Dimorphism in the Skull Form of Golden Jackals (Canis aureus Linnaeus, 1758) in the Western Balkans: A Geometric Morphometric Approach

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ABSTRACT

Geometric morphometrics analyses are now often used to examine morphological variation in carnivore skull. Differences in golden jackal skull shape and size due to sexual dimorphism were examined using two dimensional geometric morphometrics approach. A total of 21 landmarks described the dorsal side of the skull and lateral side of the mandible. Individual variation in skull and mandible morphology was statistically significant for the shape (p<0.01). Sexual dimorphism as main effect was highly significant for the dorsal skull shape and the mandible shape and size (p<0.01). The comparison of mandible mean shapes using discriminant function analysis and parametric test did not reveal significant differences between the sexes, while dorsal mean skull shape was statistically significant (p<0.05). The shape comparison between males and females using permutation tests with procrustes distance yielded with significant results for both skull and mandible shape. Further studies are required with increased sample size and number of landmarks so as to obtain a more accurate expression of form and better differentiation between the sexes.

INTRODUCTION

The difference in body size between the sexes is a common feature of many organisms, from vascular plants to invertebrates and vertebrates, including mammals (Hood, 2000). Sexual dimorphism is a source of intrapopulation variation that continues to be extensively studied in multiple taxa, not only for the role of sexual selection, but also for ecological processes that can amplify or reduce morphological and behavioural differences between the sexes (Spoljaric and Reimchen, 2008). Cranioometric characteristics and sexual dimorphism were described in carnivore species in Europe (Petrov et al., 1992; Gittleman and Van Valkenburgh, 1997; Eisenmann and Van der Geer, 1999; Christiansen, 2008; Schutz et al., 2009; Hartová-Nentvichová et al., 2010; Milenková et al., 2010). Despite numerous studies of sexual dimorphism in carnivore skull size, few studies have aimed to examine dimorphism in the overall cranial shape of golden jackals (Canis aureus Linnaeus, 1758). According to Van Valkenburgh and Wayne (1994), the extent of sexual dimorphism varies in jackal species among regions, and is not as pronounced as is typical of canids. A comparative biometrical evaluation (Eisenmann and Van der Geer, 1999) found that jackals exhibit weak sexual dimorphism. Male jackal skulls are larger than those of females, but with great overlap, in particular in juveniles and sub adults (Stoyanov, 2012).

The golden jackal is a territorial, medium-sized, generalist carnivore that is widespread in southeastern and central Europe, South Asia and in north-eastern Africa (Šálek et al., 2014). Golden jackal populations are increasing across the northern and western Balkan Peninsula, central Europe and on the Indian Subcontinent, where it holds the status of Least Concern (Katuwal and Dahal, 2013). In Europe, it is distributed in small and scattered populations, mainly along the Mediterranean and Black Sea coasts of the Balkan Peninsula (Giannatos et al., 2005; Krofel, 2008). In recent decades, the jackal’s range...
has expanded broadly within Croatia, Serbia, Bulgaria and Romania, and vagrants occasionally appear in northeastern Italy, Slovenia, Hungary, Austria, Slovakia, and Macedonia (Kryštufek et al., 1997; Zachos et al., 2007; Stoyanov, 2012). Expansion of the species started in the early 1980s, when the golden jackal colonized the Istrian Peninsula, expanding its Balkan range due likely to the strong increase of its Dalmatian and Bulgarian populations (Lapini et al., 2009). In Greece, its population has been declining over the past three decades (Krofel, 2008).

This study aims to characterize and quantify sexual form variation in skulls of golden jackal using geometric morphometric methods that are powerful tools in describing and analyzing biological forms (Bookstein, 1991; Adams et al., 2013). These methods permit a rigorous quantification of shape variation using homologous or equivalent landmarks for all specimens. Another advantage is that the results can be visualized and interpreted directly in their anatomical context (Drake and Klingenberg, 2008; Klingenberg, 2013). Primarily, measures of body size, skull length, body length or body mass are taken. Although these size descriptors are useful when discussing overall body size dimorphism, they do not represent shape (Hartová-Nentvichová et al., 2010). The advantage of geometric morphometric approaches over traditional morphometrics is that the analysis result may be visualized as configurations of landmarks back in the original space of the organism and can be represented as actual shapes or shape deformations rather than only as statistical scatter plots (Nacua et al., 2012).

