REVIEW ARTICLE

GEOMETRIC MORPHOMETRY IN VETERINARY ANATOMY

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ABSTRACT

Geometric morphometry is a shape analysis method based on the analysis of landmarks, curves and contours, all geometric data from two or three-dimensional Cartesian coordinates. In this analysis, analysis is made on 2 or 3 dimensional samples using Landmark processes. After the Landmark processes, Generalized Procrustes Analysis is applied to standardize the coordinate data before statistical analysis. Then, with Procrustes Analysis, the points are superimposed, and, then, the average shape is obtained. With Principal Component Analysis, shape variations are obtained for all samples. Shape differences between groups can be revealed using Discriminant Function Analysis and Canonical Variate Analysis. In recent years, shape analysis has been used in veterinary anatomy, and shape variations between samples have been revealed. Geometric morphometry, which includes more interpretation features than linear measurements, has brought a different perspective to veterinary anatomy. It contains useful reference information, especially in the field of gender analysis and taxonomy.

Keywords: Canonical variate analysis, discriminant function analysis, morphology, principal component analysis, shape analysis

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1. INTRODUCTION

Geometric morphometry (GM) is a method based on the analysis of anatomical points, curves and contours, all geometric data taken from two- or three-dimensional Cartesian coordinates (Bookstein, 1997; Aytek, 2017). With these data, physical variations and functional differences within and between groups are explained as a result of the analysis of the shape. (Aytek, 2017; Klingenberg and Marugán-Lobón, 2013).

The basic principle of GM is shape analysis. In this context, there is a usage area on every object that has a shape (Klingenberg, 2015). There is a field of use on the samples obtained as a result of GM physical residues (Aytek, 2016; Caruana and Herries, 2021). In recent years, it has been used especially on plant and animal materials, and shape variations of biological samples have been revealed. Also, Geometric morphometrics is an important additional approach for discrimination among populations and individuals in forensic (Manthey and Ousley, 2020)

GM studies have been used instead of linear studies in recent years. As a result of linear studies, we obtain the distance or angle between two points. In linear methods, the explanation part of the comments is limited. In GM, on the other hand, a shape between the determined points emerges, and variation of the shapes within the group is obtained (Jashari et al., 2022). GM gives more results in terms of interpretation. The GM method, which allows to examine a large number of shape variables together, is also supported by statistical data; it presents the results in a graphical representation with numerical and figural analyzes (Mitteroecker and Gunz, 2009).

The aim of this review is to present an overview of geometric morphometrics as an additional approach to morphometric studies in veterinary anatomy, explain how to discriminate shape data and their groups from one another, clarify the issue of sample and landmark numbers that should be used in morphometric studies and define frequently used programs and analyzes in the field of in GM.

2. LANDMARKS

In the GM, anatomical points are determined and marked on the shape of the sample. The anatomical points in question are landmarks (Bookstein, 1997; Aytek, 2017). Landmarks are homologous, they can be found in the same way in every repetition, they stand in the same plane (Bookstein, 1997). A Landmark must be a dot in each instance within or between groups. The data required for GM is obtained from landmarks defined in 2-D or 3-D samples (Adams et al., 2018; Berio and Bayle, 2020).

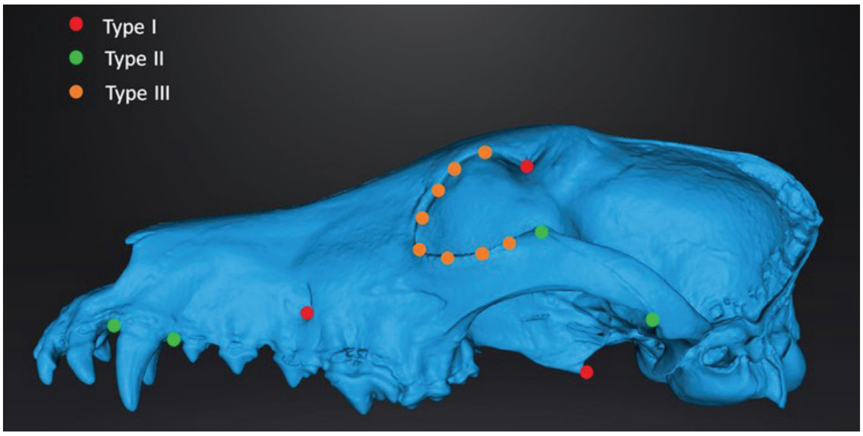


Figure 1
Type of Landmarks

Bookstein classified landmarks into 3 groups (Figure 1):

Type I: Landmarks are determined by biological position. Their locations are obvious. It is the most suitable type for landmark studies because it is very easy to define.

