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Sexual dimorphism and population differentiation of the wolf (*Canis lupus*) based on morphometry in the Central Balkans

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Abstract. Previously described morphological and genetic differentiation of the wolf populations in the region of the Central Balkans was tested using morphometric characteristics. Morphometric analyses of sexual dimorphism and population differentiation of the Dinaric-Balkan wolf population in the Central Balkan region was based on five parameters of external morphology and body mass. In total, 128 wolves collected at 35 localities from three populations (Bosnia and Herzegovina, eastern Serbia and western Serbia) were included in the analyses. Male adult wolves from the Central Balkans were significantly weightier (Student's $t = 4.931$, $p < 0.000$), longer (HBL $t = 4.060$, $p < 0.000$; TL $t = 2.141$, $p < 0.035$; EL $t = 4.152$, $p < 0.000$) and higher at the shoulder ($t = 4.152$, $p < 0.000$) than adult females. There was no significant difference between sexes in ear length ($t = 1.870$, $p < 0.065$). Sexual dimorphism in subadult wolves (age 6 to 9 months) was not found. Canonical discrimination analysis of morphological data showed that the three populations were differentiated into an "eastern" (eastern Serbia and western Serbia) and "western" subpopulation (Bosnia and Herzegovina). Our results support the hypothesis that the Drina River separates populations in the Central Balkan region into bigger and weightier wolves from Bosnia and Herzegovina and smaller and lighter ones from Serbia. The apparent steady differentiation of the Dinaric-Balkan wolf's population into an "eastern" and "western" subpopulation can be important in conservation and management strategies especially at the transboundary level.

Key words: wolf, *Canis lupus*, morphometry, population differentiation, Central Balkans, Serbia, Bosnia and Herzegovina.

Introduction

Within the wolf's global distribution, different populations are characterized by notable phenotypic variation in external morphology (color, body size and body mass) and skull features (Boitani 1995, Mech 1970). Except for a few published results from the Central Balkans (Djan et al 2014, Milenković 1997, Milenković et al. 2010), data on morphological variation or population differentiation from this region are very limited. In particular, more information is needed on wolf body size and body mass. According to analyses of linear cranial characteristics of skulls from the territory of the former Yugoslavia, Milenković (1997) found significant differences between populations. The author suggested that biogeographic features of the Central Balkan region, specifically Morava-Vardar valley, influence differentiation within the Dinaric population. In this region, human development induced changes in the landscape which became unsuitable for wolves. This habitat transformation separated the wolf population in Serbia into a western and an eastern subpopulation (Milenković et al. 2007). Based on geometric morphometrics of 54 skulls that belong to the Carpathian and Dinaric–Balkan populations from Serbia and Bosnia and Herzegovina, Milenković et al. (2010) found that Carpathian wolves were larger than the Dinaric-Balkan wolves. Also the authors found significant diffferences in shape, especially in the cranial flexion which was observed for the first time in a comparative study of a wolf population. According to the analyses the Balkan-Dinaric wolves had a more prominent snout and sagittal crest than Carpathian wolves. Analyses of population differentiation based on mtDNA sequence variability suggest that the Dinaric-Balkan wolf population is differentiated into "western" (Croatia/Bosnia and Herzegovina) and "eastern" (Serbia/Macedonia) subpopulations (Djan et al. 2014). Contrary to the morphological findings from Milenković (1997), genetic results suggest the river Drina (which is also the border between Serbia and Bosnia and Herzegovina) acts as a barrier to wolf movement in the Central Balkan region (Djan et al. 2014).

The wolf (*Canis lupus*) is a highly mobile

predator with a complex social structure and flexible adaptations to very different habitats, from arid deserts in the south to tundra in the north (Boitani 2000). Its distribution includes the whole of the Palearctic and Nearctic regions. It is a widespread large carnivore in Serbia and Bosnia and Herzegovina (B&H). In Serbia, its distribution includes mountain regions in the western, eastern and southern part of the country. Along the valley of the Velika Morava River (centre of Serbia) wolves are absent or only occasionally present. Beside the mountain regions, a small isolated population inhabits the south-eastern part of the Pannonian plain in the Vojvodina province (Milenković 1997, Milenković et al. 2007, Milenković et al. 2010). In Bosnia and Herzegovina its distribution includes the whole mountainous region in the southern and central part of the country. The wolf is absent or sporadically present only in the north, along the Sava river valley (Posavina region). Wolves from Serbia and from Bosnia and Herzegovina belong to the widely distributed Dinaric-Balkan wolf population (Boitani 2000, Cahapron et al. 2014).