Our objective was to verify the existence of sexual differences in the skull form of golden jackals in the Western Balkans, as previous studies (Petrov et al., 1992; Gittleman and Van Valkenburgh, 1997; Schutz et al., 2009; Hartová-Nentvichová et al., 2010; Milenković et al., 2010; Stoyanov, 2012; Drake and Klingenberg, 2010) have indicated that dimorphism in this body region is potentially affected by sexual selection.

**MATERIALS AND METHODS**

**Study area and sample collection**

The study was carried out over the entire Croatian territory (N 46°33’ – 42°23’; E 13°30’ – 19°27’), over a total surface area of 56,542 km². Skulls (26 males, 24 females) and mandibles (13 males, 27 females) were collected during the regular hunting season (2009-2012), pursuant to hunting regulations (year round, with the exception of females in advanced gravidity). Animal age was estimated using patterns of tooth replacement and eruption, and based on counting dentine layers on longitudinally sanded canine roots (Lombaard, 1971; Rouichová and Andéra, 2007). Specimens older than one year were used for statistical analyses.

**Acquiring landmark data**

High resolution photographs of specimens were taken with a Sony (DSC-HX300/B) digital camera. The dorsal side of the skull and left side of mandible were photographed. Each skull and mandible was placed on a stand alongside graph paper for scale. To describe skull form of golden jackal specimens, a total of 21 landmarks were chosen on the dorsal side of the skull and on the lateral side of the mandible (Fig. 1, Table 1). The x,y coordinates of landmarks were extracted from a digital image of a specimen using the digitalization software tpsDig version 2.17 (Rohlf, 2013). The entire procedure of image acquisition and landmark digitization was repeated twice to estimate measurement error. Obtained coordinate data was imported into the MorphoJ software version 1.06a (Klingenberg, 2008) which was then used for further shape analyses.

**Fig. 1. Landmarks recorded on the dorsal side of the skull and on the lateral side of the mandible (for anatomical definitions, see Table 1).**

**Statistical analyses**

Shape and size variation in the skulls of golden jackals was quantified using the geometric morphometrics method. The first step in morphometric analysis is to remove all non-shape variation from the data with a Procrustes superimposition (Klingenberg, 2011). A full Procrustes fit was performed to separate size and shape variation from the coordinate data using a procedure without the object symmetry of the skull (Drake and Klingenberg, 2010).
Sexual Dimorphism in the Skull Form of Golden Jackals

Table I.- List of landmarks recorded on dorsal view of skull and on the lateral side of the mandible.

<table>
<thead>
<tr>
<th>Dorsal side of skull</th>
<th>Mandible</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Anterior midline point on suture between the premaxillaries</td>
<td>1 Anterior rim of Canine alveoli</td>
</tr>
<tr>
<td>2 Anterior point of midline between nasals</td>
<td>2 Top of Canine</td>
</tr>
<tr>
<td>3 Posteriormost point of midline between nasals</td>
<td>3 Posterior rim of Canine alveoli</td>
</tr>
<tr>
<td>4 Meeting of frontoparietal suture</td>
<td>4 Posterior edge of the carnassial</td>
</tr>
<tr>
<td>5 Suture between parietal and interparietal</td>
<td>5 Top of the coronoid process</td>
</tr>
<tr>
<td>6 Distalmost point of interparietal</td>
<td>6 Point at mandibular notch</td>
</tr>
<tr>
<td>7 Point at occipital-parietal-temporal suture</td>
<td>7 Distalmost point of condyloid process</td>
</tr>
<tr>
<td>8 Posterior end of zygomatic arch</td>
<td>8 Distalmost point of angular process</td>
</tr>
<tr>
<td>9 Point at orbital process</td>
<td>9 Anterior rim of massesteric fossa</td>
</tr>
<tr>
<td>10 Extension of Canine alveoli</td>
<td></td>
</tr>
<tr>
<td>11 Premaxillary - maxillary - nasal suture</td>
<td></td>
</tr>
<tr>
<td>12 Point at postorbital process</td>
<td></td>
</tr>
</tbody>
</table>

Size was measured for each specimen as the centroid size, i.e. the dispersion of landmarks using a function of their distances from the centroid which is the “baricenter” of a configuration. Procrustes ANOVA was used to quantify relative amounts of variation in sample with sexual dimorphism as the additional main effect. The variance explained by main effects is measurement error and it was calculated as ratio between the sum of squares of an effect and the total sum of squares multiplied by 100 (Viscosi and Cardini, 2011).

Principal components analysis (PCA) was performed to illustrate the main features of shape variation within a sample and as an ordination method. The first principal component (PC1) was interpreted and accounts for the maximum variance in the sample.

