DOI:10.31196/huvfd.1012563

Geometric Morphometric Analysis on the skull of the Red Fox (Vulpes Vulpes)

İftar GÜRBÜZ[1,a,*], Yasin DEMİRASLAN[1,b], Funda AKSÜNGER KARAAVCI[2,c], Osman YILMAZ[3,d] , İsmail DEMİRCİOĞLU[4,e]

[1]Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Anatomy, Burdur, Türkiye.
[2] Bingöl University, Faculty of Veterinary Medicine, Department of Anatomy, Bingöl, Türkiye.
[3] Van Yüzüncü Yıl University, Faculty of Veterinary Medicine, Department of Anatomy, Van, Türkiye.
[4] Harran University, Faculty of Veterinary Medicine, Department of Anatomy, Şanlıurfa, Türkiye.
[a]ORCID: 0000-0001-9460-0645, [b]ORCID: 0000-0003-3612-6142, [c]ORCID: 0000-0001-8311-1723, [d]ORCID: 0000-0003-2013-9213, [e]ORCID: 0000-0002-0724-3019

Geliş Tarihi: 21.10.2021 **Kabul Tarihi: 08.11.2022**

Abstract: This study was carried out to determine the shape of the fox skull by the geometric morphometric method according to gender. For this purpose, four female and nine male fox skulls were used. The skulls were photographed from the dorsal and lateral aspects. Eleven homologous landmarks on the dorsal aspect and 19 homologous landmarks on the lateral aspect were marked on the photographs of the skull. Principal component analysis and Relative warp analysis were performed on the coordinate values of the images. In addition, MorphoJ software was used to determine the shape differences and directions of the landmarks. According to principal component analysis, male and female individuals were mainly concentrated to the *y* axis's right on lateral images, and female and male individuals were not concentrated in one region on dorsal images. Therefore, according to the principal component analysis, no significant gender difference was found. The consensus graph determined that the oral edge of the incisive tooth and the cranial edge of the canine tooth in females were ventrally directed compared to males. It is thought that the findings obtained as a result of the study will contribute to zooarchaeological and taxonomic research.
Keywords: *Geometric morphometry, MorphoJ, Red fox, Skull.*

Kızıl Tilkilerde (*Vulpes vulpes*) Kafatasının Geometrik Morfometrik Analizi

Özet: Bu çalışma, tilki kafatasının cinsiyete göre geometrik morfometrik yöntemle şekilsel durumunun belirlenmesi amacıyla yapıldı. Bu amaçla 4 dişi ve 9 erkek tilki kafatası kullanıldı. Kafatasları dorsal ve lateral yönlü olacak şekilde fotoğraflandı. Kafataslarının dorsal görüntüleri üzerinde 11, lateral görüntüleri üzerinde 19 homolog landmark işaretlendi. Görüntülerden elde edilen koordinat değerlerinde temel bileşenler analizi ve Relative warp analizi yapıldı. Ayrıca işaretlenen landmarkların şekil farklılıklarını ve yönlerini belirlemek amacıyla MorphoJ yazılımı kullanıldı. Temel Bileşenler analizine göre, lateral görüntülerde dişi ve erkek bireylerin büyük oranda *y* ekseninin sağına toplandığı, dorsal yönlü görüntülerde ise dişi ve erkek bireylerin bir bölgeye yoğunlaşmadığı tespit edildi. Dolayısıyla Temel Bileşenler analizine göre belirgin bir cinsiyet farklılığına rastlanmadı. Konsensus grafiğine göre ise, dişilerde incisiv dişin oral kenarı ile canin dişin cranial kenarının erkeklere göre ventral yönlü olduğu belirlendi. Çalışma sonucunda elde edilen bulguların zooarkeolojik ve taksonomik araştırmalara katkı sağlayacağı düşünülmektedir.
Anahtar kelimeler: *Geometrik morfometri, Kafatası, Kızıl tilki, MorphoJ.*

Introduction

The red fox (*Vulpes Vulpes*) belongs to the order Carnivora. Between the carnivorous, its habitat has one of the most common geographical intervals, and they are located in Europe, Asia, North Africa, and North America (Wozencraft, 2005). The red foxes are strict protection by law in Turkey (Animal Protection Law, 2004).