Type II: These are the points that express the most recessed-protruding or the most extreme point of the shape.

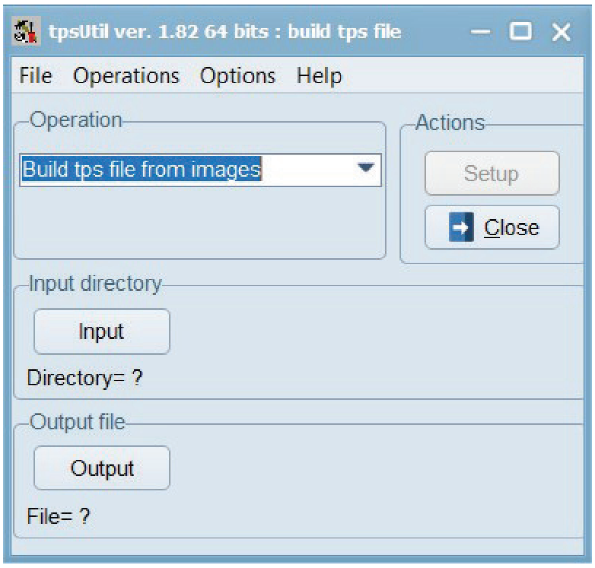
Type III: In this landmark type, the points are determined based on the other landmarks. Semi-landmark points along a curve are also called Type III landmarks.

The number of landmarks taken from the materials in the GM studies must be the same. If the material is damaged and its form is distorted, the number of landmarks is reduced by considering the regions where the symmetry line is missing, or it is possible to take landmarks from these regions by reconstructing the missing regions.

2.1. LANDMARK OPERATIONS IN TWO-DIMENSIONAL EXAMPLES

The GM method can be applied to 2D samples. Photographs or x-rays can be used for this purpose (Duro et al., 2021; Gündemir et al., 2021; Gurbüz et al., 2022).

First, the photographs of the samples are obtained. Then, the photos must be converted to "tps" format for Landmarking operations. For this, the tpsUtil program can be used (Rohlf, 2018).



tpsUtil ver. 1.82 64 bits : build tps file

Operation: Build tps file from images

Directory= ?

File= ?

Figure 2 TpsUtil

The stages of introducing visuals to the program with TpsUtil (Figure 2):

1. After logging into the program, select 'Build tps file for images' from the 'Operations' section at the top.
2. The file containing the sample images to be introduced in the 'Input' section is selected.
3. The tps file to be created in the 'Output' section is given a name.
4. 'Setup' is selected from the 'Actions' section.
5. Introducing the samples to the program is completed by selecting 'Create' with all files marked in the window that opens.

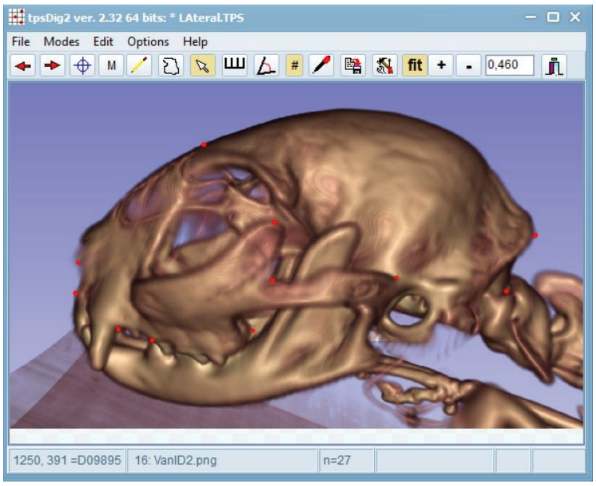


Figure 3 TpsDig

Then, landmark marking is done on the "tps" files by using the "TpsDig2" program (Rohlf, 2004) (Figure 3).

