 1. The number of specimens (n), mean length (X in mm) and standard deviation (SD) separately for each craniometric parameter in both sexes (male – Me, female -Fe) of common red deer (*Cervus elaphus*) group from the whole territory and in the investigated localities in Bulgaria: Population 1 – Central Stara Planina Mountains; Population 2 – Strandzha-Sakar area; Population 3 – Ludogorie-Dobrudzha area; Population 4 – Vitosha Mountain. Symbols of the characters are the same as in Fig. 2

Sex		V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16	V17	V18	V19
Common red deer group from the whole territory of Bulgaria																				
Me	n	58	57	56	56	58	73	74	73	74	74	74	74	74	58	74	58	74	73	70
	X	458.3	435.4	409.4	262.4	149.8	117.4	79.2	78.2	187.3	174.5	136.0	108.8	63.3	270.0	172.4	131.4	66.0	93.5	102.8
	Sd	22.2	20.4	20.2	31.5	10.0	6.2	5.8	3.6	11.9	13.1	10.2	6.4	7.3	20.2	12.5	9.2	5.3	6.2	7.6
Fe	n	19	19	19	19	19	19	19	19	19	19	19	19	19	19	19	19	19	19	19
	X	389.0	375.2	349.8	228.2	181.4	106.8	55.0	62.8	149.3	144.4	105.7	88.2	50.1	233.2	136.5	127.4	51.8	71.5	83.4
	Sd	8.2	7.2	6.2	8.3	249.9	4.5	5.0	4.7	3.3	3.4	4.2	3.9	2.4	5.3	6.4	24.8	6.2	3.7	4.3
Red deer from population 1																				
Me	n	23	22	21	22	23	28	29	28	29	29	29	29	29	23	29	23	29	29	29
	X	453.0	431.6	404.1	255.7	147.2	119.3	78.1	77.4	182.9	170.4	133.2	107.6	59.9	266.0	168.0	127.2	65.5	93.3	101.4
	Sd	24.3	22.0	23.9	21.7	13.1	5.2	6.6	2.9	9.4	8.0	13.1	5.6	7.3	23.4	13.7	10.6	5.2	6.6	7.8
Fe	n	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
	X	392.4	378.2	351.2	227.5	277.1	104.2	50.7	62.5	152.0	144.2	108.2	84.4	50.4	236.4	138.0	114.0	55.7	70.5	83.0
	Sd	6.0	5.2	5.5	4.9	412.7	1.6	0.8	6.8	3.1	4.0	4.1	1.8	1.2	4.7	2.9	4.3	4.2	1.5	3.6
Red deer from population 2																				
Me	n	9	9	9	9	9	14	14	14	14	14	14	14	14	9	14	9	14	14	14
	X	469.3	437.9	417.8	263.9	156.3	115.6	79.8	80.3	191.3	177.3	139.1	110.2	67.4	281.7	179.1	136.3	67.9	96.3	105.4
	Sd	16.5	18.4	10.8	17.0	5.5	7.5	5.7	4.3	8.5	7.8	6.6	5.6	7.5	11.2	8.6	5.6	6.7	5.8	7.3
Red deer from population 3																				
Me	n	12	12	12	11	12	17	17	17	17	17	17	17	17	12	17	12	17	16	16
	X	466.5	443.3	413.6	260.4	150.9	118.5	81.5	77.8	188.9	176.6	137.9	109.5	66.2	271.5	176.1	135.1	67.2	93.8	105.9
	Sd	24.2	23.5	21.5	12.8	6.7	6.7	4.7	4.0	16.2	4.8	7.3	6.3	6.4	24.8	8.6	6.7	4.9	6.3	5.8
Fe	n	12.0	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
	X	387.1	373.5	349.0	228.6	125.6	108.3	57.5	62.9	147.7	144.6	104.3	90.5	49.9	231.3	135.6	135.2	49.5	72.1	83.6
	Sd	8.9	7.8	6.7	10.0	6.0	5.0	4.6	3.2	2.2	3.2	3.7	2.9	2.9	4.8	7.8	28.6	6.1	4.6	4.8
Red deer from population 4																				
Me	n	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	11
	X	452.8	433.2	408.2	273.6	148.8	114.3	77.9	78.2	190.6	177.9	136.4	109.1	62.5	267.9	170.6	131.9	63.7	90.9	98.4
	Sd	16.2	15.8	16.3	53.9	6.8	4.6	4.7	3.1	11.7	25.9	8.4	8.4	4.6	11.2	13.7	7.9	3.5	4.5	7.7

