Pakistan J. Zool., pp 1-6, 2022. DOI: https://dx.doi.org/10.17582/journal.pjz/20220613180648

Comparison of Cranium Shape in Hamdani and Awassi Sheep using Dorsal and Lateral Landmarks

İsmail Demircioğlu[1], Yasin Demiraslan[2], Barış Can Güzel[3]*, Ali Koçyiğit[4] and Ayşegül Demircioğlu[5]

[1]*Department of Anatomy, Faculty of Veterinary Medicine, Harran University, Harran university, TR-63200 Eyyubiye, Sanliurfa, Turkey*
[2]*Department of Anatomy, Faculty of Veterinary Medicine, Burdur Mehmet Akif Ersoy University, TR-15030 Burdur, Turkey*
[3]*Department of Anatomy, Faculty of Veterinary, Medicine Fırat University, TR-23100, Elazig, Turkey*
[4]*Harran University Laboratory and Veterinary Health Vocational School, TR-63400, Birecik, Şanlıurfa, Turkey*
[5]*Bursa Uludağ University Institute of Health Sciences, 16059, Bursa, Turkey*

Article Information
Received 13 June 2022
Revised 03 July 2022
Accepted 25 July 2022
Available online 28 October 2022 (early access)

Authors' Contribution
BCG, AD and AK collected material. ID and YD conceived the idea and planned the article. ID and YD wrote the manuscript. BCG edited the manuscript.

Key words
Awassi, Geometric morphometry, Hamdani, Sheep, Procrustes coordinates

ABSTRACT

The aim of this study is to determine whether or not kinship relations, and breed factors of Hamdani and Awassi sheep have an effect on the shape by using geometric morphometry method through their crania. For this purpose, heads of 7 adult Hamdani sheep and 10 adult Awassi sheep were used for the dorsal analysis of cranium and heads of 8 adult Hamdani and 9 adult Awassi sheep were used for lateral analysis of cranium. In the dorsal and lateral comparison of the breeds, the first principal component (PC1) explained 41.905% and 39.078% of the total shape difference, respectively. When the procrustes coordinates were examined, it was found that the samples were both dorsally and laterally mostly similar to the samples in their own group. Consequently, it is thought that the shape analysis of the crania of Hamdani and Awassi sheep breeds was made in detail by geometric morphometry method; thus, yielding the results that may be basic data for many disciplines, especially zooarchaeology, taxonomy, and forensic sciences were presented.

INTRODUCTION

Local sheep breeds in Turkey show morphological differences in order to adapt to the climatic conditions of the relevant region. Even though Akkaraman, Morkaraman and Awassi sheep are generally reared in the eastern and southeastern regions of Turkey, kangal, ayvaz, hamdani, asurani, karakaş, and norduz sheep breeds and types are locally reared in the region (Aytek, 2017; Bärmann *et al*., 2013).

Cranium is a part of the skeletal system that is frequently used in taxonomic classification of living creatures. There are numerous intraspecific polymorphism in sheep (Duro *et al*.,2021; Sosyal *et al*., 2003). Species detection based on the cranium morphology is quite difficult due to the intraspecific variety (Kaymakçi, 2010). Conventional/ classical morphometry remains incapable in such situations. Thus, in recent years, morphometric geometry method has been started to be preferred (Demircioglu *et al*., 2021).

Geometric morphometry is a method that determines the shape differences of the objects via the landmark (LM) coordinates and reveals the amount of shape change (Slice, 2007; Viscosi and Cardini, 2011). In the analysis made by aligning the coordinates on the Cartesian coordinate plane of the determined LMs, inter- and intra-group differences and similarities of the structure are revealed. While LM is being determined, the points that are present at the same location in all the samples are found (Aytek, 2017; Bigoni *et al*., 2010). LMs are grouped in three types according to their anatomic locations. First group (Type I) LMs are the most suitable group with easy repeatability for geometric

* Corresponding author: bcguzel@hotmail.com
0030-9923/2022/0001-0001 $ 9.00/0

morphology. These are the points with clear localizations and identifications that are easy to find. Second group (Type II) LMs are the points placed at the tip or most significant (protrusion) parts of anatomic structures. Third group (Type III- semilandmark) LMs are the points placed based on other LMs (Jashari *et al*., 2022). The increasing number of studies revealing shape differences using geometric morphometry method especially in the field of zoology in recent years has showed the importance and usability of the method (Demircioglu *et al*., 2021; Gündemir *et al*., 2020; Gürbüz *et al*., 2020, 2022; Szara *et al*., 2022).