Stages of landmark marking with TpsDig2:

1. Select 'Input source' from the 'File' section.
2. The tps file, where the samples to be landmarked are saved, is selected.
3. Using the 'Digitalize landmark' command in the 'Modes' tab, landmarks are marked. At this stage, the landmarks need to be marked in the same order because only then the coordinates can be determined without error.
4. Save data by selecting 'Save data' from the 'File' section. No changes are made to the filename part.
5. In the 'File exist' field that appears on the screen, the 'Overwrite' option is checked.
6. Landmarks are saved as text file.

2.2. LANDMARK OPERATIONS IN THREE- DIMENSIONAL EXAMPLES

The three- dimensional (3D) technology had made large use in GM in many studies. In this section, Stratovan Checkpoint, which is used in GM studies with 3D samples, will be mentioned (Kraatz and Sherratt, 2016). Stratovan Checkpoint is a software package for GM that allows to mark, manipulate and analyze landmarks and semi-landmarks in 3D (Figure 4).

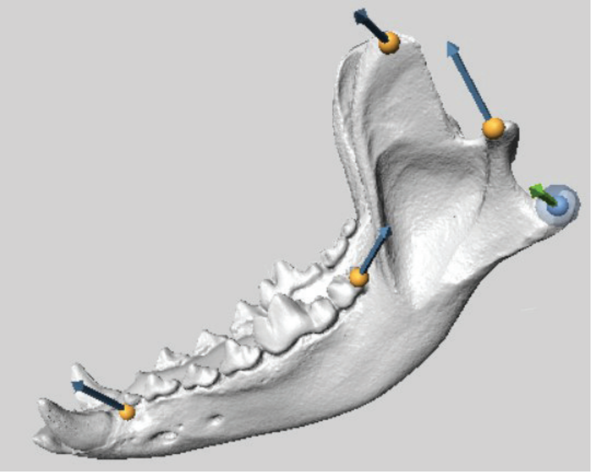


Figure 4 Landmark Operations in Mandible

Stages of landmark marking with Stratovan Checkpoint:

1. 'Browse' is marked. The file where the 3D samples to be used are saved is selected.
2. After a sample is selected, 'Open Spacemen' is ticked.
3. Click on 'Surface'. Landmarks are marked.
4. In the 'Landmark' section, the landmarks can be numbered or named.

3. STATISTICAL ANALYSIS IN GEOMETRIC MORPHOMETRIC

3.1. GENERALIZED PROCRUSTES ANALYSIS

It is necessary to take an overview of the concept of 'shape' to understand Generalized Procrustes Analysis. Shape is all the geometric features of an object other than its size, position, and direction. The first step of GM studies is to extra ct shape data from the object. (Fig. 5A). All objects are scaled to the same size (Fig. 5B) by dividing all landmark coordinates by the size of the centroid of the respective structures. Thereafter, all objects are centered on the same center of gravity (Fig. 5C). Finally, the object is rotated around this common center of gravity until the sum of squared deviations between corresponding landmarks is minimal (Fig. 5D) (Manthey and Ousley, 2020)

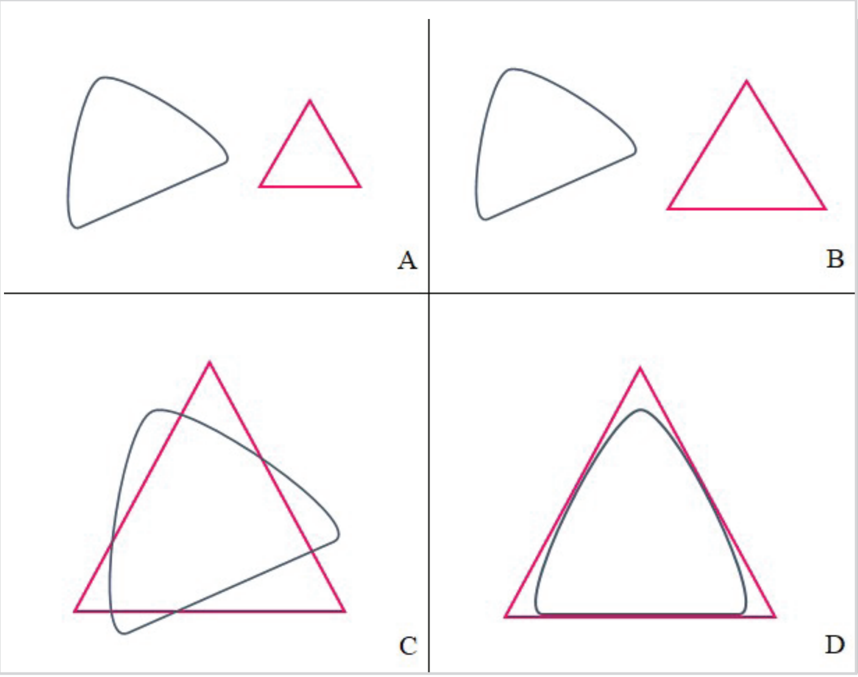


Figure 5
(A) Original configurations,
(B) scaling to the same size,
(C) translation to the same location,
(D) rotation to optimal fit

