Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2020) 44: 396-403
© TÜBİTAK
doi:10.3906/vet-1907-86

Cranial size and shape sexual dimorphism in the Kangal dog from Turkey

Pere Miquel PARÉS-CASANOVA[1], **Abu Bakar SIDDIQ**[2,*], **Vedat ONAR**[3]
[1]Department of Animal Science, University of Lleida, Lleida, Spain
[2]Department of Anthropology, Mardin Artuklu University, Mardin, Turkey
[3]Department of Anatomy, Faculty of Veterinary Medicine, İstanbul University-Cerrahpaşa, İstanbul, Turkey

Received: 19.07.2019 • **Accepted/Published Online:** 28.01.2020 • **Final Version:** 06.04.2020

Abstract: This study has so far been the first attempt to characterize and quantify skull sexual variation in Kangal dog, by means of geometric morphometric techniques. A sample of 16 adult Kangal crania has been analyzed with this purpose. To obtain a full image of morphological pattern, digital pictures were taken from the ventral, left lateral, and dorsal sides of each skull, and a total of 16, 15, and 16 landmarks respectively were obtained on each image. Skull size and shape differed significantly in all aspects among different sexes, male skulls being bigger. Shape differences were observed mainly on zygomatic arch and muzzle on the dorsal view, pterygoid bone and articular surface to mandibular condyle in the lateral aspect, and cranial width and maxillary bone on the ventral view. Although the sample was comparatively small in number, being the first geometric morphometric approach applied on the Kangal dog, the obtained results will add vital information particularly to understand the cranial shape sexual dimorphism of this unique dog breed in Turkey.

Key words: Geometric morphometrics, sexual dimorphism, skull morphometry, Kangal dog, Turkey

1. Introduction

The phenotipic variation in the skull and skeleton among Kangal dogs is superior to all other species of the family Canidae [1,2]. This variation can be due to sexual dimorphism [3]. Sexual dimorphism occurs when males and females of the same species exhibit characteristic differences beyond their sexual organs e.g., when they differ in external appearance or other features [4,5]. If the body size presents sexual dimorphism, it frequently appears in shape dimorphism, too [5]. Therefore, apart from sexual differences in total body size, the variation of relative size and shape of skeletal parts must be of special interest in the study of sexual dimorphism. Moreover, these types of studies can reveal differential selection criteria acting on distinct body parts of each sex. Therefore, the main point can be that the degree of sexual dimorphism is the result of the difference between the sum of all selective pressures affecting the male and the sum of those affecting the female.

Some dog breeds present a male-biased sexual dimorphism in the skull. However, skull sexual dimorphism has still been less researched although many studies have been done on different aspects of the skulls of different dog breeds around the globe [6-12]. On the other hand, the Kangal dog, also known as Karabaş ("Blackhead"), Sivas Çoban Köpeği (the shepherd dog of Sivas), or Anatolian Shepherd dog, is a large and massive dog breed in Turkey, originally found in the Central Anatolian provincial regions of Sivas, Tokat, and Yozgat. The breed has been selectively bred since ancient time especially for guarding livestock. It is dolichocephalic, with mastiff-like phenotypic appearance and a massive head, and has been categorized in the molosser category/group [8]. Although this is a unique dog breed and has been living in human society for millennia, there are only a few studies that came to light on the Kangal dog so far. Moreover, no study on sexual dimorphism in this dog breed has been carried out until today. This study, therefore, attempts to enrich the scientific knowledge about this unique dog breed in Turkey.

Traditional linear measurement analysis is limited to capture substantial information of morphology [13]. In contrast, landmark-based morphometric methods of geometric morphometric techniques are advantageous for separating shape information from size variation and moreover in providing a visual representation of shape variation in their anatomical context [14-16]. They permit a rigorous quantification of shape variation using homologous landmarks [15]. Geometric morphometrics could be assumed to have a higher sensitivity and detect finer differences since a wide variety of variables should be taken into account [15]. Geometric methods allow the

* Correspondence: abubakarsiddiq@artuklu.edu.tr

investigation of the morphology of complex structures, such as mammalian skulls [5] but until now, they have not been extensively applied to study the external morphology of domestic mammals, and very few authors have applied such methods in dogs [17].