The effect of size on shape (i.e., allometry) within each sex was tested using multivariate regression of the shape variables onto natural log-transformed centroid size. Permutation test with 10000 runs was used to test significance of allometric effect. Before performing regression, the similarity of regression slopes was evaluated using VecCompare - IMP software (Sheets, 2010). This program tests the differences of allometric models by comparing the angle between group-specific regression vectors (Zelditch et al., 2003; Frédéric et al., 2008) and statistically tests whether that angle exceeds those that can be obtained by resampling within each group. The between-group angle is obtained as the arccosine of the signed inner products between normalized regression vectors and is compared with the upper 95% confidence interval of within-group angle ranges assessed by a bootstrapping approach (N = 900) (Rodríguez Mendoza, 2013). If the between-group angle exceeds the 95% confidence interval of the two within-group angles, the difference is judged statistically significant at the 0.05 level. If the intergroup angle is larger than one intragroup range, the bootstrapping approach is not informative, because while the one sample yields smaller ranges, the other group with the large range could still cover the angle range between groups. If the intergroup range is smaller than both within-group ranges, no difference exists in the regression slopes (Rosas and Bastir, 2004).

Skull and mandible mean shape differences between males and females were analyzed using DFA to determine the presence of sexual dimorphism. For this analysis, Procrustes and Mahalanobis distances, parametric T-square test results are reported. A permutation tests (using Procrustes distance and the T-square statistic) were performed to test the null hypothesis of equal group means. Cross-validation scores were used to assess classification accuracy. Wireframe graphs of the mean and displaced landmarks described the most significant shape difference.

**RESULTS**

**Measurement error and variation of sample**

Procrustes ANOVA was performed on all specimens, including replicas, to test the amount of measurement error. The total amount of measurement error for size and for observed aspects was 2.59% of total sum of squares. Size for main effect (sexual dimorphism) and individual variation was statistically significant only for mandible (p<0.01).

For shape, the measurement error explained 3.21% of total sum of squares for studied aspects in which lateral side of mandible amounted 3.19% of total sum of squares. For main effect, Procrustes ANOVA resulted with statistically significant variation of dorsal shape of skull (F=3.81, p<0.01) and lateral shape of mandible (F=8.55, p<0.01). The individual shape variation was statistically significant (p<0.01) in both structures.
Principal components analysis (PCA)

The first two PCs explained 43.68% of total shape variation of the dorsal aspect of skull (Fig. 2A). Along the PC1 axis (accounting for 23.36% of the variation), the most notable changes were a difference at the relative position of the frontoparietal, parietal and interparietal, and occipital-parietal-temporal sutures (LM 4–7), i.e. the surface of braincase was longer and zygomatic arch was wider (LM 8 and 9). A small differences were recorded in orbit shape (LM 11 and 12). The PC1 and PC2 of mandible accounted for 55.90% of the variation. Females were generally aligned with positive values of PC1 (accounting for 50.05% of the variation) and males with negative values (Fig. 2B). The canines (LM 1-3) were wider and carnassial were little longer and placed posterior (LM 4). The most noticeable changes were reduction in size of coronoid and angular processes and masseteric fossa (LM 6–9).

The similarity of regression slopes and multivariate regression

The comparison of the angles between and within sexes yields a clear indication of significant differences in the slopes of the regression models. The angle between sexes was larger than the 95% confidence interval of the two within-sex angles, so difference is judged statistically significant at the 0.05 level (Table II).

Table II.- Results of between and within groups angles ranges assessed by a bootstrapping approach with 900 runs using VecCompare7 (IMP software).

<table>
<thead>
<tr>
<th>Comparisons</th>
<th>Dorsal side of skull</th>
<th>Mandible</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between sexes</td>
<td>96.3</td>
<td>122.2</td>
</tr>
<tr>
<td>Males</td>
<td>76.8</td>
<td>105.4</td>
</tr>
<tr>
<td>Females</td>
<td>91.1</td>
<td>118.4</td>
</tr>
</tbody>
</table>

The multivariate regression method did not determine the presence of allometry. The percentages of predicted shape variation related to size were not statistically significant (Table III). Based on multivariate regression results and on different angles of regression vectors between sexes, the “size-correction” method is not performed and original variables were used for DFA.

