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CRANIAL VARIABILITY AND SEXUAL DIMORPHISM OF GOLDEN JACKAL IN BULGARIA

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Abstract

Craniometric characteristics and sexual dimorphism have been described for many carnivore species in Europe. However, very few studies have focused on cranial variability of golden jackal (*Canis aureus* Linnaeus, 1758) in Europe, despite its expanding distribution in recent decades. Although numerous works have addressed sexual dimorphism in carnivore skull size, only few studies have attempted to study dimorphism in overall cranial shape. The present study proposes the first comprehensive analysis of golden jackal skull morphometry in Bulgaria, trying to clarify shape and size related cranial variability and differentiation. Extensive morphometric data of jackal skulls were analysed by applying recently developed statistical tools to answer the following questions: (i) is there a geographic variation in skull size and shape among golden jackal population in Bulgaria, (ii) are there age-related cranial differences, and (iii) how pronounced is the sexual dimorphism in skull shape and size? A total of 176 skulls of golden jackals, collected all over the country, were analysed by univariate and multivariate statistical methods. Principal component analysis and linear discriminant analysis were applied on the standardized and log-transformed ratios of the original measurements to clearly separate specimens by shape and size. Skulls of golden jackal in Bulgaria show considerable individual variability but weak intrapopulation differentiation. The differences in shape and size of the jackal skulls, as far as they exist, are age-related, but only juveniles younger than 11 months could be easily distinguished. Subadult and adult jackals largely overlap in skull size and shape. Sexual dimorphism in jackal skulls is weakly pronounced, with older males a little bit larger than females. The results of the present research are consistent with recent genetic and morphological studies and give new insights on patterns in cranial variability and population structure of golden jackal in Bulgaria.

Key words: *Canis aureus*, cranial variability, geographic variation, sex dimorphism, skull morphometry, skull shape.

Introduction

Craniometric characteristics and sexual dimorphism have been described for many carnivore species in Europe (Petrov et al. 1992, Gittleman and Valkenburgh 1997, Milenković et al. 2010). Sexual size dimorphism is common among vertebrates, with males usually being the larger sex (Ralls 1977). In the end of the last century, the extreme dimorphism in Mustelidae (Moors 1980, Wiig 1986), Felidae (Wiig and Andersen 1986) and Pinnipedia (Stirling 1975), and the reversed dimorphism in predatory birds (Andersson and Norberg 1981, Pleasants 1988) attracted particular interest, and new theories were proposed, associating the sexual dimorphism with divergent selection pressures on males and females (Moors 1980, Wiig 1986, Wiig and Andersen 1986). However, sexual dimorphism in Canidae, when present at all, is usually minimal, with males being slightly larger than females (Jolicoeur 1959, Hell et al. 1989, Simonsen et al. 2003, Schutz et al. 2009, Sillero-Zubiri 2009). In African jackals (*Canis lupaster* Hemprich and Ehrenberg, 1832; *Lupulella adusta* (Sundevall, 1847); and *Lupulella mesomelas* (Schreber, 1775)) sexual dimorphism varies among regions and is even less pronounced than in other canids (Van Valkenburgh and Wayne 1994). Up to date, very few studies have focused on cranial variability of golden jackal (*Canis aureus* Linnaeus, 1758) in Europe (Kryštufek and Tvrtković 1990, Stoyanov 2012, Markov et al. 2017, Rezić et al. 2017, Krendl et al. 2018), despite its expanding distribution in recent decades.

Golden jackal is one of the most widely distributed canid species and is found in many areas of Europe and Asia (Jhala and Moehlman 2004, Arnold et al. 2012, Hoffmann et al. 2018, Moehlman and Hayssen 2018, Spassov and Acosta-Pankov 2019). Since 1980s, jackals have increased in their distribution and abundance in what is arguably the most dramatic recent expansion among native predators on the continent (Jhala and Moehlman 2004, Šálek et al. 2014, Koepfli et al. 2015, Trouwborst et al. 2015). The jackal expansion in the last two decades has been rapid and still ongoing. The jackals reached Switzerland, Lichtenstein, Germany, Denmark, Poland, France, Netherlands, Baltic states, Belarus, and, in 2019, also Finland (Pyšková et al. 2016, Krofel et al. 2017, Potočnik et al. 2019). The ongoing expansion of the species in Europe has caused concerns regarding possible negative effects its presence could exert, due to excessive predation of other wildlife species or livestock, and the transmission of pathogens (Rutkowski et al. 2015, Ćirović et al. 2016). In addition, there are several uncertainties regarding jackal management and policies, often in association with the unknown origins of jackal populations (Trouwborst et al. 2015). Jackal expansion in the last decades has triggered research interest in Europe. Many aspects of golden jackal's ecology, diet, population density, genetics, legal implications of range expansion, and management have been studied in the last two decades in Europe (see full review in Potočnik et al. 2019). Bulgarian territory is considered the core area of golden jackal distribution in Europe with the highest population density (Stoyanov 2013, Spassov and Acosta-Pankov 2019), but morphometric studies, including skulls from Bulgaria, were very scarce and local so far (e.g. Markov et al. 2017, Krendl et al. 2018). Despite numerous studies of sexual dimorphism in carnivore skull size, only few studies have attempted to study dimorphism in overall cranial shape (Schutz et al. 2009, Rezić et al. 2017).

The present study is the first comprehensive analysis of golden jackal skull morphometry in Bulgaria, trying to clarify shape and size related cranial variability and differentiation. Extensive morphometric data of jackal skulls were analysed by applying recently developed statistical tools to answer the following questions: (i) is there a geographic variation in skull size and shape among golden jackal population in Bulgaria, (ii) are there age-related cranial differences, and (iii) how pronounced is the sexual dimorphism in skull shape and size? Although modern genetic methods have been applied recently in phylogeny and taxonomy, understanding patterns in cranial variability of golden jackal still provides very valuable insights on population structure. Furthermore, it is not only crucial for understanding the phylogeny, but also for management and conservation. Moreover, integration of genetic techniques and morphometrics represent a valuable tool in the resolution of taxonomic uncertainty.

Material and Methods

A total of 176 skulls of golden jackal from Bulgaria were analysed. The sample comprised of 84 specimens, collected between 1998 and 2007 from 20 different sites all over the country, but most of them coming from three main regions with highest jackal's population density: Yambol,