tistically significant classification functions (Wilks' Lambda = 0.07373, approx. F (13, 55) = 53.155, p < 0.0000), which discriminated the skulls of Bulgarian red deer between both sexes:

$$Y_{\text{Male}} = 1.889 V7 + 4.960 V8 - 0.190 V16 + 6.369 V6 + 3.828 V17 - 3.389 V19 - 0.306 V18 + 0.579 V4 - 0.563 V14 + 2.105 V11 - 0.406 V13 + 0.194 V15 - 0.093 V10 - 709.561$$

and

$$Y_{\text{Female}} = 0.722 V7 + 3.643 V8 - 0.002 V16 + 5.610 V6 + 3.125 V17 - 2.705 V19 - 0.446 V18 + 0.472 V4 - 0.181 V14 + 1.563 V11 + 0.120 V13 - 0.137 V15 + 0.109 V10 - 503.806.$$

The total correct distribution of the studied specimens according to their previously known gender was 100%.

The checking of the significance of differences in the mean values (Breakdown & one-way ANOVA) of the studied parameters in the male red deer from four geographic populations resulted in the effective hypothesis decomposition concerning their equality. Parameters V6, V13, V15, V16 and V19 were distinguished by significance at p < 0.05.

The multivariate analysis of the craniometric differences (Stepwise Analysis; Wilks' Lambda = 0.5738; approx. F (6, 43) = 5.3213; p < 0.0004) among males inhabiting the mountainous regions (Group H – including specimens from the Central Stara Planina and Vitosha mountains) and hilly landscapes (Group L – including specimens from the Ludogorie-Dobrudzha and Strandzha-Sakar areas) determined correctly 84.905% of the studied speci-

Table 2. Mean values [in mm] of some craniometric characteristics (V1 – Total length of the skull; V2 – Condylbasal length of the skull, and V9 – Orbital breadth) and their ratios (in %) in adult males from the studied operational units of red deer in Bulgaria (Pop1 – Central Stara Planina Mountains; Pop2 – Strandzha-Sakar area; Pop3 – Ludogorie-Dobrudzha area; Pop4 – Vitosha Mountain) and the subspecies of the red deer *Cervus elaphus hippelaphus* (C. e. h.) and *Cervus elaphus pannoniensis* (C. e. p.) from Central Europe, as determined craniometrically by BALIŠ (1980)

Unit	V1	V2	V9	V9/ V1	V9/ V2	V2/ V1	V1 unit / V1 C.e.p.	V2 unit / V2 C.e.p.	V9 unit / V9 C.e.p.	V1 unit / V1 C.e.h.	V2 unit / V2 C.e.h.	V9 unit / V9 C.e.h.
C.e.h.	410.3	398.3	100.00	42.0	43.0	97.0	86.02	88.33	90.79	100.00	100.00	100.00
C. e. p.	477.0	450.9	110.14	39.0	42.0	95.0	100.00	100.00	100.00	116.26	113.21	110.14
Pop1	453.0	431.6	107.21	40.0	42.0	95.0	94.97	95.72	97.34	110.41	108.36	107.21
Pop2	469.3	437.9	112.13	41.0	44.0	93.0	98.39	97.12	101.81	114.38	109.94	112.13
Pop3	466.5	443.3	110.73	40.0	43.0	95.0	97.80	98.31	100.53	113.70	111.30	110.73
Pop4	452.8	433.2	111.72	42.0	44.0	96.0	94.93	96.07	101.44	110.36	108.76	111.72