Discriminant function analysis (DFA)

As there are only two groups and a single axis of shape difference, scores with histogram bars were
shown proportional to their frequency (Fig. 3). The shape comparison of the dorsal aspect of the skull using parametric tests with Procrustes and Mahalanobis distances and T-square test yield a significant difference between the mean shapes of males and females (p<0.05). Classification success from the jackknifed cross-validation score amounted to 54.16% for females and 73.07% for males (Fig. 3A). The permutation tests showed similar results (p<0.05). The differences recorded between dorsal female and male mean skull shapes are little longer nasals (LM 3), wider posterior end of zygomatic arch (LM 8) and more extended canine alveoli (LM 10). The values of Procrustes and Mahalanobis distances and parametric T-square test did not show significant differences between female and male mandible mean shapes. Only a permutation test using Procrustes distance was statistically significant (p<0.01; Table IV). The most noticeable shape differences between the sexes were in position of carnassial (LM 4) which was placed posterior in males, then, females had slightly greater coronoid process (LM 5), angular process (LM 8) and masseteric fossa (LM 9). The classification rate from cross-validation test showed 70.37% correct classification for females and 53.84% for males (Fig. 3B).

**Table III.- Testing the relationship between size and shape (allometry) using multivariate regression method.**

<table>
<thead>
<tr>
<th>Observed aspect</th>
<th>Sample size</th>
<th>% of predicted shape variation related to size</th>
<th>p-value</th>
<th>p-value (perm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dorsal side of skull</td>
<td>f=24</td>
<td>6.361</td>
<td>0.153</td>
<td></td>
</tr>
<tr>
<td></td>
<td>m=26</td>
<td>4.767</td>
<td>0.287</td>
<td></td>
</tr>
<tr>
<td>Mandible</td>
<td>f=27</td>
<td>3.423</td>
<td>0.479</td>
<td></td>
</tr>
<tr>
<td></td>
<td>m=13</td>
<td>15.828</td>
<td>0.086</td>
<td></td>
</tr>
</tbody>
</table>

Table IV.- Results of parametric T-square testing of the difference between group means and comparisons between the mean shapes of males and females based on Procrustes and Mahalanobis distances. Permutation tests with 10,000 runs were used to test the null hypothesis of no mean difference between the sexes.

<table>
<thead>
<tr>
<th>Observed aspect</th>
<th>Procrustes distance (Pd)</th>
<th>Mahalanobis distance</th>
<th>T-square test</th>
<th>p-value</th>
<th>p-value (perm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dorsal side of skull</td>
<td>0.013</td>
<td>2.319</td>
<td>67.127</td>
<td>0.040</td>
<td>0.036</td>
</tr>
<tr>
<td>Mandible</td>
<td>0.030</td>
<td>2.230</td>
<td>43.645</td>
<td>0.056</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Fig. 3. Cross-validated discriminant analysis of the skull and mandible of golden jackals. Histograms show the values of the cross-validation scores. Wireframe deformations are used to demonstrate shape changes. A, Skulls in dorsal view; B, Lateral view of mandibles. Symbols: dark grey bars, females; light grey bars, males.
DISCUSSION

The sources of measurement error such as positioning and digitizing are commonly tested by using an analysis of variance–ANOVA (Viscosi and Cardini, 2011). The estimate of the total shape variability and the amount of measurement error by Procrustes ANOVA resulted with greater value of explained percentage of total sum of squares for mandible shape. For size, amount of measurement error was 2.21% of total sum of squares for dorsal aspect and was negligible for mandible. This seems as a higher amount of error and it could lead to an important reduction of discriminative power. In our case, a significant larger sample of specimens would be needed to yield a better estimate of total shape variability and amount of error present. The main cause of larger values of measurement error percentage is that using 2D pictures to test morphological variation in a 3D object (skull) is an approximation that inevitably implies measurement error, i.e. a loss of information and a degree of inaccuracy in estimating size and shape. This approximation of a 3D object with a 2D picture is called Two to Three Dimensional approximation - TTD (Cardini, 2014). According to this author, the ANOVA provides an objective and essential information that 2D data of the 3D object are accurate in relation to the amount of variation in study sample, but an exclusive focus on statistical significance may be unwise because results could be highly significant even if TTD is relatively large and 2D data are therefore not as good as it might be desirable. This might require cautions interpretations and clear warning to readers about potential inaccuracies and TTD should be assessed at least preliminary in a 2D study of 3D structures.