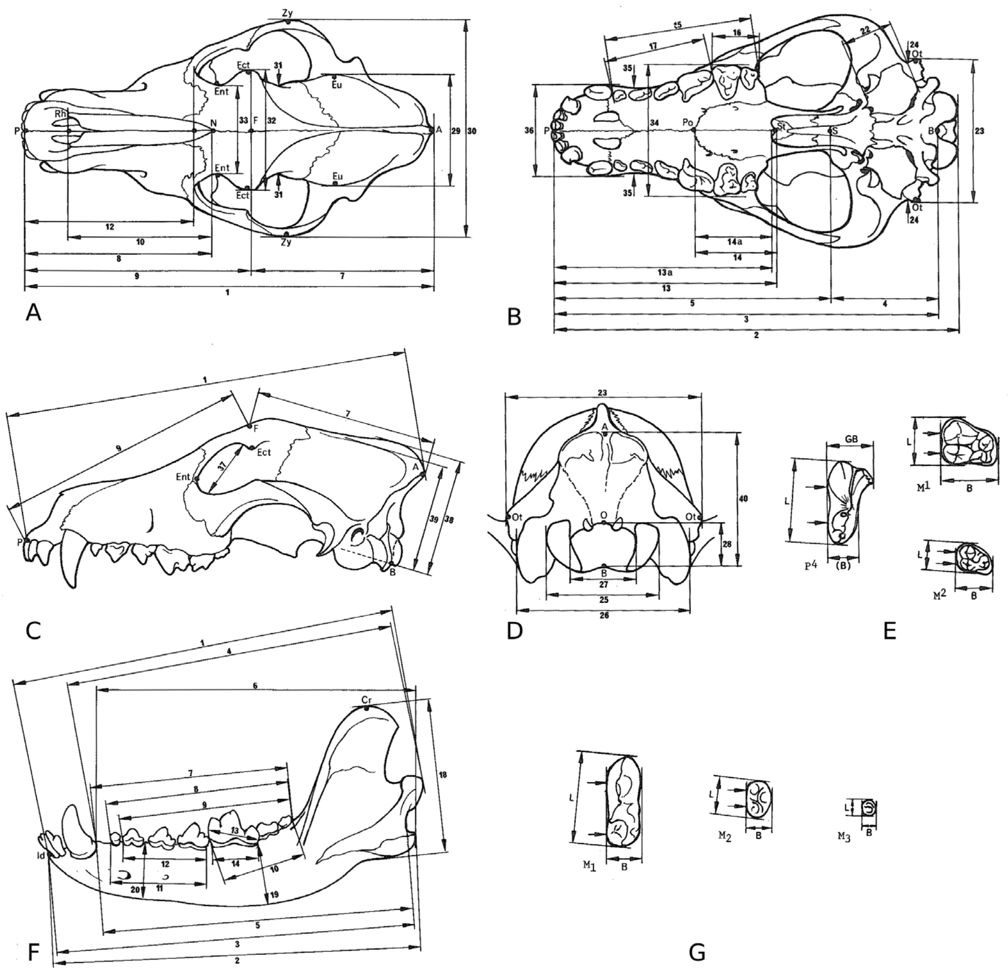

Fig. 1. Skull measurements employed in the analyses (following von den Driesch 1976).

Note: See measurements description in Table 1: A. *Canis* cranium, dorsal view; B. *Canis* cranium, basal view; C. *Canis* cranium, left side view; D. *Canis* cranium, nuchal view; E. *Canis* maxillary teeth (P^4, M^1 and M^2), length (L) and breadth (B); F. *Canis* mandible, left side, lateral view; G. *Canis* mandibular teeth (M_1–M_3), length (L) and breadth (B).

Veliko Tarnovo and Burgas. It also included 57 skulls, collected by Stoyan Vassilev in the end of 1980s, 20 specimens from the scientific collection of the National Museum of Natural History dating back to the last century, and 15 skulls, collected between 2008 and 2012, measured at a national trophy exhibition in 2012. The skulls with unknown sex were excluded from the analyses. The age of jackals was determined in consideration of upper incisive teeth wear (Lombaard 1971) and for some individuals also by counting the annual cementum layers in canines (Klevezal and Kleinenberg 1967). Both methods are reliable enough for the purposes of the study and provide accurate results, with precision up to one year for the first one (Harris et al. 1992, Rajchev 2002). The skulls were assigned to three age groups: juveniles, subadults and adults. Juveniles were defined as individuals with fully developed second dentition but less than 10 months of age, subadults as individuals older than 11 months, when they reach sexual maturity, but less than two years of age, and adults as two years old and over. On each specimen 70 measurements were taken by digital sliding calliper, i.e. 47 cranial and 23 from mandibles (see Fig. 1 and Table 1), following von den Driesch (1976).

Table 1. Description of *Canis* skull measurements (following von den Driesch 1976).

No	Measurements description	Abbreviation
	Cranium	
1	Total length: Akrokranion – Basion	Tl
1a	Greatest skull length: Akrokranion – front border of the Incisivi Anteriori	Maxl
2	Condylobasal length: aboral border of the occipital condiles – Prosthion	Cbl
3	Basal length: Basion – Prosthion	Bl
4	Basicranial axis: Basion – Synsphenion	Bca
5	Basifacial axis: Synsphenion – Prosthion	Bfa
6	Neurocranium length: Basion – Nasion	Ncl
7	Upper neurocranium length: Acrocranion – Frontal midpoint	Uncl
8	Viscerocranium length: Nasion – Prosthion	Vcl
9	Facial length: Frontal midpoint – Prosthion	Fl
10	Greatest length of the nasals: Nasion – Rhinion	Nasl
11	Length of braincase	Brcl
12	Snout length: oral border of the orbits – Prosthion	Snl
13	Medial palatal length: Staphilion – Prosthion	Mpl
13a	Palatal length: median point joining deepest intersection Choanae – Prosthion	Pl
14	Length of the horizontal part of the palatine: Staphylion – Palatinoorale	Mplh
14a	Length of the horizontal part of the palatine corresponding to 13a	Plh
15	Length of the cheektooth row	Lp1m2
15a	Length from oral border of C^1 to aboral border of M^2	Lc1m2
16	Length of the molar row	Molr
17	Length of the premolar row	Prmr
18	Length of the carnassial, measured at the cingulum	Lp4
18a	Greatest breadth of the carnassial	Bp4
19	Length of the carnassial alveolus	Lp4a

No	Measurements description	Abbreviation
20	Length of M^1, measured at the cingulum	Lm1
20a	Breadth of M^1, measured at the cingulum	Bm1
21	Length of M^2, measured at the cingulum	Lm2
21a	Breadth of M^2, measured at the cingulum	Bm2
22	Greatest diameter of the auditory bulla	Bull
23	Greatest mastoid breadth: Otion – Otion	Mst
24	Breadth dorsal to the external auditory meatus	Mstau
25	Greatest breadth of the occipital condyles	Occb
26	Greatest breadth of the bases of paraoccipital processes	Poprb
27	Greatest breadth of the foramen magnum	Fmagb
28	Height of the foramen magnum: Basion – Opisthion	Fmagh
29	Greatest neurocranium breadth: Euryon – Euryon	Skb
30	Zygomatic breadth: Zygion – Zygion	Zyg
31	Least breadth of skull: breadth at the postorbital constriction	Pob
32	Frontal breadth: Ectorbitale – Ectorbitle	Fb
33	Least breadth between the orbits: Entorbitle – Entorbitale	Iob
34	Greatest palatal breadth: measured across the outer borders of the alveoli	Palb
35	Least palatal breadth: measured behind the canines	Lpalb
36	Breadth at the canine alveoli	Rb
37	Greatest inner height of the orbit	Orb
38	Skull height	Skh
39	Skull height without the sagital crest	Skhs
40	Height of the occipital triangle: Akrokranion – Basion	Otrh
	Mandible	
1	Total length: from condyle process – Infradentale	Mand
2	Length: the angular process – Infradentale	Mlapid
3	Length: the indentation between condyle process and angular process – Infradentale	Mlapcpid
4	Length: the condyle process – aboral border of the canine alveolus	Mlcpca
5	Length: the indentation between the condyle process and the angular process – aboral border of the canine alveolus	Mlapcp-ca
6	Length: the angular process – aboral border of the canine alveolus	Mlapca
7	Length: the aboral border of the alveolus of M_3 – aboral border of the canine alveolus	Mlcam3
8	Length of the cheektooth row, M_3–P_1, measured along the alveoli	Mlp1m3
9	Length of the cheektooth row, M_3–P_2, measured along the alveoli	Mlp2m3
10	Length of the molar row, M_1–M_3, measured along the alveoli	Mmolr
11	Length of the premolar row, P_1–P_4, measured at the cingulum	Mprmr
12	Length of the premolar row, P_2–P_4, measured at the cingulum	Mlp2p4
13	Length of the carnassial, measured at the cingulum	Mlm1
13a	Breadth of the carnassial, measured at the cingulum	Mbm1
14	Length of the carnassial alveolus	Mlm1a
15	Length of M_2, measured at the cingulum	Mlm2