This procedure usually requires a generalized Procrustes fit when more than two configurations are involved. Thus, one configuration becomes the target configuration, and all other configurations are superimposed on it. The superimposed configurations, including the target, are averaged and rescaled to a center size of 1.0. It results in a consensus configuration that is, then, used as the target configuration for a second round, where each individual is placed on the new target, and a new consensus configuration is then calculated, similar to the first round. This time, the target is excluded as it does not belong to the bookmark configurations in the dataset. The whole procedure is repeated until the consensus no longer changes. This usually happens after just a few rounds. As a result, all examined configurations are superimposed as close as possible to the average shape of individuals (Dryden and Mardia, 2016). All remaining information is such that it can be used for actual analysis later (Manthey and Ousley, 2020).

Generalized Procrustes Analysis is one of the methods used to standardize coordinate data (Özkoçak and Alkaya, 2017). With the Procrustes Analysis, based on the value of "center of gravity" (the square of the distance of all landmarks from the center of gravity), the samples are superimposed over the distance of this point to the tangent plane and the differences can be observed (Özkoçak and Alkaya, 2017) (Figure 6).

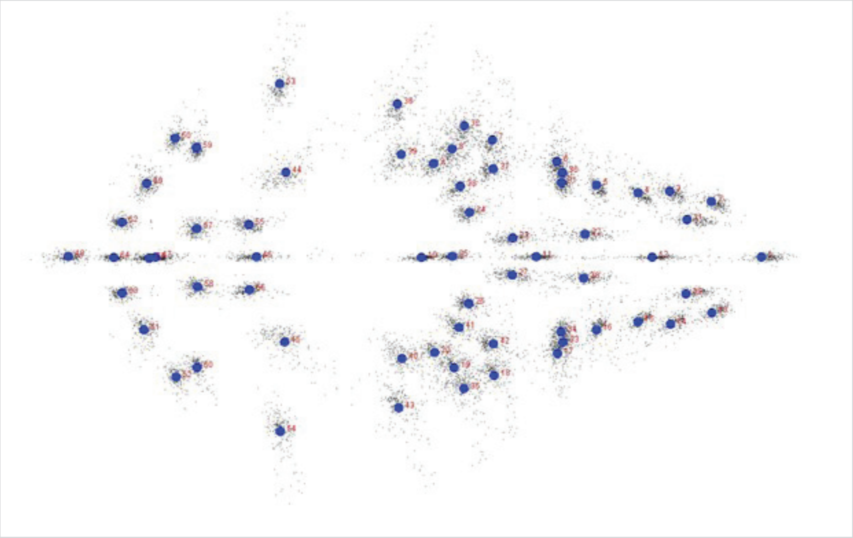


Figure 6 Procrustes Fit

3.2. PRINCIPAL COMPONENT ANALYSIS

Principal component analysis (PCA) is one of the most widely used methods for multivariate analysis. It can be used as an analysis method to view the main features of shape change in a dataset and also to discover relationships between the observations. It reduces the dimensionality of the dataset by converting it to a new coordinate system, where the variation in the data can be defined with fewer dimensions than the original data (Bro and Smilde, 2014; Jolliffe, 2005).

Although different statistical analyzes are used in GM studies, the most frequently used analysis is PCA. This application can also be realized with many softwares. PCA reveals shape variations, not between-group differences. Variation is analyzed in various aspects depending on the number of variables in the data. The direction of shape change, which can be expressed by a linear equation using weights for each landmark coordinate, includes the largest possible change and is called the first major PC1 (Manthey and Ousley, 2020).