Using geometric morphometric methods, this study aims to characterize and quantify sexual form variation in skulls of the Turkish Kangal dog. Although the skull typology of adult male Kangal dogs has been carried out so far [8], there is still a lack of data regarding the skull-based sexual dimorphism in this dog breed. Therefore, this has been the first attempt of geometric morphometric approach to understand the cranial shape sexual dimorphism of Kangal shepherd dogs in Turkey. With the obtained result on Kangal specimens, the study will further contribute to the better understanding of factors relating to the evolution of sexual dimorphism molosser dogs.

2. Materials and methods

2.1. The sample

A sample of 16 crania of Kangal dogs were analyzed in this study. Sex of each specimen was previously known. Eleven of these specimens were the skulls of male Kangal dogs and 5 were the skulls of female Kangal dogs. Among the 16 specimens, 11 male skulls and 3 female skulls were accessed from the reference collection of the Department of Anatomy, Faculty of Veterinary Medicine, İstanbul University-Cerrahpaşa; and the other 2 female skulls were accessed from the Department of Anatomy, Faculty of Veterinary Medicine, Adnan Menderes University, Turkey. Notably, no living dog was killed to obtain any of the skulls used in this study since all of the specimens belonged to the reference collections of these two distinct universities. Providing that sexual dimorphism pattern was the primary focus of this study, only the skulls of adult individuals were selected for the analyses. Therefore, only the adult specimens with fully erupted upper second molars (m^2) were included in the sample of this study.

2.2. Image-capturing and landmark digitizing

Specimens were photographed with a high-resolution Nikon (D5100) digital camera and an AF-S DX Micro Nikkor 40mm f/1·2.8G lens. To obtain a full image of morphological patterns, the ventral (including upper tooth row, auditory bulla, as well as palatine, basisphenoid and basioccipital bones), dorsal (including the nasal, frontal and parietal bones), and left lateral (including premaxilla, maxilla and temporal bones) aspects of each skull were photographed. For each position, every specimen was placed on a stand in a standardized position with a ruler placed alongside the skull. In setting up the digital camera, care was taken to mount it firmly in place being attached to a tripod stand and set at its maximum zoom.

The *x*, *y* coordinates of 16 (Figure 1), 15 (Figure 2), and 16 (Figure 3) landmarks for the ventral, the left lateral, and the dorsal views were respectively extracted from images of each specimen using the digitalization software TpsDig v. 1.40 [18] Those landmarks were chosen in order to have a good representation of the overall skull form and in a way that allowed observing important features of the skull anatomy on different views. Most of the landmarks were chosen from von den Driesch's guide [19] and it can be considered that they summarize sufficiently the morphology of the head structures. To study the sagittal crest, a subset of 16 semilandmarks was put along the crest on the lateral view. Semilandmarks are points which slid along the outline configuration until they match as well as possible [15]. The Cartesian *X Y* coordinates of all landmarks were digitized using TpsDig, v. 2.26 software [18]. This set was further standardized by the generalized Procrustes analysis (GPA). GPA begins by reflecting landmark configurations from one of the sides and superimposing them by their centroid (midpoint of a configuration of anatomical landmarks). Then, each landmark configuration was rotated such that the squared distances between homologous landmarks were minimized and Procrustes coordinates obtained [20]. As a result of all of these calculations, the distances between the superimposed configurations of left and right were obtained.

Centroid size (CS), the square root of the sum of the squared variances of the landmarks to the centroid point in *x*- and *y*-directions [21], was used as size measure. Each configuration was digitized twice (by first author) to estimate the error and Procrustes ANOVA was performed to test this amount.