No	Measurements description	Abbreviation
15a	Breadth of M_2, measured at the cingulum	Mbm2
16	Length of M_3, measured at the cingulum	Mlm3
16a	Breadth of M_3, measured at the cingulum	Mbm3
17	Greatest thickness of the body of jaw below M_1	Mjaw
18	Height of the vertical ramus: basal point of the angular process – Coronion	Manh
19	Height of the mandible behind M_1, measured on the lingual side	Mhm1
20	Height of the mandible between P_2 and P_3, measured on the lingual side	Mhp2

Statistical methods

All measurements were tested for normality by QQ plots and Shapiro-Wilk test. Differences in size of skull between age groups were tested by one-way analysis of variance (ANOVA) and visualized by applying Tukey's Honestly Significant Difference test (Tukey's HSD). Statistical significance of the difference in means between males and females for each cranial measurement was examined by using Student's t-test. Multivariate analyses were employed in order to explore the most significant variation in size and shape of skulls. Shape in general tends to provide more reliable information than size on the morphology of organisms (Jolicoeur and Mosimann 1960). Size is often considered as a nuisance because it is strongly dependent on ecological factors (McCoy et al. 2006), but separation of size and shape in multivariate studies of morphological data is problematic (Claude 2008).

This problem was addressed by using principal component analysis (PCA). The first principal component of PCA is usually considered as a general size axis, while the remaining principal components represent the shape space. However, it also includes size related shape information (Jolicoeur and Mosimann 1960) and has been identified by Jolicoeur (1963) heuristically as a multivariate allometric size axis. The mixture of size and size related shape information in the first component makes the interpretation of the other components of a PCA rather difficult. Baur and Leuenberger (2011) have developed new methods allowing interpretation of principal components in terms of ratios and clear separation of size and shape. The authors defined an isometric size axis, called 'isosize', as the geometric mean of the original measurements and thus comprising only differences in scaling. For the exact definition of 'isosize', see Baur and Leuenberger (2011). Allometry free shape variables could be obtained by projecting the measurements orthogonal to isosize. A PCA calculated on the covariance matrix of these shape variables then accounts solely for differences in proportions. Baur and Leuenberger (2011) suggested to plot the isosize against each significant shape component in order to assess the amount of allometry in the data.

Hence, for clear separation of shape and size, the PCA was applied on the standardized (dividing each measurement by geometric mean) and log-transformed ratios of the original measurements (Claude 2008, Baur and Leuenberger 2011). To examine how well the skulls of males and females are separated, the data were subjected to a linear discriminant analysis (LDA). The performance of the LDA was assessed by means of cross validation (Rencher 2002), where one specimen is omitted from the analysis and classified according to the discriminant function found for the remaining specimens in the data set.

Geometric interpretation of PCA and LDA was made by using graphical tools developed by Baur and Leuenberger (2011). The 'PCA ratio spectrum' was applied for the interpretation of principal components in shape space, and the 'LDA ratio extractor' was used for finding the best ratios that separate the skulls of males and females. The amount of allometry in the data was assessed by the 'allometry ratio spectrum'. For detailed mathematical description and statistical framework of the applied methods see Claude (2008) and Baur and Leuenberger (2011).

All statistical and graphical analyses were performed with R, version 3.6.1 (R Core Team 2019). Slightly modified versions of the R-scripts provided by Baur and Leuenberger (2011) and Claude (2008) were employed for calculations. PCA and LDA were performed by using package MASS (Venables and Ripley 2002).

Ethics Statement

The skull samples used in this study were obtained from individuals that died in vehicle collisions, due to natural causes or as a result of legal hunting. Specimens from the National Museum of Natural History and private collections were measured, as well. No animal was killed for the purpose of this study.

Results

Shapiro-Wilk tests and QQ plots showed that all measurements did not deviate significantly from normal distribution. It allowed applying t-test and one-way ANOVA. However, for most of the following statistical methods the assumption of normally distributed data is not strongly suggested. The results from ANOVA and Tuckey's HSD test showed that in the most skull traits juveniles, i.e. jackals between 7–10 months of age, differed from the older animals (Fig. 2). Only in two measurements, zygomatic breadth and least breadth between the orbits, there were significant differences between subadult and adult specimens. Hence, summary statistics of the skull measurements were calculated for joint group of subadult and adult jackals. Sexual dimorphism in skull size was examined by t-test (Table 2). Almost in all skull measurements differences in means between males and females were statistically significant. However, there is a large overlap in all skull traits, therefore both sexes could be hardly differentiated only by skull size (Fig. 3). The very high level of statistical significance, as demonstrated by t-test, is due to a large sample size.

The PCA was applied on specimens from all age groups. The first principal component in shape space accounted for 17.98 % of the variance. Projecting the data along isosize and first principal component in shape space did not reveal any specific patterns in distribution or clustering of the individuals (Fig. 4). Only 12 specimens of the whole sample belonged to juveniles, i.e. below 11 months of age. Although with some overlap, their cluster was well separated from subadult and adult animals as it is shown by the ellipses enclosing 95 % of the confidence interval for each age group (Fig. 4A). Most of subadults are enclosed by adults on a plot. The sex dimorphism in shape and size of the skull is not pronounced and there is a large overlap between males and fe-