The aim of the study is to determine the similarities and differences of the crania of Hamdani and Awassi sheep, which are morphologically similar to one another and reared at close locations, using geometric morphometry method and to reveal the shape-dependent variances according to the effect of breed.

MATERIALS AND METHODS

Samples

In the study, heads of 7 adult Hamdani and 10 adult Awassi sheep were used for the dorsal analysis of the cranium and heads of 8 adult Hamdani and 9 adult Awassi sheep were used for the lateral analysis of the cranium. Sheep heads were collected from the butchers in Siirt and Sanliurfa. Materials obtained from clinically normal sheep were boiled and macerated.

Data collection and landmarking

Cranium were photographed by using a camera (18x55 lens, Canon Eos, 600D, Japan) and keeping the focus on the same plane (camera resolution 890 x 1065 pixels). While taking photos in the dorsal direction, the contact point of os nasale and os frontale on the median plane was focused. While taking photos in lateral direction, the ventral edge of the orbit was focused. The distance between the lens and the material was detected as 30 cm. Photographs in Jpg were stored in the computer. By using these photographs, a tps file was created by using TpsUtil (Version 1.79) (Rohlf, 2017) program. Tps file was opened in TpsDig2 (Version 2.31) (Rohlf, 2018) program and 10 homologous landmarks in the dorsal direction (Fig. 1) and 13 homologous landmarks in the lateral direction (Fig. 2) were determined (Jashari *et al*., 2022; Pedrosa *et al*., 2005; Slice, 2007). Thus, *x* and *y Cartesian* coordinates of each anatomic point were obtained. Before the statistical analysis, verification test was done in TpsSmall (Version 1.34) (Slice, 2007) program for landmarks. Accordingly, uncentred correlation and root mean square error values for dorsal landmarks were determined as 0.999999 and 0.000053, respectively, and uncentred correlation and root mean square error values for lateral landmarks were determined as 1.000000 and 0.000015, respectively. These results revealed the accuracy of the landmarks.

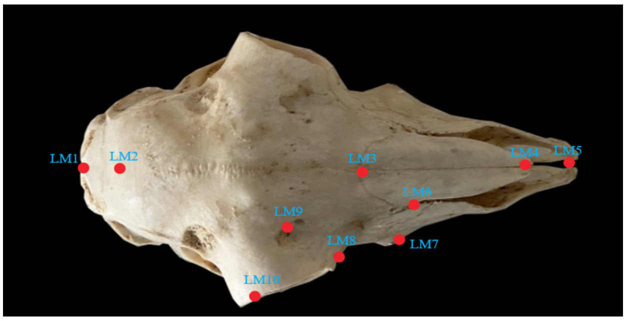

Fig. 1. Dorsal landmarks: LM1, External occipital protuberance; LM2, Junction of sutura coronalis and sutura interfrontalis; LM3, Junction of sutura interfrontalis; sutura internasalis and frontonasal suture; LM4, Anterior edge of sutura internasalis; LM5, Anterior edge of fi ssura interincisiva; LM6, Fissura nasomaxillaris; LM7, Tuber faciale; LM8, Medial angle of orbita; LM9, Foramen supraorbitale, LM10; Posterio-ventral corner of margo supraorbitalis.

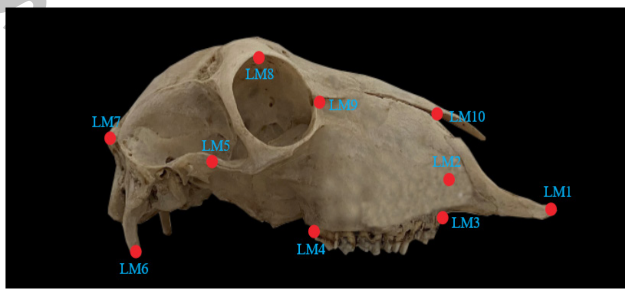

Fig. 2. Lateral landmarks: LM1, Anterior edge of incisiv bone; LM2, Infraorbital foramen; LM3, Anterio-dorsal edge of PM1; LM4, Caudal edge of M3; LM5, Middle point of zygomatic arch; LM6, External acoustic pore; LM7, Ventral edge of jugular process; LM8, External occipital protuberance; LM9, Ventral edge of occipital condyle; LM10, Middle point of margo supraorbitalis; LM11, Medial angle of orbita; LM12, Fissura nasomaxillaris; LM13, Anterior edge of septal process.