The main purposes of PCA can be listed as: reducing the size of the data, making predictions, and viewing the data set for some analysis. By looking at multidimensional data from the right angle, relationships in the data can often be explained. PCA focuses on finding that "right angle". (Koçak, 1998)

In PCA, the appropriate coordinate system is sought by the following steps:

As the 1st axis, the direction with the largest change of data is selected.

As the 2nd axis, the direction perpendicular to the previous 1st axis and at the largest change of data is selected.

As the 3rd axis, the direction perpendicular to the previous 1st and 2nd axes and which is at the largest change of the remaining data is selected.

The direction with the largest remaining change in the data is always chosen as the new axis.

PCA is an ideal method for size reduction, representing multidimensional data approximately and with less dimensional data. Multidimensional data finds the orthogonal-largest-variance-directions for the original data and displays the original data in this coordinate system. One of the most important advantages of the PCA method in geometric studies in the field of veterinary anatomy is that the little-changing PCA properties are unimportant for modelling. In this way, it can speed up the computation related to the modeling (Jolliffe, 2005).

3.3. DISCRIMINANT FUNCTION ANALYSIS

Discriminant Function Analysis (DFA) is a method for obtaining optimal decision rules for distinguishing groups. In fact, it's just the same as a two-group Canonical Variate Analysis (CVA). However, what matters in DFA is not the relative arrangement of multiple groups, as in CVA, but rather the degree of separation of the groups tends to be. Accordingly, more emphasis is placed on the possibility of correct and incorrect classification of observations (Büyüköztürk and Çokluk-Bökeoğlu, 2008).

CFA is a multivariate statistical procedure that classifies unknown individuals and the probability of them being classified into a particular group (such as gender or ancestry). It is based on building a model to predict group memberships. This model consists of discriminant functions that emerge based on a linear combination of predictive variables that provide the best discrimination between groups. These functions are derived from a sample whose group memberships are known. It can then be applied to new individuals or units with the same variables and measurements of unknown group memberships (Mundry and Sommer, 2007).

Discriminant analysis can also be defined as a set of procedures used to classify individuals or units under the populations to which they belong, with minimal error. These groups can be different treatment groups (experimental group, control group, etc.) or naturally formed groups (male, female, etc.) created by researchers in experimental research designs (Dibennardo and Taylor, 1983; Walker, 2008). Discriminant analysis attempts to identify one or more functions as a linear combination of variables that maximizes the differences between individuals in two or more groups.

Discriminant analysis can be used for the following different purposes. Classifying individuals or units, testing theories about whether it is possible to classify individuals or units based on predictions, examining differences between groups, classifying groups, determining the rate of variance explained by independent variables in dependent variables, evaluating the relative importance of independent variables in classifying according to dependent variables, and eliminating unimportant variables can be given as an example for these purposes (Diekhoff, 1992).

3.4. CANONICAL VARIATE ANALYSIS

Canonical Variate Analysis (CVA) is a widely used method for analyzing group structure in multivariate data. It is mathematically equivalent to a one-way multivariate analysis of variance and is also called Canonical Discriminant Analysis. Its purpose is to develop models whose purpose is to determine what has changed and what has not changed in group structure. Three approaches were adopted: the maximum likelihood approach, the least squares approach, and the covariance structure analysis approach. The common point of all approaches is that they assume canonical variables that are stable over time (Campbell and Atchley, 1981).

CVA is used when you want to analyze groups within samples, such as gender or ancestry. It is a multivariate procedure that maximizes the distinction between groups according to the variation within the groups and allows an unknown individual to be classified into one of the known groups. Same as Discriminant Function Analysis if there are only two groups in the analysis (Manthey and Ousley, 2020).

3.5. MULTIVARIATE REGRESSION

Multivariate regression is the method used when we need to include more than one dependent variables and an independent variable in the analysis. It can be helpful for studies on allometry or evolutionary change in shape over time (Klingenberg, 2016). For example, we can study changes in size and shape of the cranium of a certain animal group during infancy and puberty ages with multivariate regression. In this example, age is an independent variable, and size and shape of the cranium are the dependent variables. With multivariate regression, variation in the independent variable can be explained or predicted by variation in one or more of the dependent variables (Manthey and Ousley, 2020).

4. SYMMETRY AND ASYMMETRY

Symmetry is a fundamental feature of the body plans of most organisms and their many parts, and can be defined as the repetition of parts in different positions and directions relative to each other. Animals are usually bilaterally symmetrical, with their left and right sides mirror images of each other. However, bilateral symmetry is not the only type of symmetry in biological structures (Klingenberg, 2015). For example, different symmetries can be observed in organs, bones and plants.