The carnivores are highly varied in magnitude, diet, social behavior, movement and activity patterns (Kruuk, 2002). This variability is reflected in the dimensions and shape of skulls (Tamlin et al., 2009). Therefore, the morphological structure of the skull is affected by factors such as individual features, genetic, environmental impacts, growth and gender. In evaluating the morphological structure of the skull, traditional craniometric analysis is an essential tool for studies. However, morphological variations can be determined by the geometric morphometric method frequently used in recent years, which allows the skull to be evaluated in shape (Kistner et al., 2021).

Homolog landmarks are used in geometric morphometry and the geometric structure of the samples is digitized in cartesian coordinates. Thus, the sample is examined in shape (Higgins, 2000; Slice, 2007). Slice (2007) reported that the geometric morphometric method has provided more appropriate data for statistical analysis than traditional morphometric studies. Geometric morphometric studies are available in different species in the literature (Demiraslan et al., 2021; Demircioğlu et al., 2021; Duro et al 2021; Gündemir et al., 2020a; Gündemir et al., 2020b; Gürbüz et al., 2020; Yalçın et al., 2010). This study aimed to determine by the geometric morphometric analysis on the skull of adult red foxes in Turkey.

Material and Methods

Ethical approval

Ethical permission of the study was obtained from "Harran University Animal Experiments Local Ethics Committee (Date: 13.07.2020, No: 2020/003)".

Material

A total of 13 adult fox skulls, four female and nine male, were used in the study. These foxes that died in a traffic accident or poaching were obtained from Turkey's Mediterranean and Southeastern Anatolia regions between 2010-2015. The skulls were without any deformation or pathological findings.

Geometric morphometric analysis

Fox skulls were photographed lateral and dorsal at a distance of 30 cm (Canon EOS 650D, Japan). The images were saved to the computer in JPEG format. These images were converted to tps format in TpsUtil (Version 1.79) software (Rohlf, 2019). Nineteen homologous Landmarks (L) (Figs 1 and 2.) on lateral images and 11 homolog landmarks on dorsal images were marked with TpsDig2 (Version 2.31) (Rohlf, 2018) software. A homologous landmark confirmation test was performed using TpsSmall (Version 1.34) (Rohlf, 2017) software.

In the TPS small analysis, the slope and correlation values of the landmarks for lateral were found to be 0.996153 and 0.999997, respectively. The slope and correlation values for dorsal were 0.999783 and 1.0000, respectively. These values show that the landmarks are placed correctly.

Figure 1. Lateral landmarks (L) of the male one.

L1. The most cranial point of os incisivum, **L2.** The cranial point of os nasale, **L3.** The caudal point of os nasale, **L4.** The craniodorsal point of orbita, **L5.** The caudal point of margo supraorbitalis, **L6.** The caudal point of crista sagittalis externa, **L7.** The medial point of back of the cranium, **L8.** The distal point of condylus occipitalis, **L9.** Dorsal point of porus acusticus externus, **L10.** The point of processus retroarticularis, **L11.** The caudal juncture of arcus zygomaticus, **L12.** The point of processus hamatus, **L13.** The cranial juncture of arcus zygomaticus, **L14.** The point of tuberculum maxillare, **L15.** The caudal point of third molar tooth, **L16.** The caudal point of first molar tooth, **L17.** The cranial point of first molar tooth, **L18.** The caudal point of canine tooth, **L19.** The cranial point of canine tooth.

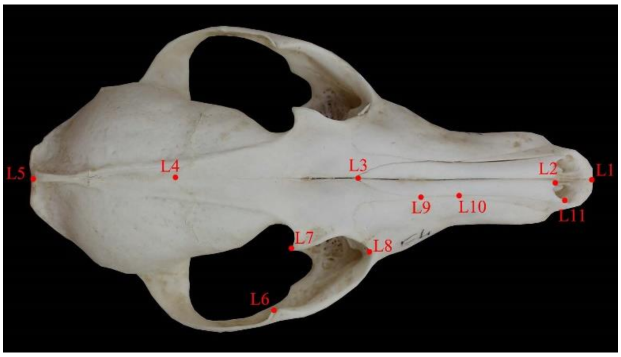

Figure 2. Dorsal landmarks (L) of the male one.