Statistical analysis

In the sheep skull photos, General Proctrustes Analysis (superimposition) was conducted due to the differences such as size, position, and direction (Hammer *et al*., 2021). PAST (Version 4.02) (Sosyal *et al*., 2003) program was used for this analysis. Principal component analysis and 2-t test according to breed groups were performed on the new coordinates that were obtained as a result of Procrustes analysis with the same program.

Thus, separation level of the samples according to the breed factor was found by using the covariance analysis (Klingenberg, 2001). MorphoJ (Zelditch *et al*., 2004) program was used to determine at which landmarks the shape differences are concentrated and proximity degree of sheep breed groups with one another.

RESULTS

Table I shows the results of the principal components analysis conducted on the LM coordinates detected in sheep cranium. Accordingly, in the dorsal and lateral comparison of the breeds, the first principal component (PC1) explained 41.905 and 39.078% of the total shape difference, respectively. The differentiation of Hamdani and Awassi sheep crania in terms of PC1, was shown in the graph in Figure 3. Accordingly, Hamdani sheep clustered to the right of the y-axis and Awassi sheep to the left of the y-axis from the dorsal and lateral directions.

Table I. Results of the principal component analysis, PC: principal component.

PC	Dorsal		PC	Lateral	
	Eigenvalue	% Variance		Eigenvalue	% Variance
1	0.00221914	41.905	1	0.0013426	39.078
2	0.00152586	28.814	2	0.000604912	17.607
3	0.000573917	10.838	3	0.000425114	12.374
4	0.000405166	7.651	4	0.000359535	10.465
5	0.000155866	2.9433	5	0.000193815	5.6413
6	0.000129684	2.4489	6	0.000169352	4.9293
7	9.35104E-05	1.7658	7	0.000113514	3.304
8	7.65435E-05	1.4454	8	6.57003E-05	1.9123
9	4.47961E-05	0.84591	9	5.4271E-05	1.5796
10	3.26157E-05	0.6159	10	3.38931E-05	0.98651
11	1.46688E-05	0.277	11	2.7343E-05	0.79586
12	1.26268E-05	0.23844	12	1.90851E-05	0.5555
13	6.93483E-06	0.13095	13	1.29498E-05	0.37693
14	3.87311E-06	0.073138	14	8.21216E-06	0.23903
15	4.18938E-07	0.007911	15	4.11283E-06	0.11971
16	1.37427E-10	2.5951E-06	16	1.23855E-06	0.03605

Figure 4 shows the graphs obtained as a result of the test conducted to determine the proximity level of the samples over the Procrustes coordinates. Thus, it was found that the samples were both dorsally and laterally mostly similar to the samples in their own group.

Figure 5 shows the graphs showing at which LMs the shape differences are concentrated. Accordingly, it was observed that shape differences became clear at the levels of LM3, LM8, LM9, and LM10 from the dorsal direction, LM1, LM4, LM5, LM7, LM8, LM9, LM10, LM11, and LM12 from the lateral direction.

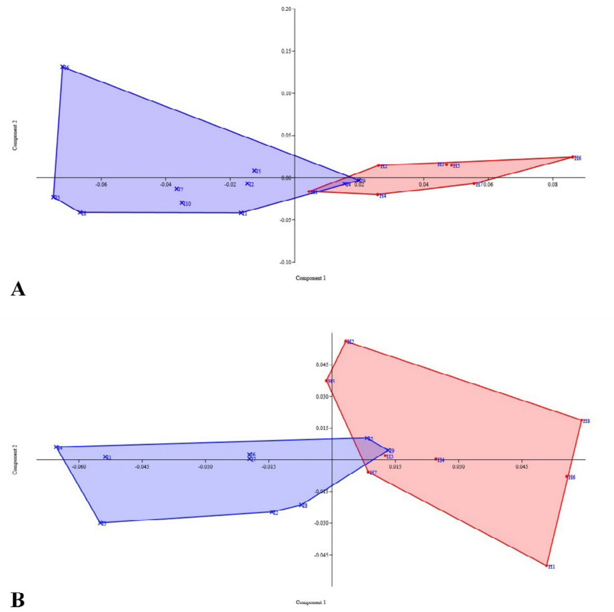

Fig. 3. Distribution of individuals on the graph based on the first principal component; A, Dorsal; B. Lateral; Red, Hamdani, Blue, İvesi.

