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Analysis of Morphometric Characteristics of Different Populations of *Tabanus bromius* Linne 1758 (Diptera: Tabanidae)

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Authors' Contribution
FA conceived, designed and performed the experiments, collected and identified samples. IE and GO analyzed the data. FA, IE and GO wrote the article.

Key words
***Tabanus bromius* L., geometric morphometric, geographic variation, Tabanidae, Diptera.**

ABSTRACT

Horse flies (Diptera: Tabanidae) are known world-wide as important mechanical vectors of viruses, bacteria, protozoans, and helminths that cause diseases in wild and domestic animals. *Tabanus bromius* L. is common species worldwide, which shows considerable variation in the size of the body due to its adaptation to different habitats. In this paper, thirteen populations of this species, which are collected from different habitats and ecological regions (forests, sea sides and alpines) at altitudes between 5-2200 meters in Anatolia and Aegean were compared using geometric morphometries. When the wing shape differences of populations were analyzed by UPGM, the cluster analyses recognized two main groups of populations, one group comprising Afyon (890 m), Amasya (800 m), Artvin (960 m), Bolu (1200 m), Eskişehir (1170 m), Kastamonu (600 m) and Zonguldak (1050 m) while the second group comprised Bursa (700 m), Giresun (150 m), Sinop (160 m), Samsun (340 m), Karabük (230 m) and Trabzon (200 m).

INTRODUCTION

Tabanus bromius is one of the most common and variable species of dipterous family Tabanidae with a wide distribution. The variability in this species is the differences between the forehead structures particularly in the females. Extremely pale examples are usually found in the south parts of distribution areas. The individuals which are distributed in this region were named as *T. bromius* var. *flavofemoratus* by Strobl.

Morphometry can be considered as the geometric examination of the variations that occur in the shapes or a combination of biological, geometrical and statistical science. Morphometry can be divided in itself into traditional morphometry which examines biological variations and similarities by linear measurements and geometric morphometry which presents the dimensions and shapes of the structures through 2 or 3 dimensional landmark data (Bookstein, 1982).

Not only 3 dimensional structures such as skull and bone but also 2 dimensional structures such as wings can be compared by means of geometric morphometry. The reliability of the landmark data obtained from 2 dimensional structures is quite high due to technical faults. However, existing differences can be made meaningful by using a sufficient number of landmarks and individuals.

Statistical shape analysis compares body forms using specific landmarks that are determined based on anatomical prominence (Ercan *et al*., 2008a). The proper use of anatomical shape information could significantly improve our understanding of evolutionary processes and anatomical changes due to pathological disorders (Fritscher *et al*., 2004).

In this study, wing shape of different populations of female *T. bromius* L., which were collected from different locality, altitude and habitats of Turkey were compared by geometric morhometric analysis.

MATERIALS AND METHODS

Female individuals of *T. bromius* registered in the inventory of the Zoology Museum of Anatolian University, Faculty of Science Department of Biology were used as the study material. These individuals were seized by using Malaysian type of trap during several field surveys. Female individuals which are study materials were purified from internal and external parasites (Mayr and Ashlock, 1991). Therefore, morphometric data was prevented to be affected by potential traumas. 211 bodies for each part

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which were collected from Afyon, Amasya, Artvin, Bolu, Bursa, Eskişehir, Giresun, Karabuk, Kastamonu, Samsun, Sinop, Trabzon and Zonguldak cities (Fig. 1) were full sample randomly selected.

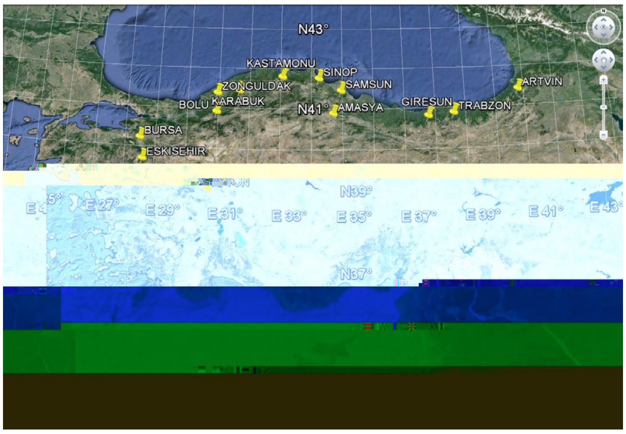

Fig. 1. Study area.