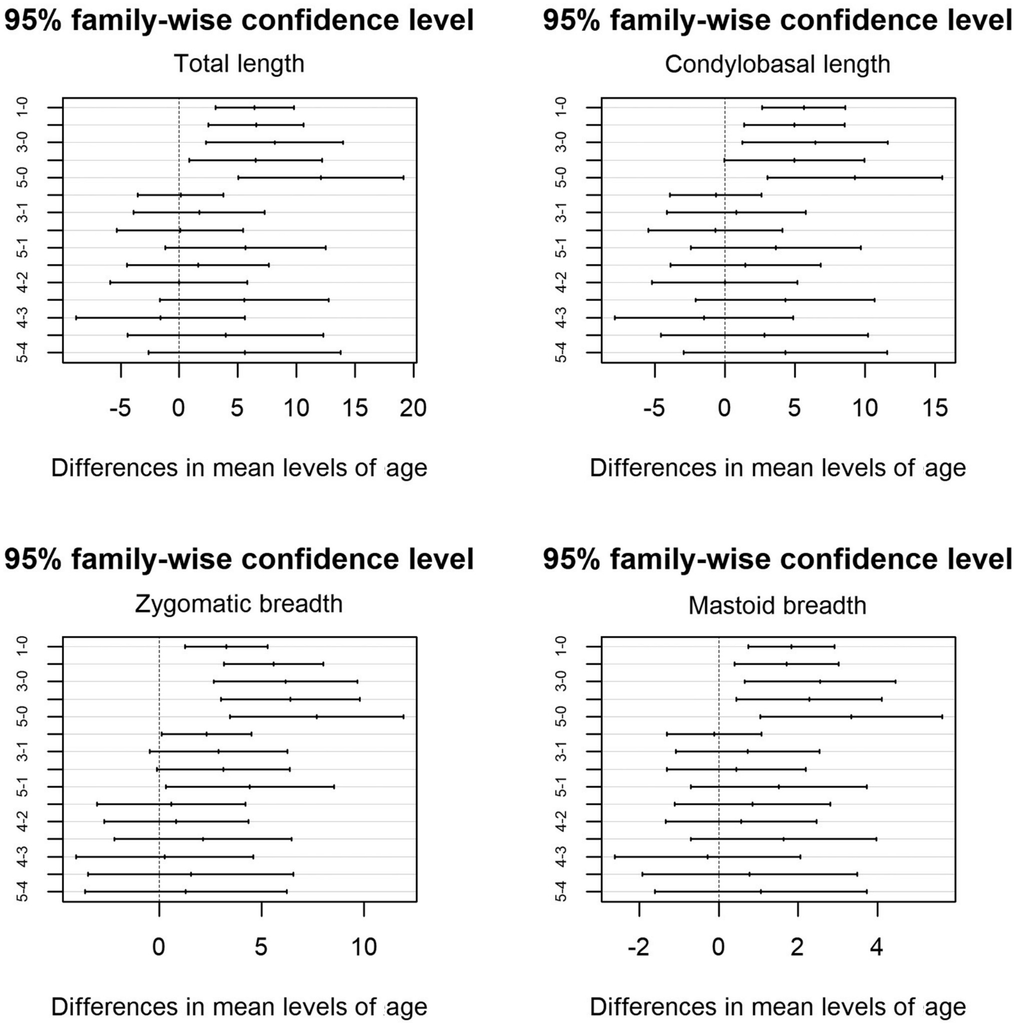

Fig. 2. Differences by age in some basic cranial measurements. Results from Tukey's Honestly Significant Difference test.

Table 2. Descriptive statistics of basic skull measurements and statistical significance of the differences examined by Student's t-test.

No	Abbreviation	Males (*n*=83)				Females (*n*=51)				*p*
		min	max	$\bar{x}$	*s*	min	max	$\bar{x}$	*s*	
					Cranium					
1a	Maxl	154.1	183.4	168.8	6.2	158.0	175.5	164.6	4.4	0.0000***
1	Tl	152.0	181.0	166.5	6.3	155.9	175.4	162.3	4.4	0.0000***

No	Abbreviation	Males (n=83)				Females (n=51)				p
		min	max	$\bar{x}$	s	min	max	$\bar{x}$	s	
2	Cbl	144.8	168.7	158.1	5.3	147.8	162.9	154.0	3.7	0.0000***
10	Nasl	52.9	68.0	59.7	3.7	50.9	64.8	58.0	3.2	0.0058**
18	P^4	15.6	18.6	17.2	0.6	15.0	18.0	16.8	0.6	0.0004***
22	Bull	22.0	28.6	25.1	1.4	21.9	27.1	24.5	1.2	0.0206*
23	Mst	51.2	61.4	56.5	2.0	52.1	60.4	55.2	1.5	0.0001***
29	Skb	49.5	56.0	52.5	1.4	47.8	54.5	51.5	1.4	0.0000***
30	Zyg	82.0	97.2	89.3	3.6	77.6	94.1	86.6	3.7	0.0001***
31	Pob	23.9	34.2	28.3	2.1	22.2	33.5	27.9	2.0	0.2609
32	Fb	36.1	51.0	42.2	3.0	35.9	46.6	41.5	2.9	0.1685
33	Iob	21.4	31.5	26.3	1.8	22.6	30.7	25.4	1.7	0.0066**
34	Palb	49.7	59.0	53.9	1.8	50.0	57.0	52.9	1.6	0.0004***
36	Rb	26.0	32.3	29.7	1.3	26.8	30.7	28.7	1.0	0.0000***
38	Skh	44.7	53.0	48.5	1.8	43.4	55.1	47.8	1.9	0.0405*
					Mandible					
1	Mand	109.5	133.4	121.9	4.5	113.0	127.6	118.9	3.3	0.0000***
8	P_1–M_3	59.1	69.1	65.6	2.0	61.1	68.0	64.6	1.8	0.0057**
13	M_1	17.6	20.9	19.2	0.7	17.6	20.0	18.8	0.6	0.0007***
18	Manh	41.8	53.4	48.7	2.5	43.1	52.9	46.8	2.2	0.0000***

Note: Level of statistical significance: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. $\bar{x}$ – mean, s – standard deviation.

males. Differences are mainly in size but not in shape. Older males have bigger skulls, but this does not depend on their exact age. Most of subadults and females could not be separated by size and shape of the skull. The differences in cranial size and shape did not depend on the geographic region as well (Fig. 4B).

The first two principal components in shape space accounted for 28.66 % of the variance (Fig. 5). Projecting the data along first and second principal components reveals only differences in skull shape. The group of juvenile jackals could be distinguished from subadults and adults only along first principal component, but with a large overlap between clusters. There are no differences in skull shape between males and females (Fig. 5A), and between jackals from different regions, as well (Fig. 5B).

The ‘PCA ratio spectrum‘ allows the interpretation of principal components in shape space (Fig. 6). It is statistically stable because of the narrow confidence intervals shown on the graph. Considering factor loadings, ratios between least breadth at the postorbital constriction (Pob), least breadth between the orbits (Iob), greatest diameter of the auditory bulla (Bull), frontal breadth (Fb), length of the nasals (Nasl), height of the mandible (Manh), and some dental measurements, such as length and breadth of the upper and lower molars, explained a large proportion of the variance of the first and

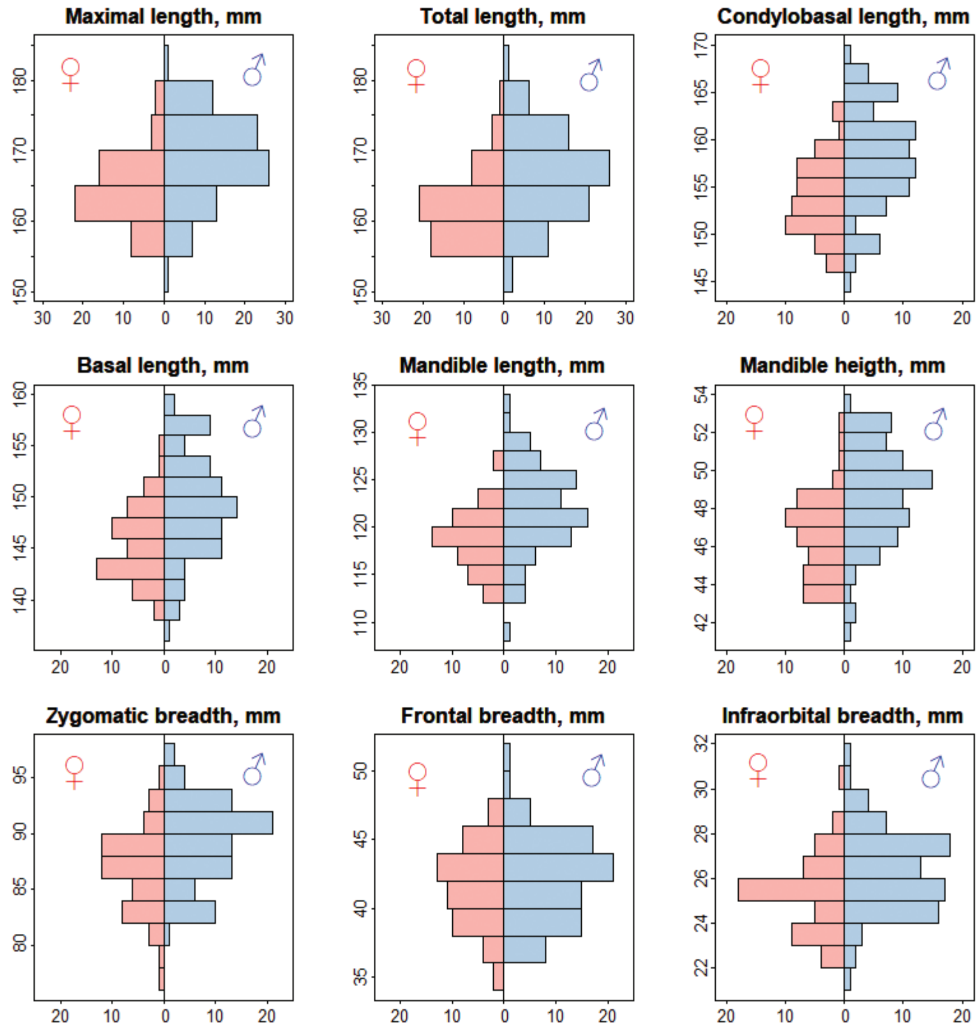

Fig. 3. Histograms showing differences in basic cranial measurements between adult males and females.