L1. The cranial point of os incisivum, **L2.** Medial point of processus septalis, **L3.** The caudal point of os nasale, **L4.** The frontoparietal juncture in the medial point, **L5.** The caudal point of crista sagittalis externa, **L6.** The medial point of arcus zygomaticus, **L7.** Processus zygomaticus of os frontale, **L8.** The most cranial point of orbita, **L9.** Juncture between os nasale, os maxillare and os lacrimale, **L10.** The point of nasoincisiva, **L11.** Lateral point of processus septalis.

Table 1. Result of principal component analysis on lateral and dorsal images of skulls.

Lateral			Dorsal		
PC	**Eigenvalue**	**% variance**	**PC**	**Eigenvalue**	**% variance**
1	0,00320545	73,609	**1**	0,000475544	38,209
2	0,000267159	6,1349	**2**	0,000196094	15,756
3	0,000217846	5,0025	**3**	0,000163131	13,107
4	0,00016198	3,7196	**4**	0,000126653	10,176
5	0,000137926	3,1673	**5**	9,04343E-05	7,2662
6	0,000110047	2,5271	**6**	7,66027E-05	6,1548
7	8,21893E-05	1,8874	**7**	4,41164E-05	3,5446
8	5,47129E-05	1,2564	**8**	3,26871E-05	2,6263
9	4,90408E-05	1,1262	**9**	2,02809E-05	1,6295
10	3,26029E-05	0,74868	**10**	1,03641E-05	0,83273
11	2,17944E-05	0,50048	**11**	4,94168E-06	0,39705
12	1,39696E-05	0,32079	**12**	3,7419E-06	0,30065

Generalized Procrustes Analysis (superimposition-GPA) was performed because of differences between the skulls in size, position and direction (Slice, 2007). PAST (Version 4.02) (Hammer et al., 2001) software was used for this analysis. Principal Component Analysis (PCA) was performed on the new coordinates obtained from the Procrustes Analysis. Thus, the degree of gender discrimination of the samples was determined by applying Covariance Analysis among the factors (Zelditch et al., 2004). In addition, MorphoJ (Klingenberg, 2011) software was used to determine the landmark levels and directions where shape differences were observed.

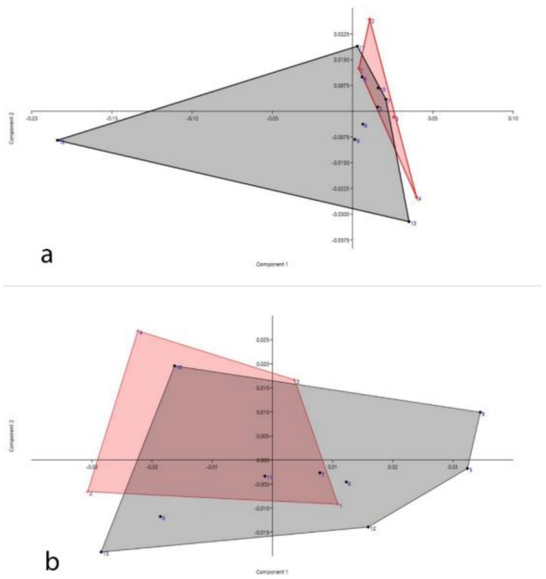

Figure 3. Graphical representation of male and female foxes according to first principal component analysis, **a.** Lateral, **b.** Dorsal.

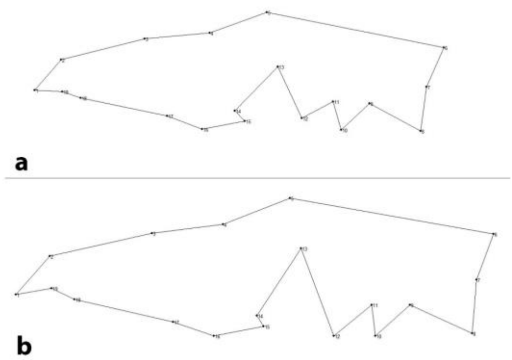

Figure 4. Lateral position of the male (a) and female (b) consensus graphic

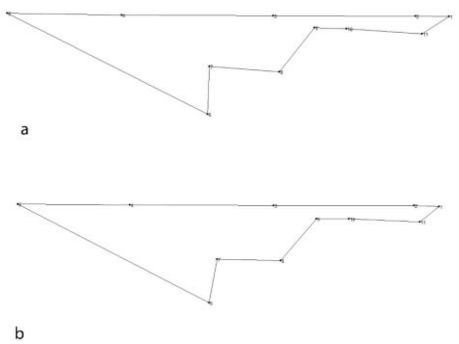

Figure 5. Dorsal position of the female (a) and male (b) consensus graphic.