2.3. Statistical analyses

The first step of statistical analysis was to remove all nonshape variation from the data with a Procrustes superimposition [22]. Sex shape differences and landmark covariation patterns were studied using a canonical variate analysis. Mahalanobis distances and 10,000 permutation rounds were used for this analysis. A two-way ANOVA (analysis of variance), with sex and aspects as factors, was employed to assess the differences in skull size. ANOVA particularly tested the null hypotheses that several univariate samples have the same mean across each of the two factors, and that there are no dependencies (interactions) between factors. Following, a Mann–Whitney *U* test was performed to assess size differences for each aspect. The two-tailed Mann–Whitney tested whether the medians of two independent samples were different. It did not assume normal distribution, but assumed equal-shaped distribution in both groups. Finally, a regression of CS against shape coordinates was performed to detect allometry. All analyses were performed respectively with

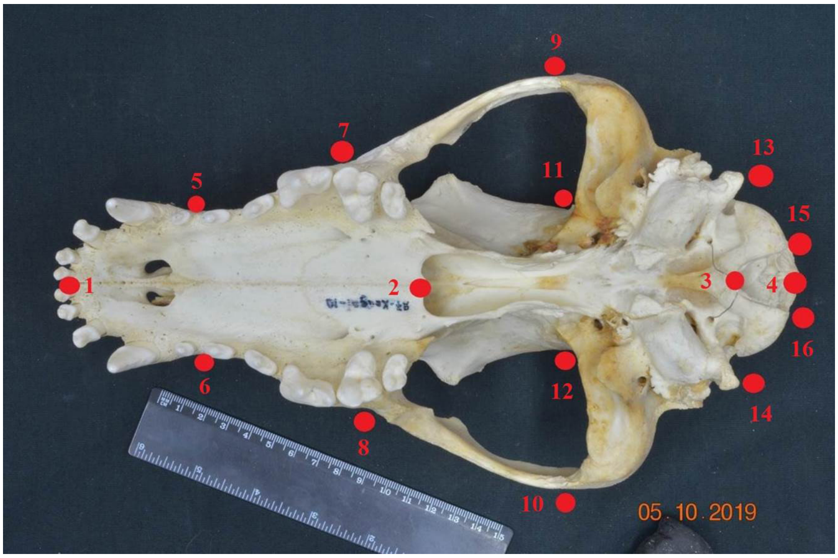

Figure 1. Positions of the 15 landmarks on the ventral view of the cranium used in this study.	
1	Most rostral tip of *corpus ossis incisivi*
2	Most caudal tip of *lamina horizontalis ossis palatini*
3	Most rostral and medial tip of *foramen magnum*
4	Most caudal and medial tip of *foramen magnum*
5, 6	Lateral narrowest points of *ossis incisivi*
7, 8	Rostral base of *arcus zygomaticus*
9, 10	Most lateral points of *arcus zygomaticus*
11, 12	Base of *fossa mandibularis ossis temporalis*
13, 14	Tips of *processus paracondylaris*
15, 16	Most lateral points of *condylus occipitalis*

the MorphoJ software v.1.06c [23] and the PAST software v.2.17c [24].

2.4. Ethics statement

This study was carried out on the skulls from existing reference collections. No living dog was killed to obtain any of the specimens analyzed here. Therefore, Ethics Committee agreement was not necessary to conduct this study.

3. Results

3.1. Measurement error and variation of sample

For size, the total amount of measurement error ranged from a 0.0002% of the total sum of squares for the lateral aspect to 0.005% for the dorsal aspect. For shape, the measurement error ranged from 0.05% of total sum of squares for the ventral aspect to a 0.43% for the lateral aspect. This suggested that measurement error was random and did not affect the outcome of ulterior analyses. It is important to note that the component of the overall variance occurred due to the imprecision of measurements in this study.

3.2. Size and shape differences

Skull size significantly differed in all aspects among different sexes ($P < 0.001$) (Table), but there were no differences if lateral and ventral aspects were considered separately ($U = 96$, $P = 0.583$ and $U = 76$, $P = 0.173$, respectively) (Figure 4). Thus, size differences were focused only on dorsal aspect, males being 6.4% bigger than females. Shape differences appeared on all aspects ($P < 0.0001$). Differences were focused maxillary width and basicranial width on the ventral aspect; occipital and interparietal crests on the lateral aspect (Figure 5); on maxillary width and length (Figure 6); and cranial vault length, on the dorsal view (Figure 7). No sexual differences appeared for sagittal crest silhouette ($P = 0.993$). Regression was significant for dorsal and lateral aspects ($P = 0.0095$ and 0.0003 respectively), but not for ventral side ($P = 0.143$).