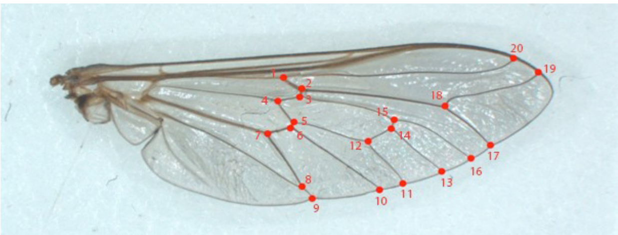

Fig. 2. Landmarks used in the present study. 1, R_s*R_{2+3}; 2, R_4*r-m; 3, M_1*r-m; 4, br*d; 5, m-cu*M_3; 6, m-cu*M_4;7, m-cu*CuA;8, A_1*CuA; 9, Basal end of CuA; 10, Basal end of M_4; 11, Basal end of M_3; 12, M_3*m-m; 13, Basal end of M_2; 14, Basal of M_2; 15, m-m*M_2; 16, Basal end of M_1; 17, Basal end of R_5; 18, R_5*R_4; 19, Basal end of R_4; 20, Basal end of R_{2+3}

For the morphometry studies which will be done on the wings, right wings were taken out and fixed by using entellan on a clean lam (Merck KgaA, Darmstadt, Germany) and labeled.

Standard anthropometric landmarks were chosen and marked on digital images as 2D coordinates for landmarks in the wing images (Fig. 2). Images of the wings were obtained with a 5 megapixel Leica DFC 480 digital camera from a distance of 10 centimeters. The landmarks were recorded using TPSDIG 2.04 software. For each wing image, 20 anthropometric landmarks on the wing were defined.

Statistical analysis

Cluster analysis was used to classify the wings. Clustering methods are designed to create the homogeneous groups of cases called clusters (Aldenderfer and Blashfield, 1989). The similarity between wings was calculated according to general and landmark-based terms. For this aim, the Procrustes distance was used to calculate the similarity between individuals. The average linkage method (also known as UPGMA) was used for merging clusters.

General Procrustes Analysis (GPA) was applied in order to examine the differences between clusters according to mean shapes. The homogeneity of the variance–covariance matrices was examined using the Box-M test (Dryden and Mardia, 1998). As the variance–covariance matrices were not homogeneous, the James Fj test was performed based on a resampling procedure was used for comparisons (Brombin and Salmaso, 2009).

The shape deformations were evaluated using Thin Plate Spline (TPS) analysis. Procrustes mean shapes were calculated for TPS analysis. Based on the results of the TPS analysis, the areas exhibiting the greatest enlargements or reductions were marked in different colors to indicate deformations.

To obtain the overall measurements of shape variability, the root mean square of Kendall's Riemannian distance to the mean shape was calculated.

For other statistical comparisons the concordance of the data to normal distribution was examined with Shapiro Wilk test and Mann Whitney test was used for between group comparisons. Data was presented with median (minimum-maximum) values.

R, PAST, ClustanGraphics 8.00 and TpsSmall 1.20 software were used for statistical analysis.

Landmark reliability

We calculated the intra-rater reliability coefficient for a two-facet crossed design ('landmark pairs-by-rater-by-subject', l x r x s) based on the generalizability theory (GT) (Ercan *et al*., 2008b). In the GT, the reliability for relative (norm-referenced) interpretations is referred to as the generalizability (G) coefficient (Dimitrov, 2006). In this study, the landmarks were marked by the same investigator. After a month, the same investigator marked the landmarks on 20 wings randomly selected from the study population. An analysis was performed to obtain a G reliability coefficient. The rating indicated a strong repeatability for subjects (G=0.9997).

RESULTS

In our study, the subjects could be split into two groups according to their similarity. Two clusters were determined at the dissimilarity level of 0.046. The wings similarity was calculated according to general and landmark-based terms.

Ninety per cent (90.05%) of the wings accumulated in the clusters, the rest were considered as outliers. A dendogram using average linkage is shown in Figure 3.