Results

Lateral

The first principal component explained 73.609% of the total shape variation according to pirincipal component analysis. Besides, from first to fifth principal components explained 91.6333% of the total shape difference (Table 1).

There was no distinctly grouping between females and males on the graphic of PCA 1. The male and female individuals mainly were gathered on the right of the *y* axis on the graphic (Figure 3). Statistical comparison of the landmark values was not made because the number of samples was considered insufficient.

In the relative warp analysis, RWA 1, RWA 2 and RWA 3 were found to be 59.75%, 29.44%, 10.82% in females, 95.54%, 2.04%, and 1.07% in males, respectively.

The consensus graph showed that the oral edge of the incisive tooth and the cranial edge of the canine tooth direction was ventrally in females. In addition, the direction between the cranial edge of the canine (L19) and the oral edge of the incisive tooth (L1) was cranioventral in females. This direction was parallel to the ground in males. L10 and L12 were on the same plane in females. In males, the LM12 was positioned more dorsal to the skull. The direction between L13-L12 that marked arcus zygomaticus was steeper in females than in males (Figure 4).

The direction of L13 was craniodorsal and L12 was caudoventral for the first principal component shown on the PC1 graph (Set scale 0.05). This change was from female to male (Figure 6).

Dorsal

According to the principal components analysis, the first principal component explained 38,209% of the total shape variation. However, from first to fifth principal components explained 84.5142% of the total shape variation (Table 1).

No significant gender difference was observed in the PC 1 graph. It was determined that male and female individuals did not congregate in one region (Figure 3). PC 1 graph showed that the 12th individual gathered in the same region with individuals 5, 6, and 7 on the dorsal aspect. At the same time, a common gathering area was not observed with the other individuals on the lateral view (Figure 3).

The result of Relative Warp Analysis, RWA 1, RWA 2 and RWA 3 were 41.59%, 37.94%, 20.47% in females, and 43.34%, 17.37%, 13.20% in males, respectively. The consensus graph explained that no significant difference was observed between the gender in the dorsal geometric analysis (Figüre 5).

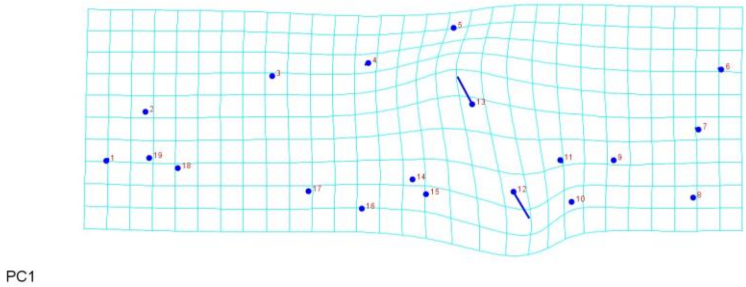

Figure 6. Landmark representation of shape differences of skull between male and female foxes for the first principal component on lateral figure of the skull.

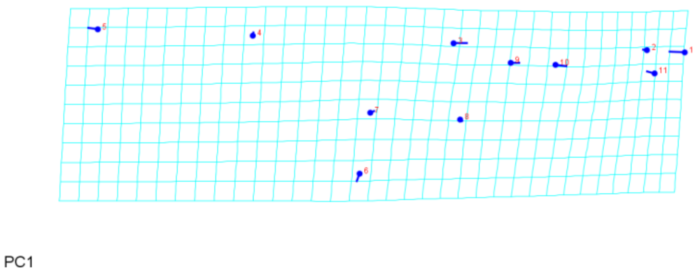

Figure 7. Landmark representation of shape differences of skull between male and female foxes for the first principal component on dorsal figüre of the skull.