4. Discussion

Sexual size dimorphism is a common phenomenon in many animal taxa in mammals [25-26]. This can occur in several ways, from anatomical to physiological traits. The

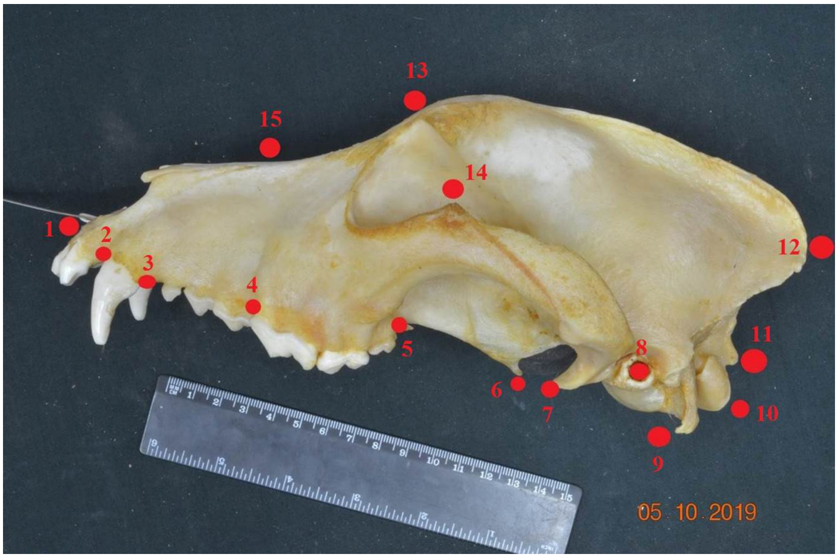

Figure 2. Positions of the 15 landmarks on the lateral view of the cranium used in this study.	
1	Most rostral tip of *corpus ossis incisivi*
2	Rostral base of canine tooth
3	Caudal base of canine tooth
4	Rostral bas of P^2
5	Caudal base of M^2
6	Tip of *os pterigoideum*
7	Tip of *processus retroarticularis ossis temporalis*
8	Middle of *porus acusticus externus*
9	Tip of *processus paracondylaris*
10	Caudo-ventral tip of *condylus occipitalis*
11	Most caudal tip of *os occipitale*
12	Most caudal tip of *crista nuchae*
13	Frontal projection of *processus zygomaticus ossis frontalis*
14	Tip of *processus frontalis ossis zygomatici*
15	Fronto-nasal suture

determination of sexual dimorphism other than body mass requires complex measurement techniques, for instance, those related with geometric shape. Particularly shape analysis [14] allows a deeper understanding of mechanisms underlying sexual dimorphism, because different parts of the body can serve multiple functions and be under distinct selective regimes [27]. However, although shape can contribute meaningfully to various functions such as feeding, mating, parental care, and other life history characteristics, patterns of sexual shape dimorphism have historically received considerably less attention than sexual size differences. Besides, the examination of both size and shape of traits together provides a much more complete quantification of sexual dimorphism, since the two components are necessarily related to one another [27].

Using geometric morphometric techniques [2,13,15,16,21], this study scientifically demonstrates that the skulls of male individuals are generally larger than those of the female individuals of the Kangal dog breed in Turkey. Remarkably it also reveals shape sexual differences in Kangal dogs, mainly related to aspects of the face and cranial vault, where important masticatory muscles are attached, like *temporalis* and *masseter* [28]. It appears that male individuals perhaps have greater estimated bite force than the females. This may result in stronger bony points for attachment of masticatory muscles among male individuals, which eventually caused the shape differences between male and female Kangals. If head dimensions are directly related to the jaw musculature, bigger head will increase the jaw force. Moreover, higher jaw force would