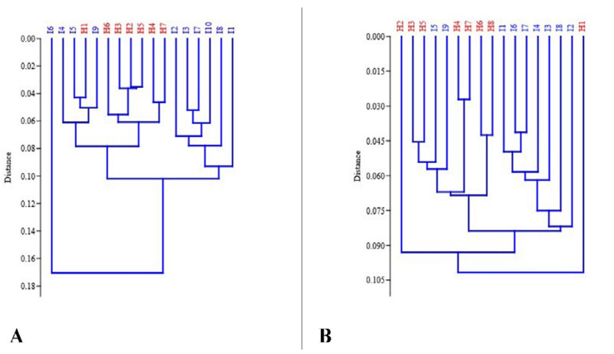

Fig. 4. Graphical representation of hierarchical closeness of individuals. A, Dorsal; B, Lateral, Red, Hamdani; Blue, İvesi.

Table II shows the statistically significant results according to the 2-t test conducted on the Procrustes coordinates according to the sheep breed groups. Accordingly, significant results according to the breed groups both in dorsal and lateral directions were above the values representing the x coordinates of the landmarks.

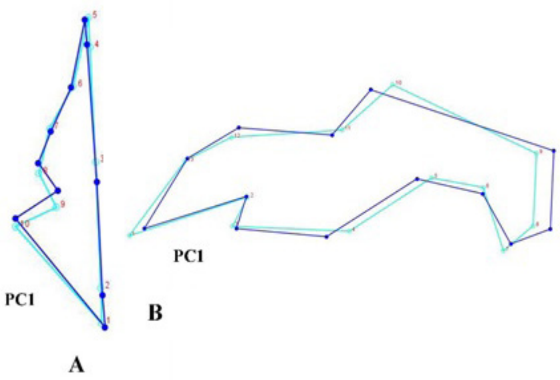

Fig. 5. Wireframe graphical view of shape differences according to the first principal component A. Dorsal, B. Lateral, dark blue color represents the average shape based on the primary principal component.

Table II. Significant (S) or nonsignificant (NS) results of the 2-t test, NA: Not account.

Landmarks	Dorsal		Lateral	
	x	y	x	y
1	S	NS	NS	NS
2	NS	S	S	S
3	S	NS	NS	NS
4	S	NS	NS	S
5	S	NS	NS	NS
6	NS	NS	S	NS
7	S	NS	NS	NS
8	NS	S	S	S
9	NS	NS	S	NS
10	NS	S	S	NS
11	NA	NA	NS	NS
12	NA	NA	NS	NS
13	NA	NA	S	NS

DISCUSSION AND CONCLUSION

Classical morphometry examines the varieties and differences of the shape (Rohlf, 2018). However, this method cannot give the full information of the shape as the measurements are limited (Zeder, 2005). Thus, instead of classical morphology, geometric morphometry is started to be used in some disciplines conducting anthropology, zooarchaecology, and taxonomy studies. The sheep breed, which began to be domesticated approximately 11.000 years ago in Turkey and western Iran, has survived to the present day by undergoing an evolutionary process, primarily according to the changes in climatic conditions and the region where it was raised.

There are evidences about domestication of sheep in Asian, Anatolia, Central and Eastern Mesopotamian regions (Pedrosa *et al*., 2005; Vaughan *et al*., 2005; Zeder, 2008). Together with domestication, it was reported that the first evolutionary change was observed in the horns and cranium (Szara *et al*., 2022). The Mesopotamian region has hosted many civilizations and has an important place in the historical process. Revealing the evolutionary process and cranium characteristics of Awassi and Hamdani sheep raised in Mesopotamia, including Turkey, contributes to zooarchaeological studies in the region.