Note: Bars represent number of animals falling in each interval on the measurement scale.

second shape principal components. The same ratios, however, showed the most distinctive allometric behaviour as could be seen from the 'allometry ratio spectrum' (Fig. 7). Presence of allometry could be assessed as well, while projecting the first shape principal component orthogonal to the isometric size (Fig. 4A). Judging from the graph, there is only a very moderate correlation between shape and size. Hence, allometric variation was of marginal importance concerning my data set.

Following the distribution patterns from PCA, only adult and subadult jackals (83

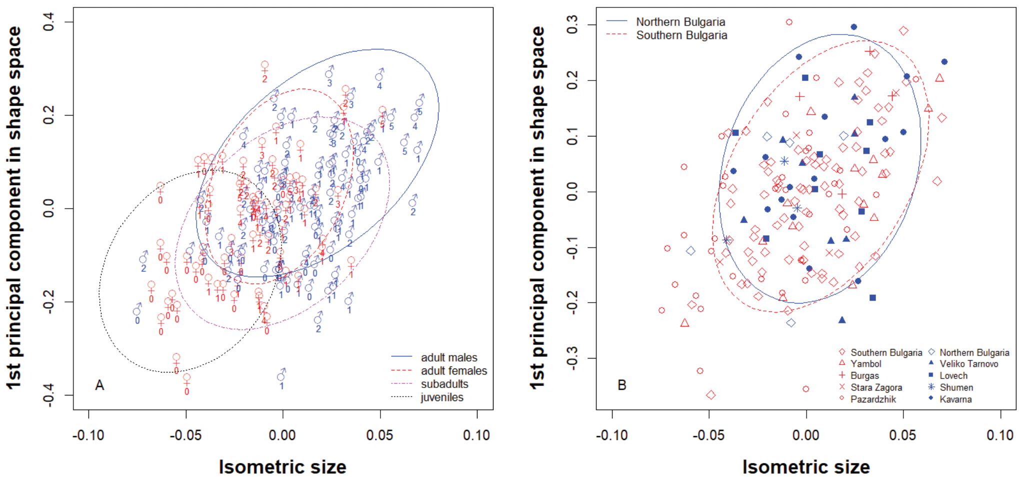

Fig. 4. Principal component analysis. Projection of individuals along isometric size and first principal component in shape space.

Note: Ellipses enclose 95 % confidence interval for each group. A. Sex and age of each individual are shown. Numbers represent age in years. B. Collection sites of individuals are shown.

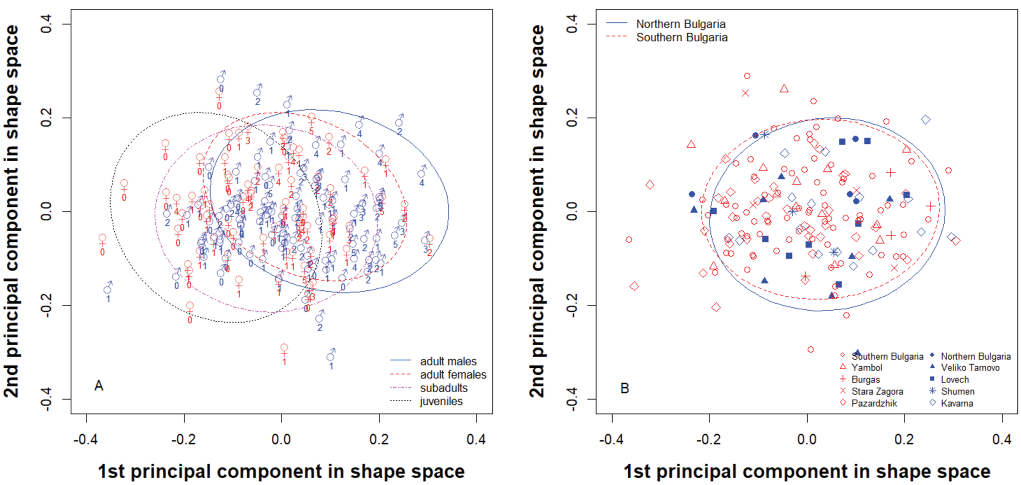

Fig. 5. Principal component analysis. Projection of individuals along first two principal components in shape space.

Note: Ellipses enclose 95 % confidence interval for each group. A. Sex and age of each individual are shown. Numbers represent age in years. B. Collection sites of individuals are shown.

males and 51 females) were subjected to linear discriminant analysis in order to separate males from females. The LDA indicated the presence of differences between sexes (Wilks λ=0.372, F=1.52, df=1, 132, p=0.047, D^2=2.66) and their possible separation by shape and size of skull (Fig. 8). However, the LDA per-

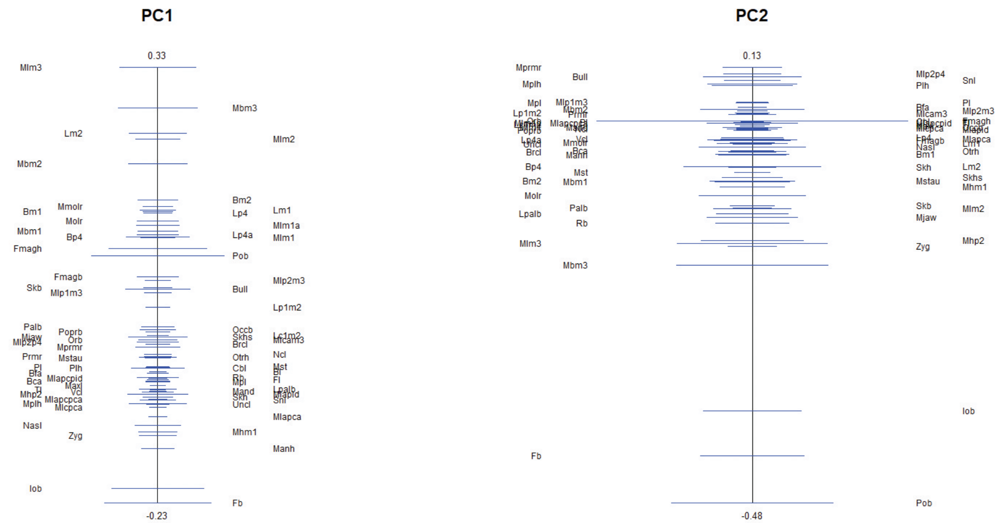

Fig. 6. PCA ratio spectrum for the first and second principal component in shape space.
Note: Bars represent 68 % confidence intervals based on 500 bootstrap replicates.