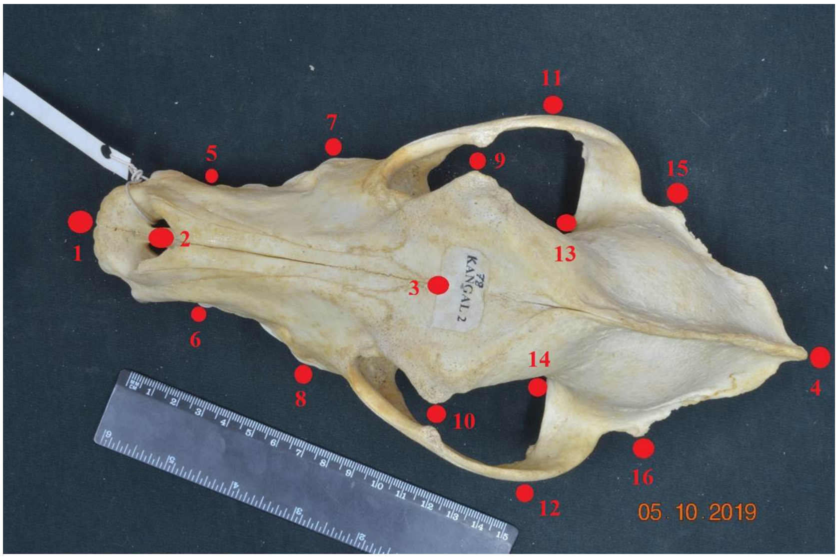

Figure 3. Positions of the 16 landmarks on the dorsal view of the cranium used in this study.	
1	Most rostral tip of *corpus ossis incisivi*
2	Most rostral tip of *os nasale*
3	Fronto-nasal suture
4	Most caudal part of *os interparietale*
5, 6	Lateral narrowest points of *ossis incisivi*
7, 8	Widest points of *maxilla*
9, 10	Most lateral points of *processus zygomaticus ossis frontalis*
11, 12	Most lateral points of *arcus zygomaticus*
13, 14	Base of *fossa mandibularis ossis temporalis*
15, 16	Widest part of cranial vault

Table. Results of two-way ANOVA with centroid size as the dependent variable and sex and aspect as factors, for the sample of 16 crania from adult Kangal dogs. Sums of squares and mean squares are in units of Procrustes distances (dimensionless).

Source	Sum of squares	Degrees of freedom	Mean square	F	P
Sex	1.61E+04	1	1.61E+04	26.03	1.92E-06
Aspect	4.08E+04	2	2.04E+04	32.9	2.15E-11
Interaction	1716	2	858.2	1.384	0.256
Residual	5.46E+04	88	620.2		
Total	1.12E+05	93			

implicate not only feeding power, but also an antipredatory behavior and, if the skull size is related to body size [8, 26], bigger males would be more fitted to win fights for copulation as well to a better protective role for livestock or caprine herds. Consequently the sexual selection, acting via male–male combats and female choice, would eventually favor bigger males. It appeared that there was no enthesis on ventral aspect, which actually caused the lack of allometry on this side. However, because of the presence of enthesis (*temporalis*, *masseter*, *buccinator*), there were allometry on the dorsal and lateral aspects.

On the other hand, artificial selective pressures [9,26] could also have acted on the male individuals to have bigger size. If the breeders select the animals according to