In most parts of its Serbian range, the wolf is a game species without any protection (even closed season) except for the isolated population in Vojvodina where the wolf has a status of a strictly protected species. Limited distribution and small size of local population were reason for its protection. According to recent estimations, there are 700-800 individuals in Serbia and the population is believed to be increasing slowly (Boitani 2000, Cahapron et al. 2014, Milenković et al. 2007). Annual harvesting on the average is about 200 wolves, which represents 25-28% of the estimated population size. On the territory of Bosnia and Herzegovina the wolf is also a game species but with different status in two administrative parts. In the Federation the wolf is protected with a closed hunting season (only for female and pups), while in the Republic of Srpska it has no protection. Population size was estimated at 400 wolves in the whole country. In the last decades the wolf population has been declining in B&H (Boitani 2000, Cahapron et al. 2014).

The aims of our study were to 1) describe the size and body mass of wolves in the region of the Central Balkans; 2) to examine the extent of sexual dimorphism; 3) to examine population differentiation based on morphometric characters and body mass; and 4) to test the hypothesis whether the Drina river or Velika Morava river represents the barrier which separates the Dinaric-Balkan population.

Material and Methods

Sampling

Population differentiation and sexual dimorphism of the wolf in the Central Balkans were studied during 2004-2014. Bodies of 128 specimens (50 from eastern Serbia, 40 from western Serbia and 38 from Bosnia and Herzegovina) from 35 localities were collected in cooperation with local hunting organizations during 9 winter seasons (December – February) throughout its range in Bosnia and Herzegovina and Serbia (Fig. 1). In total, we collected 65 males (21 from eastern Serbia, 28 from western Serbia and 16 from Bosnia and Herzegovina) and 63 females (19 from eastern Serbia, 22 from western Serbia and 22 from Bosnia and Herzegovina). Among a total of 128 measured wolves, 25 of them (21 from Serbia and 4 from Bosnia and Herzegovina) were subadults (age 6 to 9 months). The

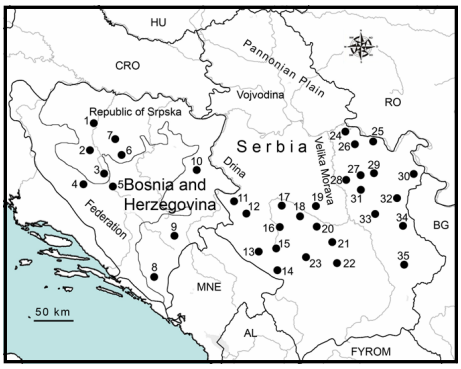

Figure 1. Localities where the wolves were collected. Localities were organized in three samples group (according to geographic location): Bosnia and Herzegovina, Western Serbia and Eastern Serbia. (Bosnia and Herzegovina: 1 Banja Luka (N=(11), 2 Mrkonjić Grad (N=4), 3 Šipovo (N=1), 4 Kupres (N=12), 5 Donji Vakuf (N=1), 6 Kneževo (N=2), 7 Kotor Varoš (N=2), 8 Trebinje (N=2), 9 Tjentište (N=1), 10 Vlasenica (2). Western Serbia: 11 Kremna (N=3), 12 Zlatibor (N=2), 13 Sjenica (N=31), 14 Tutin (N=2), 15 Ivanjica (N=1), 16 Dubac (N=1), 17 Čačak (N=1), 18 Kraljevo (N=2), 19 Županjevac (N=1), 20 Vrnjačka Banja (N=2), 21 Trstenik (1) 22 Aleksandovac Župski (N=2), Raška (N=1). Eastern Serbia: 24 Veliko Gradište (N=1), 25 Dobra (N=2), 26 Braničevo (N=2), 27 Ćovdin (N=3), 28 Svilajnac (N=2), 29 Žagubica (N=5), 30 Negotin (N=1), 31 Resavica (N=1), 32 Zaječar (N=2), 33 Boljevac (N=5), 34 Knjaževac (N=5), 35 Bela Palanka (11). Number of measured wolves per locality is given in brackets (N)).