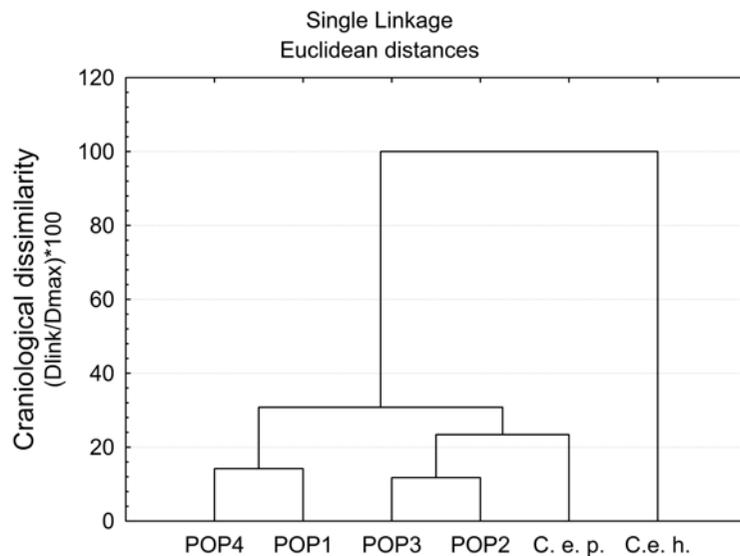


Fig. 3. Phenotypic craniological interpopulation similarity in the males from four main localities of red deer (*Cervus elaphus*) in Bulgaria (Pop1 – Central Stara Planina Mountains; Pop2 – Strandzha-Sakar area; Pop3 – Ludogorie-Dobrudzha area; Pop4 – Vitosha Mountain) and subspecies of the red deer *Cervus elaphus hippelaphus* (C. e. h.) and *Cervus elaphus pannoniensis* (C. e. p.)

mens, according to the habitat they came from. The correct classification of the specimens from mountainous populations was 87.87%; the percentage of correct determination of the specimens from hilly landscapes was 80.00%.

Six craniometric parameters were involved in the classification functions:

$$Y_{\text{Group H}} = 0.771 V19 + 2.162 V16 + 8.627 V8 - 1.108 V10 - 1.178 V5 + 1.258 V15 - 432.206;$$

and

$$Y_{\text{Group L}} = 0.907 V19 + 2.320 V16 + 9.024 V8 - 1.214 V10 - 1.313 V5 + 1.368 V15 - 478.909.$$

A detailed evaluation of the differences among all means of the craniological parameters in the females within two populations (the mountainous population from the Central Stara Planina – Population 1, and the lowland population from the Ludogorie-

Dobrudzha region – Population 3) was obtained by applying a T-test for independent samples by groups with “population” as a grouping variable. Significant differences ($p = 0.05$) were found in seven parameters (V7, V9, V11, V12, V14 and V17) or in 31.6% of all studied characters.

In order to assess the population differentiation in the females from the mountainous and lowland populations, a discriminant analysis (forward stepwise) was carried out under the same initial conditions as in males (Tolerance 0.010 and stepwise options: F to enter 1.00 and F to remove 0.00). Here the stepwise discriminant function analysis was not able to “build” a model of discrimination and to choose variables to be included in the model, therefore H_0 (the null hypothesis that there is no discrimination among groups) should not be rejected.

The craniometric comparison of the examined red deer populations from Bulgaria (Table 2) with the eastern European red deer (*Cervus elaphus hippelaphus*) and south-eastern European red deer (*Cervus elaphus pannoniensis* = *Cervus elaphus montanus*) on the basis of similarity in the total length and maximal width of the skull and their ratios as described by BALIŠ (1980) showed that: (i) The highest values of craniometric parameters – total length of the skull (V1), condylobasal length of the skull (V2) and orbital breadth (V9) among the studied operational units were found in the representatives of the south-eastern European red deer; (ii) The values of the total length of the skull (V1) in the Bulgarian red deer were by 6-12% lower than in the south-eastern European red deer and by 10-14% higher than in the eastern European red deer; and (iii) The values of the condylobasal length of the skull (V2) in the Bulgarian red deer showed the same trend – they were lower than in the south-eastern European red deer and higher than in the eastern European red deer; the values of the orbital breadth (V9) were very similar to those of the south-eastern European red deer.