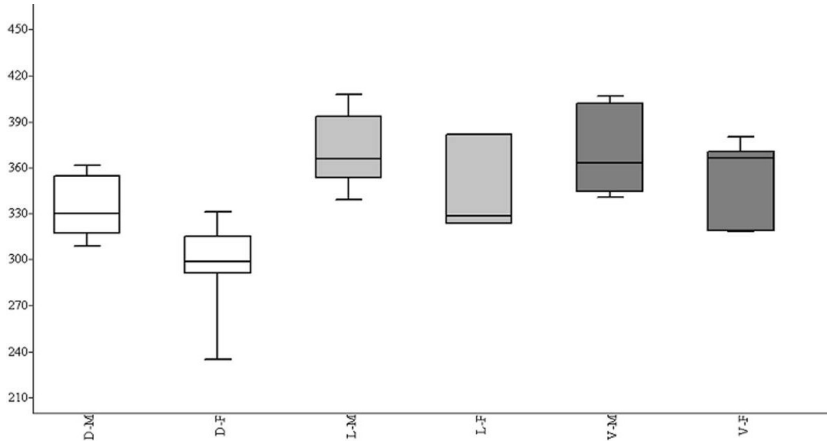

Figure 4. Box plot of skull size for every aspects of the 16 Kangal skulls (11 males and 6 females) examined in this study: Dorsal D, Lateral L, Ventral V, Male M and Female F.

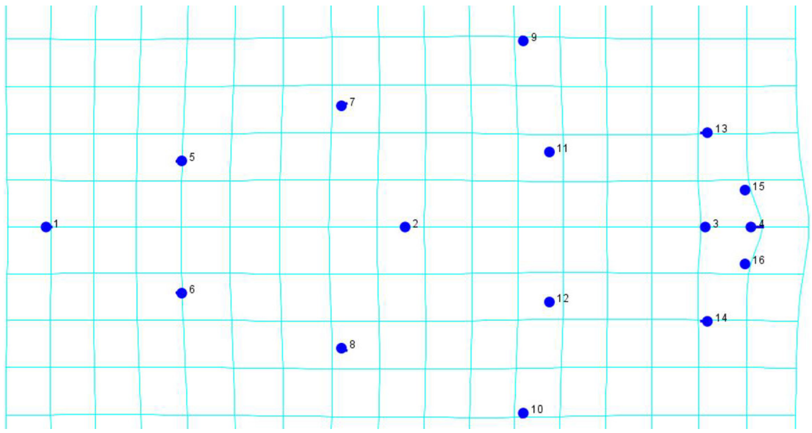

Figure 5. Deformation grid for ventral aspect.

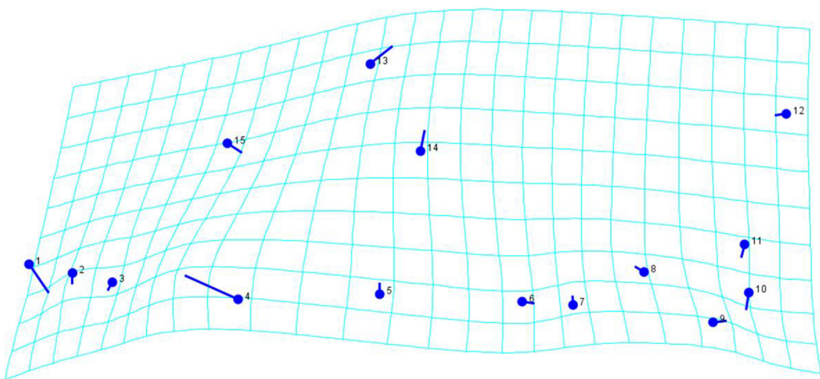

Figure 6. Deformation grid for lateral aspect.

other distinctive morphological and behavioral traits [26], the favorable condition of breeding the larger males by sexual selection might be reinforced. Therefore, selectively breeding for guarding purposes–since bigger animals are better fitted for this purpose–may have been the case in cranial size and shape sexual dimorphism in this local dog breed in Turkey.

It can be concluded that, this study has so far been the first attempt of skull-based sexual dimorphism in Kangal dogs. It has also been the application of geometric