PC1 graph (Set scale 0.005) showed that the direction of L1, L5 were cranial, L3, L9, L10 were caudal, L11 was craniodorsal and L6 was cranioventral (Figure 7).

Discussion and Conclusion

In the study, the skulls of red foxes living in Turkey were examined by the geometric morphometric methods between the gender. In literature, morphometric (Dobrowolska et al., 2018; Munkhzul et al., 2018; Onar et al., 2005; Trut et al., 1991) and geometric morphometric (Kistner et al., 2021) studies on the skulls of the foxes obtained from various regions at different times (Ancient era, the new age) are available. However, no study about the skulls of the red foxes in Turkey was analyzed by geometric morphometric method. Thus, considering that morphological structures are affected by environmental factors, this study investigates the skull shape of red foxes in Turkey. Nevertheless, this study includes some limitations in the absence of biometric data and a few materials. The skulls used in the study were obtained from foxes that died due to traffic accidents or natural causes. Therefore, the biometric data of the foxes (head-tail length, weight, etc.) could not be obtained, and a large number of materials could not be reached because the species is under protection. Since only adult fox skulls were used in the study, shape comparisons with young fox skulls could not be made.

Kistner et al. (2021) reported geometric morphometric studies using different fox skull landmarks. Accordingly, wild and farmed foxes were clearly separated, but male and female foxes did not in PC 1 graph. Similar to the literature (Kistner et al., 2021), there was no distinction of gender according to the PC 1 graph in the study.

Parsons et al. (2020) were reported a morphological study on a total of 111 red fox skulls. This study shows that the skulls of foxes living in urban and rural areas were different in shape. They (Parsons et al., 2020) stated that, habitat and gender greatly affected the shape of the skull. In addition, the gender effect was more pronounced, especially on the dorsal views of a skull in PC analysis. In our study using a limited sample and different landmarks from the literature (Parsons et al., 2020), no significant difference was observed in the geometric analysis of the dorsal aspect between the gender shown in the consensus graph.

Anatomically, female foxes have a shorter regio zygomatica and a longer squama temporalis. Therefore, the distance between the arcus zygomaticus and the os frontale is longer. Male foxes have a longer regio nasale (Parsons et al., 2020). Many of these morphological changes may be related to the development of jaw muscles that have changed with nutrition and domestication (Adams and Rohlf, 2000; Wroe et al., 2007). A wide attachment area of the temporal muscle indicates a high bite force, and a thin zygomatic arc indicates a weak masseter muscle. Indeed, finite element modeling of bite in canids, particularly high stresses occur in the zygomatic region (Wroe et al., 2007). So that morphological variation might occur in arcus zygomaticus with jaw movement (Gürbüz et al., 2020, Milencovic et al., 2010). The changes in the zygomatic arc between male and female foxes were remarkable in the study.

Mammalian skulls are highly informative, long-term preservation structures. Therefore, geometric morphometric and traditional morphometric studies are powerful tools for biogeographic, phylogenetic, and taxonomic research, especially in the absence of molecular research (Loy, 2007). Such studies about carnivorous skulls in museums and private collections can reveal a wealth of information and adaptations of these animals (Munkhzul et al., 2018). Therefore, data obtained from archaeological bone material is essential for fauna identification, comparison with other historical processes, and estimation of morphological characteristics of animals (Clark, 1995; Onar and Belli, 2005). In conclusion, we believe that the data of this study will provide official information on the archaeological excavations.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Ethical Approval

This study was approved by the Harran University Animal Experiments Local Ethics Committee (06.02.2020 date and 9196 Number Ethics Committee Decision). In addition, the authors declared that Research and Publication Ethical rules were followed.

Funding

This work is not supported by any Project.

Similarity Rate

We declare that the similarity rate of the article is 1% as stated in the report uploaded to the system.

Author Contributions

Motivation/Concept: İG, YD, FAK, OY, İD
Design: İG, YD, FAK, OY, İD
Control/Supervision: YD
Data Collection and / or Processing:İG, YD, FAK, OY, İD
Analysis and / or Interpretation: YD, İG
Literature Review: İG
Writing the Article: İG, YD, FAK, OY, İD

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***Correspondence**: İftar GÜRBÜZ

Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Anatomy, Burdur, Türkiye.

e-mail: iftargurbuz@mehmetakif.edu.tr