Testing the angles between regression slopes was done to perform a “size-correction” method in case if significant allometry is present. According to Rosas and Bastir (2004), three different kinds of size-shape relationships should be distinguished (i.e., ontogenetic, static, and evolutionary allometry). The central assumption of all the size-correction methods is that the groups in the analysis must have the same allometric trajectories (Klingenberg, 1996) and in cases where significant allometry is detected and regression slopes are not different, we can perform the pooled within-group regression which considers the group structure and it actually enhances group separation by eliminating the within-group allometric variation. Using the residuals of pooled within-group regression in the DFA reflect in a higher correct classification rate and a higher amount of shape variation than the overall multivariate regression (Rodríguez Mendoza, 2013). In our study we tested the angle between two trajectories as a measure of difference in allometric trajectories. Slopes are drastically different, as well the results of multivariate regression of size on shape were not statistically significant for observed dorsal aspect of skull and mandible and ‘size-correction’ method is not performed.

Geometric morphometrics analysis indicated that golden jackal males and females have morphological differences but they are not as expressed as they are in some members of Canidae family, for example in grey wolf (Milenković et al., 2010). Eisenmann and Van der Geer (1999) found female skulls of golden jackal to be smaller, particularly in the condylobasal, post-palatal and palatal lengths. The result is that the female skull is somewhat wider, though the difference in proportions between male and female are not significant. Kryštufek and Tvrtković (1988) studying variability of the Dalmatian golden jackals, found that female developed a sagittal crest of the same size as males. Also, they found that males had greater braincase length, interorbital constriction and condylobasal length, but there were no significant differences in skull shape except slightly greater relative height of the skull in two females. Bošković (2012) stated that sexual dimorphism in golden jackals is not expressed and there is no characteristic by which to differentiate the sexes.

According to Stoyanov (2012) golden jackal skulls from Bulgaria showed homogeneity in size and shape. In that study, the sexes showed significant differences in virtually all skull measurements though with an overlap that did not permit for clear differentiation. The author indicated that such sexual dimorphism of golden jackal skulls, with males slightly larger than females, could be explained due to the monogamous reproductive system of golden jackal and the presence of male parental care. In a study of size and shape in the cranium of grey foxes (Urocyon spp.) (Schutz et al., 2009), little to no differentiation in cranial shape between the male and female grey foxes is apparent. The little differentiation that is present in these subspecies is centered on the narrower and slightly shorter pre-maxilla, the narrower palate and zygomatic region.

In the present study, the DFA differentiated between the sexes based on the mean skull and mandible shapes. The parametric and permutation tests of dorsal shape of the skull were significant at 0.05 levels. The mandible shape difference between sexes based on Procrustes and Mahalanbis distances, the T-square statistic and associated parametric p-value were not statistically significant, while permutation test using Procrustes distance had significant value (Table IV). The results of parametric p-values are presented as well as permutations which are performed because of a small sample size and because this test do not assume normally distributed data. Although permutations...
can be performed with sample sizes too small for parametric tests, it will inevitably reduce statistical power and increase inaccuracies in estimating group means and variances (Viscosi and Cardini, 2011). The cause of the large distances of specimens from the mean shape could be different types of measurement error, overestimation because small sample size or true differences in shape. The shape changes between the sexes were recorded in the form of canines and carnassials, and significant changes were in the shape of the coronoid process, angular process and masseteric fossa (LM 5, 8, 9). According to Davies et al. (2007), canines and carnassials may be under strong sexual selection, though canines display greater intraspecific variability than carnassials. Gittleman and Van Valkenburgh (1997) stated that dimorphism is more pronounced in both the upper and lower canine size and strength than in carnassial size, skull dimensions and biomechanical features, though all dimorphism indices covaried in that study. In a study of skull variation in grey wolves (Milenkovic et al., 2010) the most marked non-allometric shape change between the sexes was an anterior–posterior shifting of the zygomatic arches, which were shifted anteriorly in males but posteriorly in females. Under the zygomatic arches, the jaw muscles from the lower jaw reach the back of the skull. Activation and coordination of masticatory muscles determine the direction of jaw movement and control occlusal force, and they comprise the major loading on the skull, influencing its growth and morphology. The division of labour between the sexes, with males more highly specialized for capturing and killing large prey and females more specialized for a nurturant role, is the most plausible explanation for the evolution of sexual dimorphism in these canids. It is evident that these results should be interpreted and generalized with some caution because small and non-uniform sample and further studies are required. The number of landmarks should be increased to obtain a more accurate expression of form and use of semi-landmarks should be employed to better describe differences in shape between the sexes. Also, further studies on larger samples are required with usage of tools for 3D measurements (3D digitizers etc.) to explore the biological meaning of the observed sexual dimorphism.

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Conflict of interest statement

We declare that we have no conflict of interest.

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