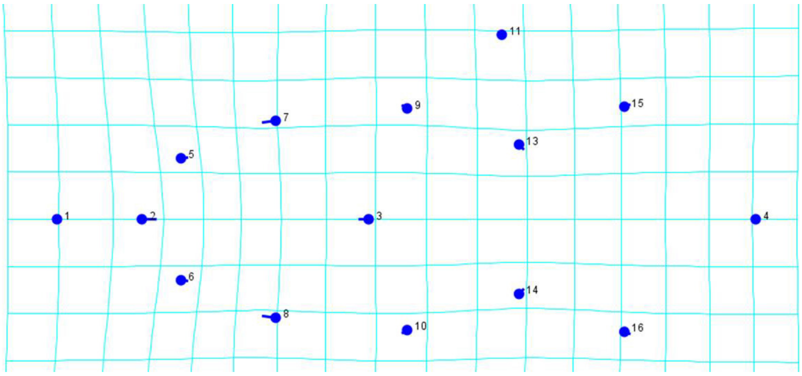

Figure 7. Deformation grid for dorsal aspect.

morphometric techniques ever applied on any of scientific issues related to this unique dog breed. The study was carried out with a sample particularly very standard for sexual dimorphism. However, depending on the maximum accessibility, it was not possible to bring a balance between the ratio of two sexes. Nevertheless, with the highest number of specimens that could possibly be obtained in Turkey, the study ultimately offers a very significant addition to the Kangal dog breed as well as enriching the knowledge of canine species in Turkey. Overall, further advances of this field apparently depend very much on the availability of a collection of Kangal and other molosser specimens of different age groups.

Acknowledgments
The authors are grateful to the authority of the Department of Anatomy, Faculty of Veterinary Medicine, İstanbul University-Cerrahpaşa, and Prof. Dr. Erkut Kara of the Department of Anatomy, Faculty of Veterinary Medicine, Adnan Menderes University, for granting access to the reference collections. The authors would like to thank the anonymous reviewers for their valuable comments for improving the manuscript.

Conflict of interest
The authors declare no conflict of interest to be disclosed regarding this study.

Supplementary material
The contents of all supporting data are the sole responsibility of the first author. The datasets generated, analyzed, and used in this study will be provided by the corresponding author on request.

References

1. Evans HE, de Lahunta A. Miller's Anatomy of the Dog. China: Elsevier, 2013.
2. Thuller MAO, Jangarelli M, do Couto DM, Araújo AHB. Sexual dimorphism of labrador retriever dogs by morphometry. Bioscience Journal 2015; 31 (5): 1475-1487. doi: 10.14393/BJ-v31n5a2015-26496
3. Abdel-Rahman EH, Taylor PJ, Contrafatto G, Lamb JM, Bloomer P et al. Geometric craniometric analysis of sexual dimorphism and ontogenetic variation: A case study based on two geographically disparate species, *Aethomys ineptus* from southern Africa and *Arvicanthis niloticus* from Sudan (Rodentia: Muridae). Mammalian Biology 2009; 74 (5): 361-373. doi: 10.1016/j.mambio.2008.06.002
4. Bidau CJ, Martinez PA. Sexual size dimorphism and Rensch's rule in Canidae. Biological Journal of the Linnean Society 2016; 119 (4): 816-830. doi: 10.1111/bij.12848
5. Yazdi FT, Alhajeri BH. Sexual dimorphism, allometry, and interspecific variation in the cranial morphology of seven *Meriones* species (Gerbillinae, Rodentia). Hystrix, the Italian Journal of Mammalogy 2018; 29 (2): 162-167. doi: 10.4404/hystrix-00018-2017
6. Brehm VH, Loeffler K, Komeyli H. Schädelformen beim Hund. Anatomia, Histologia, Embryologia 1985; 14 (4): 324-331 (in German). doi: 10.1111/j.1439-0264.1985.tb00828.x
7. Onar V. A morphometric study on the skull of the German shepherd dog (Alsatian). Anatomia, Histologia, Embryologia 1999; 28 (4): 253-256. doi: 10.1046/j.1439-0264.1999.00202.x
8. Onar V, Özcan S, Pazvant G. Skull typology of adult male Kangal dogs. Anatomia, Histologia, Embryologia 2001; 30 (1): 41-48. doi: 10.1046/j.1439-0264.2001.00292.x
9. Drake AG, Klingenberg CP. Large-scale diversification of skull shape in domestic dogs: disparity and modularity. The American Naturalist 2010; 175 (3): 289-301. doi: 10.1086/650372

10. Onar V, Cakirlar C, Janeczek M, Kiziltan Z. Skull typology of Byzantine dogs from the Theodosius Harbour at Yenikapı, Istanbul. Anatomia, Histologia, Embryologia 2012; 41 (5): 341-352. doi: 10.1111/j.1439-0264.2012.01143.x

11. Andrews K, Lowe J, McCormick WD. Skull morphology of the domestic dog in relation to cephalic index. 69th AVTRW Annual Conference, London, UK: Royal Veterinary College; 2015.