Size and shape covariances (allometries) of cranium provide important findings for revealing the evolutionary and developmental changes (Pares-casonova and Sabote, 2013). In the study, total shape difference of PC1 was found as 41.905% in dorsal and 39.078% in lateral. These results show that the crania of Hamdani and Awassi sheep have different shapes in terms of breed. In addition, the proximity levels of the samples were examined on the procrustes coordinate plane in the present study and as a result of the examination, it was found that the samples were mostly similar to the samples in their own groups. In other words, although the conditions of the regions where the two breeds were raised were close to each other, significant differences were determined between the groups in terms of shape. In their study (Pares-casonova and Sabote, 2013), stated that PC1 was 79.4% in domestic sheep and 40.1% in wild sheep and argued that this difference could be caused by the breeding conditions. Demircioglu *et al*. (2021) conducted a geometrical morphometry study on the cranium of Awassi sheep and compared sexes both from the dorsal and lateral directions, the PC1 total shape difference was reported as 37.719% and 44.238%, respectively, and they also stated that the crania of female and male individuals clustered significantly in both dorsal and lateral directions. The findings of the present study also support this opinion.

Zooarchaeological remains are important in terms of the estimation of morphological characteristics of the animals, determination of fauna, or for enabling socio-economic comparisons (Clark, 1995; Gündemir *et al*., 2020; Onar and Belli, 2005). Morphological data to be obtained from the cranium of living mammalians by geometrical morphometry method can be used to reveal the phylogenetic relation (Marcus *et al*., 2000). Zeder (2006) reported that the comparison of zooarchaecological findings could be more accurate by examining the animals in the same region. Thus, it is important to reveal the geometrical morphometric characteristics of animal breeds of the eastern and southeastern regions of Turkey, where archaeological excavations are gradually increasing.

Consequently, in the present study, the detailed shape analysis of the crania of Hamdani and Awassi sheep breeds, which are reared in the eastern and southeastern regions of Turkey and whose phenotype and yield characteristics are close to each other, was realized by using the geometric morphometry method. It is thought that these results can be principal data for many disciplines, especially zooarchaeology and taxonomy sciences.

Availability of data and materials

The authors declare that data supporting the study findings are also available to the corresponding author**.**

Funding support

There is no funding source.

Statement of conflict of ınterest

The authors have declared no conflict of interest.

REFERENCES

Aytek, A.İ., 2017. Geometrik morfometri. *Masrop E-Dergi*., **11**: 1-7.

Bärmann, E.V., Wronski, T., Lerp, H., Azanza, B., and Börner, S., 2013. Morphometric and genetic framework for *Gazella*. *Zool. J. Linn. Soc*., **169**: 673-696. https://doi.org/10.1111/zoj.12066

Bigoni, L., Velemínská, J., and Brůžek, J., 2010. Three-dimensional geometric morphometric analysis of cranio-facial sexual dimorphism in a Central European sample of known sex. *HOMO,* **61**: 16-32. https://doi.org/10.1016/j.jchb.2009.09.004

Clark, K.M., 1995. The later prehistoric and protohistoric dog: The emergence of canine diversity. *Archaeozoologia*, **7**: 9-32.

Demircioğlu, İ., Demiraslan, Y., Gürbüz, İ., and Dayan, M.O., 2021. Geometric morphometric analysis of skull and mandible in Awassi ewe and ram. *Kafkas Univ. Vet. Fak. Derg*., **27**: 43–49.

Duro, S., Gündemir, O., Sönmez, B., Jashari, T., Szara, T., Pazvant, G., and Kambo, A., 2021. A different perspective on sex dimorphism in the adult hermann's tortoise: Geometric morphometry. *Zool. Stud*., **60**: e9.

Gündemir, O., Özkan, E., Dayan, M.O., and Aydoğdu, S., 2020. Sexual analysis in turkey (*Meleagris gallopavo*) neurocranium using geometric morphometric methods. *Turk. J. Vet. Anim. Sci*., **44**: 681–687. https://doi.org/10.3906/vet-1910-92

Gürbüz, I., Aytek, A.I., Demiraslan, Y., Onar, V., and Özgel, Ö., 2020. Geometric morphometric analysis of cranium of wolf (*Canis lupus*) and German shepherd dog (*Canis lupus familiaris*). *Kafkas Univ. Vet. Fak. Derg*., **26**: 525–532.

Gürbüz, İ., Demiraslan, Y., Aksünger, K.F., Yılmaz, O., and Demircioğlu, İ., 2022. Geometric morphometric analysis on the skull of the red fox (*Vulpes vulpes*). *Harran Üniv. Vet. Fak. Derg*., **11**: https://doi.org/10.31196/huvfd.1012563

Hammer, Q., Harper, D.A.T., Ryan, D.R., 2001. PAST. Paleontological statistics software package for education and data analysis. *Palaeontol. Electron*., **4**: 9.