Other types of symmetry are often more complex than bilateral symmetry, for example, most of them contain more than two parts. They are called complex symmetry (Klingenberg, 2015). This term refers to all types of symmetry except bilateral symmetry. Shapes with complex symmetry requıre special methods for morphometric analysis.

Common examples in organisms are: dissymmetry or biradial symmetry as another type of reflexive symmetry besides bilateral symmetry, which describes a situation where there are (at least) two vertical axes of symmetry within an object. Rotational symmetry is often seen in flowers. Each part of the object has a median axis that rotates around the center until the parts form a complete circle. Translational symmetry, or serial homology, is the repetition of parts along an axis, such as the vertebrae in the spine (Klingenberg, 2015). All types of symmetry can manifest as either matching symmetry or object symmetry. Matching symmetry means that a structure exists in two different copies, which are mirror images of each other. In object symmetry, the object itself is symmetrical with the axis of symmetry passing through the structure, such as the human face. In either case, asymmetry can be detected by mirroring or superimposing one piece on top of the other. The important distinction is the correction of size differences. For object symmetry, the entire configuration is considered as a whole and, hence, the size differences between both sides are shape asymmetry. Matching symmetry, on the other hand, consists of two superimposed configurations that allow the size and shape differences to be viewed separately (Klingenberg, 2015).

Asymmetry is simply a deviation from symmetry (Klingenberg, 2015). There are three kinds of asymmetry: directional asymmetry, fluctuating asymmetry, and antisymmetry. They are identified within populations, not individual individuals, through measurement and analysis of samples, and can occur both exclusively and together in the same trait. Asymmetry can be viewed not as a single biological process, but as a manifestation of biological processes. (Klingenberg, 2015). An example of directional asymmetry is the arrangement of internal organs in the body. The features develop consistently differently on the right and left side of the body; for example, the lungs consist of three lobes on the right and two lobes on the left. The difference between the right and left means can be used to measure such asymmetry. There are many studies showing that directional shape asymmetry is common in the animal kingdom (Klingenberg, 2015). Fluctuating asymmetry is the amount of variation in the shape of a single individual from the genetically determined “target phenotype”. It occurs as small differences between the left and right sides due to random errors in individual development and can be seen as the differentiation of the individual from the mean directional asymmetry (Klingenberg, 2015). Antisymmetry describes a phenomenon in which the majority of individuals are asymmetrical, but there is also variation in aspects of asymmetry. This causes some individuals to be “left-sided” and others “right-sided”, a common observation in plants and animals (Klingenberg, 2015).

5. SOFTWARES

5.1. MORPHOJ

MorphoJ is a program for performing GM. The purpose of the program is to provide a platform for the most important types of analysis in GM (Klingenberg, 2011).

MorphoJ offers a variety of shape analyses starting from the raw coordinate data that can be imported from text files or files in formats of other morphometric programs. The program is written for both 2D and 3D data. Program can also search for outliers, and repair landmarks that were accidentally switched. ‘Variations’ part in the program includes ‘Procrustes ANOVA’ option, and ıt can be used to quantify measurement error if single specimens have been recorded repeatedly. After that step statistical analyses can be performed. It has multivariate statistics such as: PCA, canonical variate analysis and discriminant analysis, matrix correlation and matrix permutation test. It can also perform multivariate regression analysis to study the relationships of shape with time, size (allometry) or other variables. Program can divide the shape variation into components of symmetric and asymmetric variation and provide measures of individual asymmetry that can be correlated with other factors. Briefly, it provides various analyses concerning the quantitative genetics of shape and selection for shape.

5.2. R PROGRAMS

In this section, an adequate software, Geomorph by Dean Adams, will be mentioned. Several software packages are available for applying geometric morphometrics. Moreover, Geomorph is freely available software that implements all stages of Procrustes analysis in a single computer package, including digitization of samples and two- and three-dimensional analysis of both fixed landmarks and semilandmarks. Geomorph is a software package that performs geometric morphometric shape analysis in the R statistical computing environment. In geomorph, researcher can do all GM routines: digitizing landmarks on two and three-dimensional objects, reading and manipulating landmark data files, generating shape variables via Procrustes analysis for points, curves and surfaces, performing statistical analyzes of shape variation and covariation, and visualizing shape variations with graphical methods (Adams & Otarola-Castillo 2013).