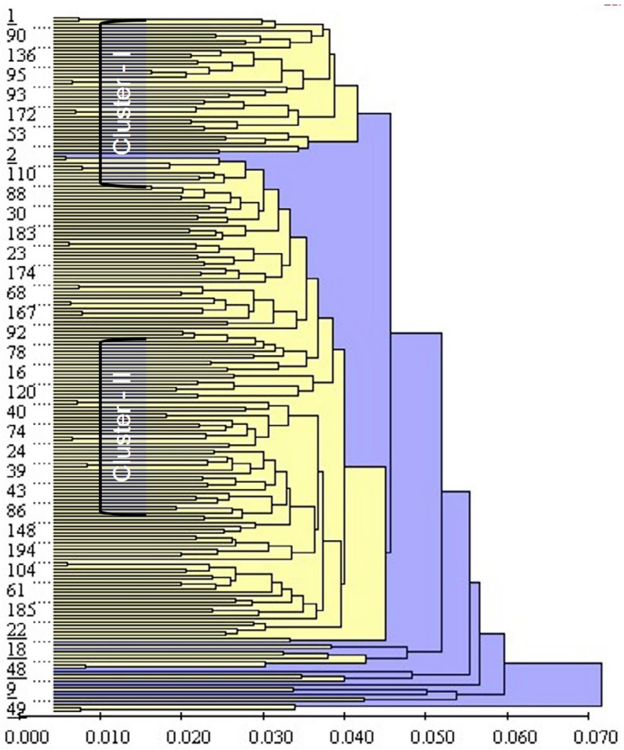

Fig. 3. Dendogram indicating the classification of wings in terms of levels of Procrustes dissimilarity.

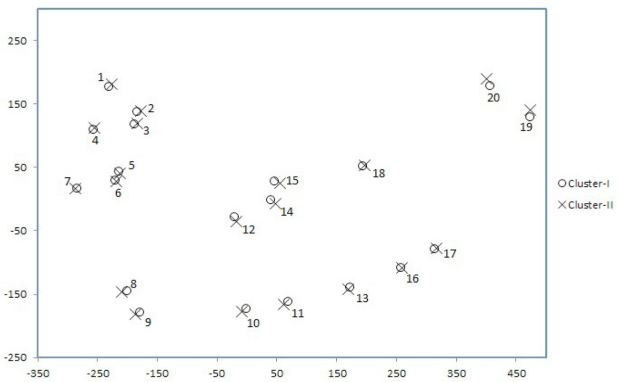

Fig. 4. The mean Procrustes shapes of cluster-I (o) and cluster-II (x).

One of these clusters (cluster-I) comprises 19.91% (42/211) and the second cluster (cluster-II) comprises 70.14% (148/211) of the total subjects.

There was a significant difference in terms of mean shapes (Fig. 4) that were found between clusters (p=0.010). To obtain the overall measurements of shape variability, the root mean square of Kendall's Riemannian distance to the mean shape was calculated. The overall measures of the shape variability of the clusters were the same (0.027).

Table I.- Comparison between clusters.

	Altitude	Precipitation	Humidity	Wind
Cluster-I	750	36	72	2.20
(n=42)	(105-1200)	(24-73)	(60-78)	(1.40-3.10)
Cluster-II	340	42	72	2.10
(n=148)	(105-1200)	(24-80)	(60-78)	(1.20-3.10)
p-value	0.012	0.007	0.656	0.031

Data are presented as median minimum-maximum.

Table I represents the comparisons according to climatic and geographic properties of the locations where the samples were collected according to the classifications of the wing shapes determined by cluster analysis.

Shape deformation of the mean shapes of the wings relative to each other (cluster-I to cluster-II) for each determined cluster was examined by using TPS analysis (Fig. 5).