Jashari, T., Kahvecioğlu, O., Duro, S., Gündemir, O., 2022. Morphometric analysis for the sex determination of the skull of the Deltari Ilir dog (*Canis lupus familiaris*) of Kosovo. *Anat. Histol. Embryol*., https://doi.org/10.1111/ahe.12807

Kaymakçı, M., and İleri, K.Y., 2010. *Meta basim matbaacilik*, İzmir.

Klingenberg, C.P., 2001. Morpho J: An integrated software package for geometric morphometrics. *Mol. Ecol. Resour*., **11**: 353–357. https://doi.org/10.1111/j.1755-0998.2010.02924.x

Marcus, L.F., Hingst-Zaher, E., and Zaher, H., 2000. Application of landmark morphometrics to skulls representing the orders of living mammals. *Hystrix*, **11**: 2747

Onar, V., and Belli, O., 2005. Estimation of shoulder height from long bone measurements on dogs unearthed from the Van-Yoncatepe early iron-age necropolis in Eastern Anatolia. *Rev. Med. Vet*., **156**: 53-60.

Parés-Casanova, P.M., and Sabaté, J., 2013. Shape, not only size, differentiate wild and domestic *Ovis*. *Indian J. appl. Res*., **3**: 633-636. https://doi.org/10.15373/2249555X/JULY2013/199

Pedrosa, S., Uzun, M., Arranz, J., Gutiérrez-Gil, B., Primitivo, F.S., 2005. Evidence of three maternal lineages in Near Eastern sheep supporting multiple domestication events. *Proc. R. Soc*., **272**: 2211–2217. https://doi.org/10.1098/rspb.2005.3204

Rohlf, F.J., Marcus, L.F., 1993. A revolution in morphometrics. *Trends Ecol. Evol*., **8**: 129-132. https://doi.org/10.1016/0169-5347(93)90024-J

Rohlf, F.J., 2018. *TpsDig version 2.31. Ecology and evolution*. SUNY at Stone Brook, USA.

Rohlf, F.J., 2017. *TpsSmall Version 1.34. Ecology and evolution*. SUNY at Stone Brook, USA 2017.

Rohlf, F.J., 2019. *TpsUtil program Version 1.79. Ecology and evolution*. SUNY at Stone Brook USA 2019.

Slice, D.E., 2007. Geometric morphometrics. *Annu. Rev.*

Anthropol., **36**: 261–281. https://doi.org/10.1146/annurev.anthro.34.081804.120613

Soysal, M.İ., Özkan, E., and Gürcan, E.K., 2003. The status of native farm animal genetic diversity in Turkey and in the World. *J. Bulgar. Anim. Sci., ,* **2003**: 11-12.

Szara, T., Duro, S., Gündemir, O., and Demircioğlu, I., 2022. Sex determination in Japanese quail (*Coturnix japonica*) using geometric morphometrics of the skull. *Animals*, **12**: 302. https://doi.org/10.3390/ani12030302

Vaughan, T.A., Ryan, J.M., and Czaplewski, N.J., 2015. Mammalian domestication. In: *Mammalogy*. 6th edition. Jones and Bartlett Publishers, Massachusetts, USA.

Viscosi, V., and Cardini, A., 2011. Leaf morphology, taxonomy and geometric morphometrics: a simplified protocol for beginners. *PLoS One*, **6**: e25630. https://doi.org/10.1371/journal.pone.0025630

Zeder, M.A., 2005. A view from the Zagros: New perspectives on livestock domestication in the fertile crescent. In: *The first steps of animal domestication: New Archaeological Approaches*. Oxford.

Zeder, M.A., 2006. Central questions in the domestication of plants and animals. *Evol. Anthropol*., **15**: 105-117. https://doi.org/10.1002/evan.20101

Zeder, M.A., 2008. Domestication and early agriculture in the Mediterranean basin: Origins, diffusion and impact. *Proc. natl. Acad. Sci*., **105**: 11597-11604. https://doi.org/10.1073/pnas.0801317105

Zelditch, M.L., Swiderski, D.L., Sheets, H.D., and Fink, W.L., 2004. *Geometric morphometrics for biologists. A primer, USA*.