6. SHAPE ANALYSIS IN VETERINARY ANATOMY

In recent years, different studies have been carried out in terms of gender analysis and taxonomy using shape analysis (Demiraslan et al., 2021; Demircioğlu et al., 2022; Gundemir et al., 2022; Hadžiomerović et al., 2022). In a study conducted in turtles, GM was applied to carapace, and it was revealed that this method was a distinctive method in males and females (Duro et al., 2021). Gender analysis has been studied in studies on skulls in turkeys and quails (İlgün et al., 2016). In foxes, skulls of males and females were examined using the GM method (Gürbüz et al., 2022). Morphological variations in the skulls of male and female fallow deer (Dama dama mesopotamica) were evaluated by the GM method (Abbasabadi et al., 2020). Radiographs of Thoroughbred horses were examined, and radiometric and geometrical characteristics were compared between male and female individuals by applying GM (Gundemir et al., 2021). In another study, Ossa coxae in horses were examined with GM, and statistically significant data were obtained in terms of male and female distinction (Gündemir et al., 2020). Sex discrimination within the same species may be possible using geometric shape analysis (Szara et al. 2022). Most recent study investigated geometric analysis of the auditory bones in the red fox as well as skull variation in different sheep breeds (Hadžiomerović et al., 2023; Gündemir et al., 2023).

In addition to sexual comparison studies, there have also been studies examining intra- and inter-species variations. Geometric morphometric analysis was performed on skull radiological images to measure intraspecific variation in craniomandibular morphology in wild and domestic rabbits (Böhmer and Böhmer 2017). In the study with bat species, traditional and geometric morphometric methods were compared (Schmieder et al., 2015). By studying the analysis of the skull and mandible of Awassi ram and sheep with geometric morphometric methods, the dimorphism of the skull between the sexes was investigated (Demircioğlu et al., 2021). The distinguishing features of the sphenoid bone between sheep and goats were investigated using the GM method (Parés-Casanova et al., 2021). In a study of Araucanian horse skulls with linear morphometry and GM, these two methods were compared, and it was revealed that GM was more distinctive and provided more information than linear morphometry (Parés-Casanova et al., 2020). In another study, the lower jawbone and skull shape of wolf and German Shepherd dogs were investigated by geometric morphometric analysis method (Gürbüz et al. 2020). There is also a study describing the differences in dorsal profiles and head shapes of donkey, pony and horse species using GM (Maśko et al., 2022).

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTIONS

Concept – OK, OG.; Design – IB, NM, TS; Supervision – GP, NGI, NH; Resources - EO; Materials – TS; Data Collection and/or Processing – EO, TS; Analysis and/or Interpretation – YA; Literature Search – IB, NM; Writing Manuscript – OG; Critical Review – NH.

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GEOMETRIJSKA MORFOMETRIJA U VETERINARSKOJ ANATOMIJI

SAŽETAK

Geometrijska morfometrija je metoda analize oblika koja se zasniva na analizi tačaka, krivulja i kontura, svih geometrijskih podataka iz dvo ili trodimenzionalnih Kartezijevih koordinata. U ovoj analizi je izvršena analiza 2 ili 3-dimenzionalna uzorka korištenjem tačaka. Generalizirane Prokrustove analize se koriste za standardizaciju koordinatnih podataka prije statističke analize. Onda se korištenjem Prokrustove analize tačke superponiraju, pri čemu se dobije prosječni oblik. Za sve uzorke su izvršene analize osnovnih komponenti i analize oblika. Razlike u oblicima između grupa se mogu dokazati korištenjem diskriminantnih analiza funkcija i kanoničkom varijantnom analizom. Zadnjih godina, u veterinarskoj analizi se koristi analiza oblika, pri čemu su otkrivene varijacije oblika. Geometrijska morfometrija koja obuhvata više interpretacijskih elemenata u odnosu na linearna mjerenja, pružila je veterinarskoj anatomiji novu perspektivu. Ona sadrži korisne referentne informacije, posebno u području rodne analize i taksonomije.

Ključne riječi: Kanonička varijantna analiza, diskriminantna analiza funkcija, morfologija, analiza glavnih komponenti, analiza oblika