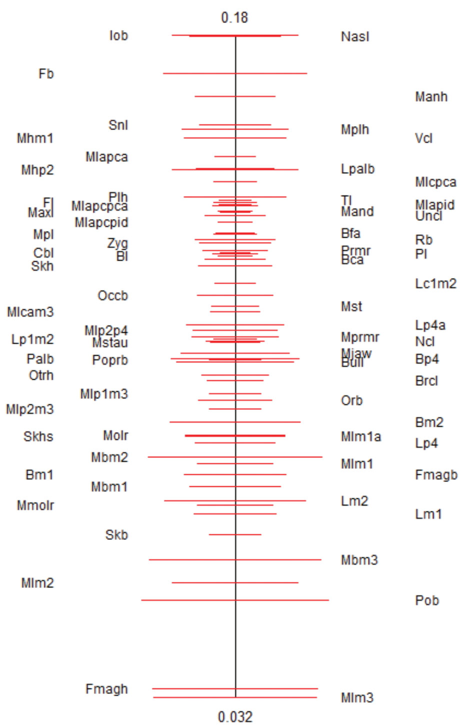

Fig. 7. Allometry ratio spectrum.
Note: Bars represent 68 % confidence intervals based on 500 bootstrap replicates.

formance estimated by cross validation was problematic. It showed that 49 % of females (25 skulls) and 35 % of males (29 skulls) were misclassified, meaning that almost 40 % of all specimens (regardless the sex) were not assigned correctly to the group they belong. Hence, the use of discriminant function for classification of jackal skulls with unknown sex is more than doubtful.

For practical reasons, a few characters that would allow quick and easy identification of most specimens might sometimes be useful, for instance in field work. One or two ratios would be preferable, as these are easily calculated and differences in proportions can sometimes even be estimated by eye (Reichenbach et al. 2012). Hence, the LDA ratio extractor was applied (Baur and Leuenberger 2011) to find the best ratios that could easily separate the skulls of male and female jackals. However, even these ratios could not clearly separate males from females (Fig. 9).

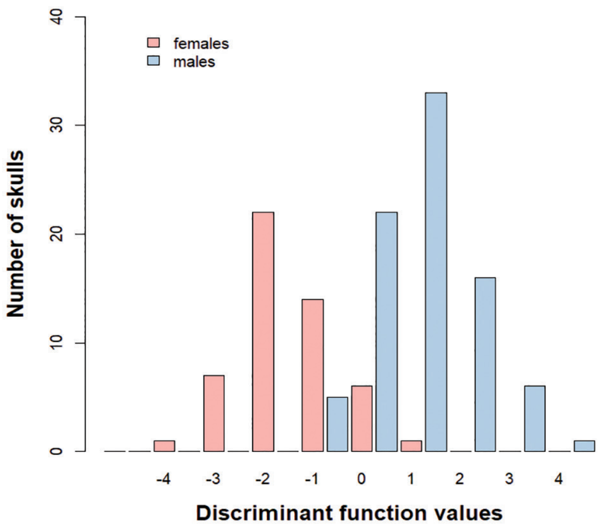

Fig. 8. Distribution of specimens along the discriminant function axis.

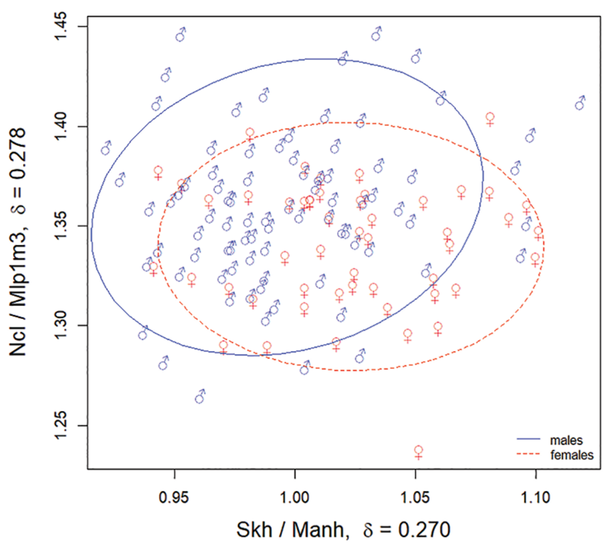

Fig. 9. Projection of individuals along best separating ratios revealed by LDA ratio extractor.

Note: The measure δ indicates how well shape discriminates in relation to size. A value of δ close to unity means that separation is mainly due to size, whereas for a value close to zero mostly shape is important. Ellipses enclose 95 % confidence interval for each sex.

Discussion

The results suggest that the differences in shape and size of the jackal skulls, as far as they exist, are age-related. However, there is no clear differentiation between subadult and adult jackals. Only juveniles, i.e. younger than 11 months, could be separated by shape and size of skull. Univariate analyses showed that in all skull traits juveniles differed from the older jackals (Fig. 2). In most canine species skull growth slows, and even stops after reproductive maturation (e.g. Larter et al. 2012). In many species, however, growth continues throughout life, so that the oldest individuals in the population are generally the largest. Golden jackals reach sexual maturity at the age of 10–11 months (Taryannikov 1976), but they rarely reproduce at this age. In Tanzania 70 % of known surviving pups were observed helping with the next year's litter and thus didn't rear their own offspring (Moehlman 1987). According to the same author, retaining helpers potentially increases the parents' reproductive success, that is, it increases the parents' chances of passing on their genes to future generations. My results showed that the jackals in reproductive age reach full growth of the skull, but some cranial dimensions continue to increase in size. Obviously, the skull breadth grows up even after the jackals reach sexual maturity. Still, most of the traits did not show any significant differences between subadults and adult jackals. Subadults differ from adults only by zygomatic breadth and least breadth between the orbits. All inferences about skull growth, based on such studies, however, should be treated cautiously. The data did not allow following ontogenetic development of jackal skulls because we compare different individuals. Usually more viable and healthy individuals reach senescence, while weaker and smaller animals die earlier and do not reach more than 2–3 years of age (Stoyanov 2013). Thus, post mortal comparison of skulls leads to biased data. On the other side, all such studies rely on samples collected post mortal and such bias could not be overcome.

Dividing the whole sample to three age groups is based on population demography of golden jackal and differences in the reproductive value of subadult and adult individuals. However, even multivariate analyses did not clearly separate subadults from adults. There was large overlap between both groups on the plots (figs 4A and 5A). The results suggest that there is no clear differentiation among Bulgarian jackals in skull size and shape, excluding juveniles. Although the sample size included in the analyses was relatively large, the projected data form a homogenous cluster but with large individual variability. Furthermore, there could be hardly seen any differences in skull shape between jackals from different regions of the country (figs 4B and 5B). The amount of geographical variation in Bulgarian population is comparable with sex and age differences. The similarities in skull morphology and morphometrics of the jackals from Bulgaria, Serbia, Hungary, Croatia and Austria were confirmed also by other studies (Markov et al. 2017, Rezić et al. 2017, Krendl et al. 2018). The results are consistent with recent genetic research as well. Studies focused on jackals in Bulgaria, Serbia, Croatia and Italy suggested a low level of genetic diversity and weakly pronounced genetic structure, with only the coastal population from Dalmatia clearly differentiated from other Balkan samples (Zachos et al. 2009, Fabbri et al. 2014, Rutkowski et al. 2015).

The sexual dimorphism in skull size was not pronounced, despite statistical significance of the differences in mean values of all measurements between males and females. The same results were confirmed by other studies, as well (Markov et al. 2017, Krendl et al. 2018). The high level of statistical significance, as demonstrated by t-test, was due to the large sample size, and could be misleading. However, there is large overlap between males and females in all skull traits, and they could be hardly differentiated only by skull size (Fig. 3). Furthermore, principal component analysis did not reveal any differentiation in skull size and shape between males and females.