The UPGMA dendrogram that summarises the craniometric relationships among the red deer populations from Bulgaria and the subspecies *C. e. hippelaphus* and *C. e. pannoniensis* is shown in Fig. 3. According to this dendrogram generated from the matrix including the total length, condylobasal length and orbital breadth of the skull of the specimens, the Bulgarian populations from lowland habitats showed the highest craniometric similarity. The populations from the mountainous habitats were united also in a group with high relative craniometric similarity. All Bulgarian populations, together with the representatives of *C. e. pannoniensis*, formed a common sub-cluster. The most diverged specimens were the representatives of *C. e. hippelaphus*, which manifested the highest levels of morphometric differentiation (about 70%) among all compared red deer populations.

Discussion

The overall information obtained in the present analysis of the sexual cranial dimorphism of the main red deer populations from Bulgaria was consistent with the previous morphological investigations of this species. This information confirmed the well-expressed craniometric differentiation of both sexes and showed that future analyses aiming to detect and assess the degree of craniometric similarity and distinction of red deer should be carried out separately by gender.

All the results obtained on examining the degree of phenotypic craniological similarity among the specimens of the same gender from the main populations of the red deer in Bulgaria revealed a high degree of phenotypic craniological similarity between the female specimens; in multivariate aspect this was less pronounced in males. The degree of phenotypic craniological dissimilarity among males from the mountainous and hilly landscapes populations demonstrated by the multivariate estimation of their skull features actually confirmed that their craniometric dissimilarity is not too high. Each population included individuals that could be incorrectly classified as belonging to another population.

These results could be explained by the fact that the red deer herds in many of the hunting areas consist of descendants of animals, which were re-acclimatised in the 1960s and 1970s from the Sherba and Palamara game breeding stations, both situated in north-eastern Bulgaria (DRAGOEV 1978). Perhaps the similar regional diversity expressed by the higher degree of similarity in the males from populations inhabiting landscapes with similar ecological conditions (lowland populations and mountainous populations) was mainly due to the trophic factor (FINDO 1993) and to the game-breeding strategies carried out to provide individuals with good trophy values.

In line with the studies of craniometric characteristics of the red deer in Slovakia, our study revealed that a greater mean maximal width of the skull was found in stags from the regions providing trophies with the highest scores from the International Council for Game and Wildlife Conservation (CIC).

The results of the cluster analysis that estimated the similarity of the craniometric features in the male red deer from four Bulgarian populations to those of the subspecies *C. e. pannoniensis* and *C. e. hippelaphus*, as determined craniometrically by BALIŠ (1980), allowed suggesting that the Bulgarian red deer is morphologically closer to *C. e. pannoniensis* than to *C. e. hippelaphus*. The populations from the lowlands showed a higher relative similarity to one another compared to that between mountainous populations. The Bulgarian lowland populations manifested more pronounced craniometric similarity to *C. e. pannoniensis* from Central Europe than the Bulgarian mountainous populations.

The mammal skulls, which are highly informative, conservative and adaptive structures, represented the most powerful tool for biogeographic, phylogenetic and systematic investigations before the molecular investigations (LOY 2007). The red deer skulls available in museum and private collec-

tions could reveal a mass of information related to the adaptations of these animals. A large part of the systematic structure of the red deer in Eurasia has been constructed on the grounds of these skulls, especially at the subspecies level.

Our results suggested that elements of the skull can be used to analyse the variability and co-variability patterns of quantitative morphological characters within the red deer natural populations. The patterns of morphological variation, interpreted in terms of response to adaptive and selective forces, ontogenetic and phylogenetic constraints depict phylogenetic relationships among the red deer population in Bulgaria that is not distant from the picture given by molecular studies and other characters. The use of morphometric data in combination with other methods of phylogenetic reconstruction may result in a revision of the subspecies level taxonomy of *Cervus elaphus* in its East-European area and may provide support for differential conservation or management strategies for some of its populations.

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