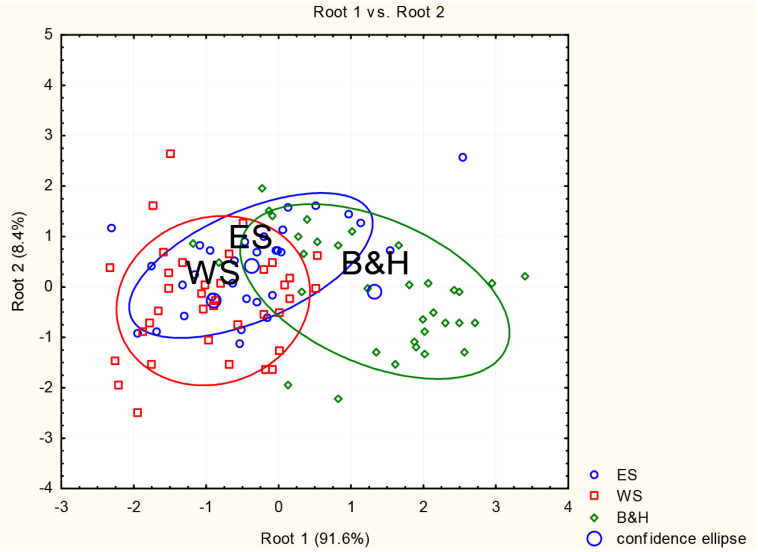

Figure 2. Canonical discriminate analysis of five body parameters and body mass of the wolves from region of the Central Balkans. Legend: B&H – Bosnia and Herzegovina, ES – eastern Serbia, WS – western Serbia, the confidence ellipse of the group discrimination.

following data were recorded for each animal: sex, date of death, and the most precise locality. Five body parameters and body mass were measured using measuring tape to 1 mm precision from each collected wolf: head and body length (HBL), tail length (TL), hind foot length (HFL), ear length (EL) and height at the shoulder (SL). Body mass (BM) was measured with a scale to an accuracy of 0.1 kg. Because all animals were collected during the winter, they were classified into two separated age categories: subadults (age from 6 to 9 months) and adults (1+ year). Age (subadults and adults) was determined according to the body size and tooth wear (Gipson et al. 2000, Mech 2006). For this reason, all skulls were cleaned of muscle tissue.

Statistical analyses

For statistical analyses, we grouped all samples into three presumed populations (Figure 1): eastern Serbia (ES), western Serbia (WS) and Bosnia and Herzegovina (B&H). Descriptive statistics (minimum, maximum and mean value, variance and standard deviation) were calculated and reported separately for adult males and females (Table 1a) for all measured body parameters and body mass across all three populations. Descriptive statistics for subadults were calculated only for the total sample due to small sample sizes (male n=7, female n=18, Table 1b). Sexual and age differences were tested using Student's t-test. Canonical discriminant analysis (CDA) was used to asses the existence of population differentiation between the three populations (3 populations × 103 adult wolves × 6 morphometric parameters). Apart from overall significance of the discrimination, we reported the contribution of individual characters (Table 2a). Canonical scores for each case were calculated, and visualized by a scatterplot of canonical scores upon which group membership was superimposed (Fig. 2). Population differentiation was assessed by Mahalanobis distances (Sneath and Sokal 1973), and the classification matrix was reported (Table 2b, 2c). All analyses were conducted using Statistica 5.1 (Statsoft, Tulsa, OK, USA).