The LDA performance in separating jackal skulls by sex was problematic. About 40 % of all specimens were not assigned correctly to the group they belong. Hence, the use of discriminant function for classification of skulls with unknown sex is more than doubtful. Even the best ratios, revealed by the LDA ratio extractor, could not clearly separate males from females. Differences between sexes on these two ratios, as far as they exist, are primarily related to the shape of the skull (δ<0.5).

Sexual dimorphism in Canidae, when present at all, is usually minimal, with males being slightly larger than females (Sillero-Zubiri 2009), although studies on wolves from the Balkans show significant sexual dimorphism in adult individuals (Trbojević and Ćirović 2016). Such sexual dimorphism of golden jackal skulls, with males a little bit larger than females, could be explained with monogamous reproductive system of jackals, and the presence of male parental care (Moore 1981, Moehlman 1987). Golden jackals form pair-bonds that are characterized by friendly behaviour and last the 6 to 8 years of their usual lifespans, there is little sexual dimorphism, either physically or behaviourally, and they share equally in most activities, such as marking and defending their territory, foraging and resting (Moehlman 1987). Such low degree of sexual dimorphism in Canidae was confirmed by other studies, as well (Jolicoeur 1959, Hell et al. 1989, Simonsen et al. 2003, Schutz et al. 2009).

Conclusion

Skulls of golden jackal in Bulgaria show considerable individual variability, but weak intrapopulation differentiation. The differences in shape and size of the jackal skulls, as far as they exist, are age-related, but only juvenile specimens younger than 11 months could be easily distinguished. Subadult and adult jackals largely overlap in skull size and shape. Sexual dimorphism in jackal skull is weakly pronounced, with older males having slightly larger skull than females. My results are consistent with recent genetic and morphological studies and give new insights on patterns in cranial variability and population structure of golden jackal in Bulgaria.

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References

Andersson M., Norberg R.Å. 1981. Evolution of reversed sexual size dimorphism and role partitioning among predatory birds, with a size scaling of flight performance. Biological Journal of the Linnean Society 15(2): 105–130. DOI: 10.1111/j.1095-8312.1981.tb00752.x

Arnold J., Humer A., Heltai M., Murariu D., Spassov N., Hackländer K. 2012. Current status and distribution of golden jackals *Canis aureus* in Europe. Mammal Review 42: 1–11. DOI: 10.1111/j.1365-2907.2011.00185.x

Baur H., Leuenberger C. 2011. Analysis of ratios in multivariate morphometry. Systematic Biology 60(6): 813–825. DOI: 10.1093/sysbio/syr061

Ćirović D., Penezić A., Krofel M. 2016. Jackals as cleaners: Ecosystem services provided by a mesocarnivore in human-dominated landscapes. Biological Conservation 199: 51-55. DOI: https://doi.org/10.1016/j.biocon.2016.04.027

Claude J. 2008. Morphometrics with R. Springer, New York. 316 p.

Fabbri E., Caniglia R., Galov A., Arbanasić H., Lapini L., Bošković I., Florijančić T., Vlasseva A., Ahmed A., Mirchev R.L., Randi E. 2014. Genetic structure and expansion of golden jackals (*Canis aureus*) in the north-western distribution range (Croatia and eastern Italian Alps). Conservation Genetics 15: 187–199. DOI: 10.1007/s10592-013-0530-7

Gittleman J.L., Valkenburgh B.V. 1997. Sexual dimorphism in the canines and skulls of carnivores: effects of size, phylogency, and behavioural ecology. Journal of Zoology 242(1): 97–117. DOI: 10.1111/j.1469-7998.1997.tb02932.x

Harris S., Cresswell W.J., Cheeseman C.L. 1992. Age determination of badgers (*Me-*

les meles) from tooth wear: the need for a pragmatic approach. Journal of Zoology 228(4): 679–684. DOI: 10.1111/j.1469-7998.1992.tb04467.x

Hell P., Paule L., Sevcenko L., Danko S., Panigaj L., Vitaz V. 1989. Craniometrical investigation of the red fox from the Slovak Carpathians and adjacent lowlands. Folia Zoologica 38(2): 139–155.

Hoffmann M., Arnold J., Duckworth J.W., Jhala Y.W., Kamler J.F., Krofel M. 2018. *Canis aureus*. The IUCN Red List of Threatened Species. 2018: e. T118264161A146194820. Available at: http://dx.doi.org/10.2305/IUCN.UK.2018-2. RLTS.T118264161A46194820.en

Jhala Y.V., Moehlman P.D. 2004. Golden jackal *Canis aureus*. In: Sillero-Zubiri C, Hoffmann M, Macdonald D (Eds) Canids: Foxes, Wolves, Jackals and Dogs Status Survey and Conservation Action Plan. IUCN/SSC Canid Specialist Group. Gland, Switzerland and Cambridge, UK: 156–161.

Jolicoeur P. 1959. Multivariate geographical variation in the Wolf *Canis lupus* L. Evolution 13(3): 283–299. DOI: 10.1111/j.1558-5646.1959.tb03016.x

Jolicoeur P. 1963. 193. Note: The multivariate generalization of the allometry equation. Biometrics 19(3): 497–499. DOI: 10.2307/2527939

Jolicoeur P., Mosimann J.E. 1960. Size and shape variation in the painted turtle. A principal component analysis. Growth 24: 339–354.

Klevezal G., Kleinenberg S. 1967. Age determination of mammals from annual layers in teeth and bones. USSR Academy of Sciences, Moscow. 143 p.

Koepfli K.P., Pollinger J., Godinho R., Robinson J., Lea A., Hendricks S., Schweizer R.M., Thalmann O., Silva P., Fan Z., Yurchenko A.A., Dobrynin P., Makunin A., Cahill J.A., Shapiro B., Álvares F., Brito J.C., Geffen E., Leonard J.A., Helgen K.M., Johnson W.E., O'Brien S.J., Van Valkenburgh B., Wayne R.K. 2015. Genome-wide evidence reveals that African and Eurasian golden jackals are distinct species. Current Biology 25: 2158–2165. DOI: 10.1016/j.cub.2015.06.060

Krendl L., Hatlauf J., Griesberger P., Heltai M., Szabó L., Stoyanov S., Markov G., Hackländer K. 2018. Craniometrical distinction: a comparison of Pannonian and Balkan golden jackal skulls. In: Giannatos G, Banea OC, Hatlauf J, Sillero-Zubiri C, Georgiadis C, Legakis A (Eds) Proceedings of the 2nd International Jackal Symposium. Hellenic Zoological Society, Marathon Bay, Attiki (Greece): 77–79.

Krofel M., Giannatos G., Cirovic D., Stoyanov S., Newsome T.M. 2017. Golden jackal expansion in Europe: A case of mesopredator release triggered by continent-wide wolf persecution? Hystrix 28(1): 9–15. DOI: 10.4404/hystrix-28.1-11819

Kryštufek B., Tvrtković N. 1990. Variability and identity of the jackals (*Canis aureus*) of Dalmatia. Annalen des Naturhistorischen Museums in Wien: 7–25.

Larter N.C., Nagy J.A., Bartareau T.M. 2012. Growth in skull length and width of the Arctic Wolf: comparison of models and ontogeny of sexual size dimorphism. Arctic 65(2): 207-213.

Lombaard D. 1971. Age determination and growth curves in the black-backed jackal. Annals of the Transvaal Museum (27): 135–169.

Markov G., Heltai M., Nikolov I., Penezić A., Lanszki J., Ćirović D. 2017. Phenetic similarity of European golden jackal (*Canis aureus moreoticus*) populations from southeastern Europe based on craniometric data. Biologia 72: 1355–1361. DOI: 10.1515/biolog-2017-0148

McCoy M.W., Bolker B.M., Osenberg C.W., Miner B.G., Vonesh J.R. 2006. Size correction: comparing morphological traits among populations and environments. Oecologia 148(4): 547–554.