12. Igado OO. Skull typology and morphometrics of the Nigerian local dog (*Canis lupus familiaris*). Nigerian Journal of Physiological Sciences 2017; 32 (2): 153-158.

13. Mitteroecker P, Gunz P. Advances in geometric morphometrics. Evolutionary Biology 2009; 36 (2): 235-247. doi: 10.1007/s11692-009-9055-x

14. Adams DC. Methods for shape analysis of landmark data from articulated structures. Evolutionary Ecology Research 1999; 1 (8): 959-970.

15. Adams DC, Rohlf FJ, Slice DE. A field comes of age: geometric morphometrics in the 21st century. Hystrix, the Italian Journal of Mammalogy 2013; 24 (1): 7-14. doi: 10.4404/hystrix-24.1-6283

16. Benítez HA. Sexual dimorphism using geometric morphometric approach. In: Moriyama H (ed.). Sexual Dimorphism. London, UK: IntechOpen Limited; 2013, pp. 35-50. doi: 10.5772/55195

17. Onar V, Siddiq AB, Asal R, Parés-Casanova PM. Craneometric canine types are well expressed at the level of the zygomatic arch conformation. International Journal of Morphology 2020; 38 (1): 78-82 (in Spanish with an abstract in English).

18. Rohlf FJ. The tps series of software. Hystrix, the Italian Journal of Mammalogy 2015; 26 (1): 9-12. doi: 10.4404/hystrix-26.1-11264

19. Von den Driesch A. A Guide to the Measurement of Animal Bones from Archaeological Sites. Boston, MA, USA: Peabody Museum of Archaeology and Ethnology Harvard University; 1976.

20. Klingenberg CP. Analyzing fluctuating asymmetry with geometric morphometrics: concepts, methods, and applications. Symmetry 2015; 7 (2): 843-934. doi: 10.3390/sym7020843

21. Zelditch ML, Swiderski DL, Sheets HD. Geometric Morphometrics for Biologists: A Primer. Boston, MA, USA: Elsevier Academic Press; 2004.

22. Rohlf FJ, Bookstein FL (eds). Proceedings of the Michigan Morphometrics Workshop. Special Publication No. 2, Michigan, MI, USA: The University of Michigan Museum of Zoology; 1990.

23. Klingenberg CP. MorphoJ: An integrated software package for geometric morphometrics. Molecular Ecology Resources 2011; 11 (2): 353-357. doi: 10.1111/j.1755-0998.2010.02924.x

24. Hammer Ø, Harper DAT, Ryan PD. Past: paleontological statistics software package for education and data analysis. Palaeontologia Electronica 2001; 4 (1): 1-9. https://palaeo-electronica.org/2001_1/past/past.pdf

25. Dale J, Dunn PO, Figuerola J, Lislevand T, Székely T, Whittingham LA. Sexual selection explains Rensch's rule of allometry for sexual size dimorphism. Proceedings of the Royal Society B: Biological Sciences 2007; 274 (1628): 2971-2979. doi: 10.1098/rspb.2007.1043

26. Van Damme R, Entin P, Vanhooydonck B, Herrel A. Causes of sexual dimorphism in performance traits: a comparative approach. Evolutionary Ecology Research 2008; 10: 229-250.

27. Berns CM. The Evolution of Sexual Dimorphism: Understanding Mechanisms of Sexual Shape Differences. London, UK: IntechOpen Limited; 2013. doi: 10.5772/55154

28. Barone R. Comparative Anatomy of Domestic Mammals. Volume 2: Artrology and Myology. Paris, France: Vigot; 2000 (In French).