Results

The average body mass for wolves from the Central Balkans was 36.89 ± 5.70 kg for adult males and 31.89 ± 4.23 kg for adult females. Average HBL of male wolves was 121.34 ± 9.41 cm and females 114.60 ± 6.75 cm (for other parameters see Table 1a). Male wolves from the Central Balkans were significant weightier ($t = 4.931$, $p < 0.000$), longer (HBL $t = 4.060$, $p < 0.000$; TL $t = 2.141$, $p < 0.035$; EL $t = 4.152$, $p < 0.000$), and higher at the shoulder ($t = 4.152$, $p < 0.000$) than females. There was no significant difference in the ear length between sexes ($t = 1.870$, $p < 0.065$). Subadult wolves were significantly lighter and smaller (BM $t = 8.389$, $p < 0.000$; HBL $t = 6.232$, $p < 0.000$; TL $t = 3.162$, $p < 0.001$; EL $t = 3.263$, $p < 0.002$; HFL $t = 3.441$, $p < 0.001$; SL $t = 6.482$, $p < 0.000$) than adults. Sexual dimorphism in subadult wolves was not found between most characters

Table 1a. Descriptive statistics of wolves from Central Balkans (BM in kilograms, HBL, TL, El, HFL, SL in cm, a. adult wolves, b. subadult wolves.

Population	Sex	Parameters	N	Mean	Min	Max	SD	Var
East Serbia	Male	BM	19	36.73	29.60	52.00	6.44	41.51
		HBL	19	116.82	106.00	125.00	5.70	32.54
		TL	19	40.65	35.00	46.00	2.72	7.42
		EL	19	11.42	10.00	12.30	0.84	0.70
		HFL	19	24.08	22.00	25.50	1.06	1.12
		SL	19	70.91	65.00	80.00	3.72	13.81
	Female	BM	12	30.82	26.80	34.80	2.88	8.27
		HBL	12	112.10	106.50	122.00	4.61	21.29
		TL	12	38.88	33.00	43.10	2.84	8.09
		EL	12	11.29	10.70	12.50	0.46	0.21
		HFL	12	23.34	21.30	25.00	0.91	0.84
		SL	12	68.88	63.00	75.00	3.79	14.37
West Serbia	Male	BM	24	34.76	27.00	44.00	4.85	23.54
		HBL	24	117.70	108.00	132.00	6.24	39.00
		TL	24	40.94	34.00	48.30	2.99	8.92
		EL	24	11.36	10.00	12.30	0.60	0.36
		HFL	23	24.74	20.00	28.00	1.65	2.74
		SL	24	73.32	68.00	82.00	4.37	19.07
	Female	BM	14	29.91	24.00	36.00	3.35	11.25
		HBL	14	112.71	107.00	120.00	3.62	13.13
		TL	14	39.84	35.00	45.00	2.81	7.91
		EL	14	10.96	10.00	12.40	0.70	0.49
		HFL	14	23.14	22.00	25.00	0.80	0.64
		SL	14	68.76	65.00	71.00	1.90	3.61
Bosnia and Herzegovina	Male	BM	15	40.52	31.50	48.00	4.27	18.20
		HBL	15	132.89	105.00	137.00	7.83	61.34
		TL	15	37.01	34.00	40.00	1.94	3.75
		EL	15	12.37	10.90	13.20	0.61	0.37
		HFL	15	24.43	22.00	25.40	1.13	1.27
		SL	15	74.27	68.00	77.00	3.03	9.16
	Female	BM	19	34.02	28.50	43.00	4.68	21.95
		HBL	19	117.57	107.00	134.00	8.53	72.69
		TL	19	37.15	30.00	42.00	3.84	14.72
		EL	19	11.68	10.00	13.00	0.75	0.56
		HFL	19	23.79	22.00	25.00	0.87	0.76
		SL	19	68.51	63.70	72.10	2.66	7.09
Central Balkans	Male	BM	58	36.89	77.00	52.00	5.70	32.46
		HBL	58	121.34	105.00	137.00	9.41	88.56
		TL	58	39.83	34.00	48.00	3.11	9.69
		EL	58	11.64	10.00	13.20	0.80	0.65
		HFL	57	24.44	20.00	28.00	1.35	1.83
		SL	58	72.78	65.00	82.00	4.02	16.18
	Female	BM	45	31.89	24.00	43.00	4.23	17.89
		HBL	45	114.60	106.50	134.00	6.75	45.59
		TL	45	38.44	30.00	45.00	3.43	11.77
		EL	45	11.36	10.00	13.00	0.72	0.52
		HFL	45	23.47	21.30	25.00	0.89	0.80
		SL	45	68.69	63.00	75.00	2.75	7.59