Milenković M., Šipetić V.J., Blagojević J., Tatović S., Vujošević M. 2010. Skull variation in Dinaric-Balkan and Carpathian gray wolf populations revealed by geometric morphometric approaches. Journal of Mammalogy 91(2): 376–386. DOI: 10.1644/09-mamm-a-265.1

Moehlman P.D. 1987. Social organization in jackals. American Scientist 75(4): 366–375.

Moehlman P.D., Hayssen V. 2018. *Canis aureus* (Carnivore: Canidae). Mammalian Species 50: 14–25. DOI: 10.1093/mspecies/sey002

Moore W.J. 1981. The Mammalian Skull. Cambridge University Press, Cambridge. 384 p.

Moors P.J. 1980. Sexual dimorphism in the body size of mustelids (Carnivora): the roles of food habits and breeding systems. Oikos 34(2): 147–158. DOI: 10.2307/3544175

Petrov I., Nikolov H., Gerasimov S. 1992. Craniometrical sex determination of wild cat *Felis silvestris* in Bulgaria. Acta Theriologica 37(4): 381–396. DOI: 10.4098/AT.arch.92-39

Pleasants J.M. 1988. Reversed size dimorphism in raptors: evidence for how it evolved. Oikos 52(1): 129–135. DOI: 10.2307/3565992

R Core Team 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rajchev E. 2002. Diet, morphology and parasitological status of red fox (*Vulpes vulpes)*, golden jackal (*Canis aureus*), wild cat (*Felis silvestris*) and stone marten (*Martes foina*) in Central Balkan and Sredna gora Mountains. PhD thesis, Thracian University Stara Zagora, Bulgaria. 151 p.

Ralls K. 1977. Sexual dimorphism in mammals: Avian models and unanswered questions. The American Naturalist 111(981): 917–938.

Reichenbach F., Baur H., Neubert E. 2012. Sexual dimorphism in shells of *Cochlostoma septemspirale* (Caenogastropoda, Cyclophoroidea, Diplommatinidae, Cochlostomatinae). ZooKeys 208: 1–6. DOI: 10.3897/zookeys.208.2869

Rencher A. 2002. Methods of multivariate analysis. Wiley Series in Probability and Statistics, New York. 708 p. DOI: 10.1002/0471271357

Rezić A., Bošković I., Lubinu P., Piria M., Florijančić T., Scandura M., Šprem N. 2017. Dimorphism in the skull form of golden jackals (*Canis aureus* Linnaeus, 1758) in the Western Balkans: a geometric morphometric approach. Pakistan Journal of Zoology 49: 989–997. DOI: 10.17582/journal.pjz/2017.49.3.989.997

Rutkowski R., Krofel M., Giannatos G., Ćirović D., Mannil P., Volokh A.M., Lanszki J., Heltai M., Szabó L., Banea O.C., Yavruyan E., Hayrapetyan V., Kopaliani N., Miliou A., Tryfonopoulos G.A., Lymberakis P., Penezić A., Pakeltyte G., Suchecka E., Bogdanowicz W. 2015. A European concern? Genetic structure and expansion of golden jackals (*Canis aureus*) in Europe and the Caucasus. PLoS ONE 10: 1–22. DOI: 10.1371/journal.pone.0141236

Šálek M., Červinka J., Banea O.C., Krofel M., Ćirović D., Selanec I., Penezić A., Grill S., Riegert J. 2014. Population densities and habitat use of the golden jackal (*Canis aureus*) in farmlands across the Balkan Peninsula. European Journal of Wildlife Research 60: 193–200. DOI: 10.1007/s10344-013-0765-0

Schutz H., Polly P.D., Krieger J.D., Guralnick R.P. 2009. Differential sexual dimorphism: size and shape in the cranium and pelvis of grey foxes (*Urocyon*). Biological Journal of the Linnean Society 96(2): 339–353. DOI: 10.1111/j.1095-8312.2008.01132.x

Sillero-Zubiri C. 2009. Family Canidae (Dogs). In: Wilson DE, Mittermeier RE (Eds) Handbook of the Mammals of the World Carnivores. Lynx Edicions, Barcelona, Spain: 352–446.

Simonsen V., Pertoldi C., Madsen A.B., Loeschcke V. 2003. Genetic differentiation of foxes (*Vulpes vulpes*) analysed by means of craniometry and isozymes. Journal for nature conservation 11(2): 109–116. DOI: 10.1078/1617-1381-00038

Spassov N., Acosta-Pankov I. 2019. Dispersal history of the golden jackal (*Canis aureus moreoticus* Geoffroy, 1835) in Europe and possible causes of its recent population explosion. Biodiversity Data Journal 7: e34825. Available at: https://doi.org/10.3897/BDJ.7.e34825

Stirling I. 1975. Factors affecting the evolution of social behaviour in the Pinnipedia. Rapports et Proces-Verbaux des Reunions/Conseil International pour l'Exploration de la Mer 169: 205–212.

Stoyanov S. 2012. Craniometric differentiation of golden jackals (*Canis aureus* L., 1758) in Bulgaria. In: International symposium on hunting 'Modern aspects of sustainable management of game populations'. Zemun - Belgrade, Serbia: 39–47.

Stoyanov S. 2013. Population ecology studies on the golden jackal (*Canis aureus* Linnaeus, 1758) in Bulgaria. PhD thesis, University of Forestry Sofia, Bulgaria. 148 p.

Taryannikov V.I. 1976. Reproduction of the jackal (*Canis aureus aureus* L.) in Central Asia. Ekologiya 55(2): 107.

Trbojević I., Ćirović D. 2016. Sexual dimorphism and population differentiation of the wolf (*Canis lupus*) based on morphometry in the Central Balkans. North-Western Journal of Zoology 12: 349-355.

Trouwborst A., Krofel M., Linnell J.D.C. 2015. Legal implications of range expansions in a terrestrial carnivore: the case of the golden jackal (*Canis aureus*) in Europe. Biodiversity and Conservation 24: 2593–2610. DOI: 10.1007/s10531-015-0948-y

Van Valkenburgh B., Wayne R.K. 1994. Shape divergence associated with size convergence in sympatric East African jackals. Ecology 75(6): 1567–1581. DOI: 10.2307/1939618

Venables W.N., Ripley B.D. 2002. Modern Applied Statistics with S. Springer, New York. 498 p.

von den Driesch A. 1976. A guide to the measurement of animal bones from archaeological sites: as developed by the Institut für Palaeoanatomie, Domestikationsforschung und Geschichte der Tiermedizin of the University of Munich. Peabody Museum of Archaeology and Ethnology, Harvard University. 137 p.

Wiig Ø. 1986. Sexual dimorphism in the skull of minks *Mustela vison*, badgers *Meles meles* and otters *Lutra lutra*. Zoological Journal of the Linnean Society 87(2): 163–179. DOI: 10.1111/j.1096-3642.1986.tb01335.x

Wiig Ø., Andersen T. 1986. Sexual size dimorphism in Norwegian lynx. Acta Theriologica 31: 147–155.

Zachos F.E., Ćirović D., Kirschning J., Otto M., Hartl G.B., Petersen B., Honnen A.C. 2009. Genetic variability, differentiation, and founder effect in golden jackals (*Canis aureus*) from Serbia as revealed by mitochondrial DNA and nuclear microsatellite loci. Biochemical Genetics 47: 241–250. DOI: 10.1007/s10528-009-9221-y