(Table 1b). According to Student's t-test there were no differences between male subadults and female subadults in BM ($t = 1.624$, $p < 0.118$), HBL ($t = 0.849$, $p < 0.405$), EL ($t = 1.568$, $p < 0.130$), and SL ($t = 0.539$, $p < 0.595$). Subadult males only had significantly longer tail ($t = 2.829$, $p < 0.009$) and hind foot length ($t = 2.256$, $p < 0.007$). In general, adult wolves were 29.4 % weightier, 11.2 % longer (HBL), and 9.6 % higher (SL) than subadult (Table 1a, b).

Table 1b. Descriptive statistics of wolves from Central Balkans (BM - body mass, HBL - head and body length, TL - tail length, HFL - hind foot length, EL - ear length, SL - height at the shoulder)

Sex	Parameters	N	Mean	Min	Max	SD	Var
Male	BM	7	26.40	21.20	29.00	2.55	6.49
	HBL	7	108.33	95.00	115.10	7.60	57.83
	TL	7	39.61	37.00	42.20	1.86	3.47
	EL	7	11.27	11.00	12.00	0.39	0.15
	HFL	7	24.30	22.70	28.80	2.09	4.36
	SL	7	65.69	55.00	74.00	6.83	46.63
Female	BM	18	23.66	17.80	27.10	2.73	7.48
	HBL	18	105.38	90.40	116.50	7.40	54.70
	TL	18	35.84	29.10	41.80	3.51	12.30
	EL	18	10.69	10.10	11.70	0.41	0.17
	HFL	18	22.35	20.00	23.40	0.90	0.81
	SL	15	64.65	58.00	69.00	3.57	12.77

Table 2. Canonical discriminate analysis of five body parameters and body mass of the wolves from region of the Central Balkans (B&H – Bosnia and Herzegovina, ES – eastern Serbia, WS – western Serbia, the confidence ellipse of the group discrimination).

a. Overall contributions of characters			
Parameters	Wilk's lambda	F	p-level
BW	**0.504**	**3.446**	**0.036**
HBL	0.489	1.914	0.153
TL	**0.609**	**13.912**	**0.000**
EL	**0.559**	**8.899**	**0.000**
HFL	0.476	0.603	0.549
SL	**0.506**	**3.646**	**0.030**

b. Mahalanobius distances between populations			
Populations	ES	WS	B&H
ES	0	2.025	**8.195**
WS		0	**14.055**
B&H			0

c. Classification matrix of populations				
Populations	ES	WS	B&H	% correct
ES	12	15	4	38.710
WS	10	26	1	70.270
B&H	10	1	23	67.647
Total	32	42	28	59.804

According to our results, adult wolves from Bosnia and Herzegovina, were weightier (male 40.52 ± 4.27 kg; female 34.02 ± 4.68 kg) and had longer body (HBL male 132.89 ± 7.83 cm; female 117.57 ± 8.53 cm) than wolves from western and eastern Serbia. Compared to wolves from eastern and western Serbia, individuals from Bosnia and Herzegovina had longer ears and were higher at the shoulder, but had a shorter tail and similarly long hind foot (Table 1a).

The overall discrimination was highly significant (Wilks' $\lambda = 0.470$, $F_{12,188} = 7.185$, $p < .0000$). Discriminant Function Analysis revealed that four of six tested parameters (BM, TL, EL, SL) had statistically significant contributions to the discrimination (Table 2a). HBL and HFL had no statistical significance, which suggests that wolves' robustness is an important discriminating factor. CDA confirmed morphological differentiation between the three populations analyzed. Almost half of the individuals from B&H were separated by the first canonical root (91.6 % of variation explained) from wolves from western and eastern Serbia (Figure 2). The wolves from B&H being the most robust, while wolves from WS being the least robust, with ES wolves scoring in between. Pairwise comparisons of Mahalanobis distances between these populations showed that significant differentiation existed between B&H and both ES and WS, but not between ES and WS (Table 2b). Our results also showed that 16 wolves from ES, 11 wolves from

WS and 11 wolves from B&H were misclassified into either population (Table 2c). The misclassification pattern for B&H could be clearly interpreted. Since the discrimination was dominated by robustness, we presume that less robust wolves from B&H accounted for this pattern. These results support the hypothesis that the Drina River separates populations in Central Balkans into the bigger and heavier form from Bosnia and Herzegovina and the smaller and lighter form from Serbia (Figure 2).

Discussion

Characteristics of adult wolves from the Central Balkans showed sexual size dimorphism, males being weightier and bigger than females. In subadults those differences were not expressed between sexes. Similar results were obtained in Croatia where wolves in age categories 6-9 months and 9-12 months showed significant differences only in one of 18 body parameters (Platiša et al. 2011). In the same study, adult wolves showed significantly larger sexual dimorphism. Wolves aged 1-2 years showed significant differences only for 6 of 18 measured parameters between sexes, while wolves older than 2 years had significant differences in fifteen body parameters. According to Platiša et al. (2011), adult wolves (older than 2 years) have clear sexual dimorphism (males are bigger and weightier than females), because males have faster growth rates (Glucksmann 1974, Mech 1970, Platiša et al. 2011). There are no significant differences in size and weight between adult specimens of the same sex (Glucksmann 1974, Mech 1970, Platiša et al. 2011). Differences between subadult and adult wolves in the Central Balkans were expected because of intensive growth in the first years. After this age wolf growth rate slows markedly (Glucksmann 1974, Mech 2006, Platiša et al. 2011).

Our study showed morphological differentiation in the region of the Central Balkans, with wolves from Bosnia and Herzegovina being weightier and bigger than those from Serbia. The presented results based on morphometrics support the hypothesis that the Drina River separates the Balkan-Dinaric population into two subpopulations. In contrast, wolves from the east and west part of Serbia, although separated by the Velika Morava river valley (Milenković 1997, Milenković et al. 2007), showed no differences in body size and mass. Similar structuring of the Dinaric-Balkan wolf population as obtained by morphological analysis was also found at the genetic level (Djan et al. 2014). Analyses based on CR-1 mtDNA of 87 wolves from the Central Balkans showed the same pattern in population structure. The differences found were mainly at the level of genetic diversity: the "eastern" subpopulation had lower genetic diversity than the "western" one. The excess of haplotypes with intermediate frequencies in "western" subpopulation might be a consequence of genetic drift after severe population bottleneck or haplotype admixture from genetically differentiated populations. Wolf population from this region always remained and still is connected with the neighboring small wolf population in Slovenia, and continuous gene flow was presumed among all other regional groups on the Eastern Balkans. Also different demographic histories of these subpopulations may explain the morphological differentiation found. Both subpopulations have gone through bottlenecks, but their timing might differ. Also population size decline probably was not as severe in "eastern" subpopulation as it was in "western" subpopulation (Djan et al. 2014). In fact, our results showing morphological difference in size and weigh could reflect the different patterns of genetic variability and demography in the two delineated subpopulations in the region of the Central Balkans.

The apparent steady differentiation of the Dinaric-Balkan wolf's population into "eastern" and "western" subpopulation can be important in conservation and management strategies especially at the transboundary level (Blanco 2012). In order to obtain better knowledge of differentiation of the Dinaric-Balkan wolf population into two subpopulations, further research on morphological and genetic variability is necessary (e.g. microsatellite markers, genomic analyses) at a larger regional scale. Further, comparison of population variability (morphological and genetic) of the Dinaric-Balkan wolves with the neighbouring Carpathian wolf populations could be very important for understanding this recently observed differentiation.

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