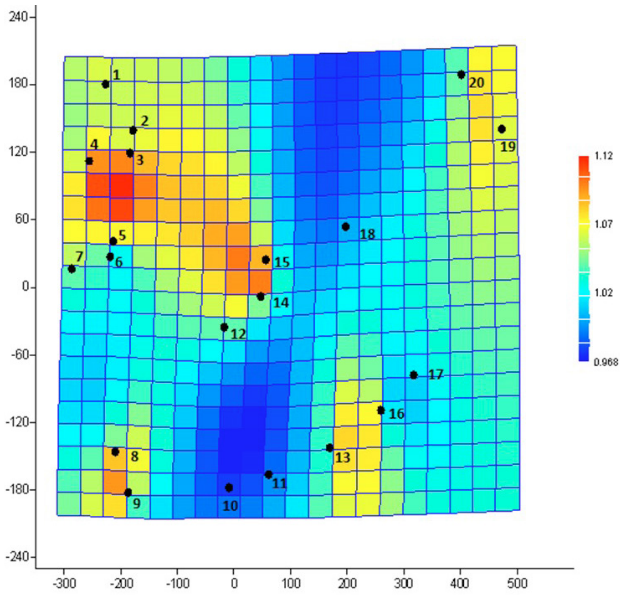

Fig. 5. Thin plate spline (TPS) graphics demonstrating the shape deformation from cluster-I to cluster-II.

DISCUSSION

The powers of the organisms to continue their living functions are associated with their capacity of accommodation to environmental conditions. The organisms which can accommodate themselves to abiotic and biotic environment in the best way are the livings which are able to continue their survival longest within the evolutionary process. Moreover, the variation of the effects of the abiotic environment on the growth and breed of the organisms are quite important because the species is affected by the variations in the distribution (Futuyma, 1998; Sinclair *et al*., 2003).

All kinds of variations which may occur in the environmental conditions affect the distribution in vector organisms. It directs the epidemiology of the parasitic diseases such as leishmaniasis and loasis. Environmental variations which may occur in natural ways or by means of human beings change the vector, host and the ambient conditions of the parasite. All kinds of vector organisms which are vectors occupy a niche in the population. Populations of these species differ in terms of behavioural and genetic characteristics because they accommodate to different environments (Patz *et al*., 2000).

Geographical variations are considered to be an indication of accommodation to micro environment. The variations between the populations in different heights can emerge as a result of the accommodation to factors such as different temperature, rainfall, wind and humidity (Karan *et al*., 2000). This can be the consequence of the variation in *T. bromius* female individuals.

Statistical studies which are done on the shape variations between the populations are important in understanding the variations in the anatomic structures and biological processes (Fritscher *et al*., 2004; Etoz and Ercan, 2012). There has been an increasing interest on statistical shape analysis in medicine and the field of biology in the last 20 years. The main reason for such widely use of statistical shape analysis is to examine the progresses in monitoring technologies, effects of diseases and environmental factors on the organ structure or the organism (Ercan *et al*., 2012).

In this study, *T. bromius* female specimens collected from 4 different regions which could vary according to climatic characteristics (Aegean, Anatolian, Black Sea and Marmara Regions) of Turkey were examined in terms of wing shape differences**.** The result of the study on analyzed samples were separated in two main groups and it was concluded that the effects of the altitude, rain and wind make difference on the shape of wing, while the effect of relative humidity does not. Size differences and wing shape differences divide populations into two main groups. Afyon, Amasya, Artvin, Bolu, Eskişehir, Kastamonu comprised the first group, while Bursa, Giresun, Sinop, Samsun, Karabük, Trabzon and Zonguldak comprised the second group. In this study, flies were clustered into two groups in regard to their wing shapes. There were significant differences between these groups according to precipitation, wind and altitude factors. So, it could be considered that these climatic features could be effective on wing morphology of the flies.

As a result of changes in populations which occur in individuals of different heights depending on the height of these differences in shape and size due to differences in the levels of height is thought to be caused by variable climatic conditions. Height with increasing temperature due to the growth periods of falling from heights among the individuals will take longer. In this case, the access time will be longer and the low height of the maturity of individual blood absorption by individuals thought will have a small body.

When comparing climatic data of populations habitats, temperature, altitude and precipitation could be considered to be more effective in differentiation of body size and shape than the other factors. In addition, body size depends upon a large number of ecological, physiological and genetic variables. To fully understand the effects of altitude and latitude on the body, all effects of genetic information and environmental components on the body must be known. For example, the body size of the larval mosquito larval habitat quality is considered to be related to the competition (Strickman and Kittayapong, 2003; Schneider *et al*., 2004).

Depending on the differences of altitude, rain and winds in habits of specimens, the regional differences of wing shape, especially basal radial cell, basal medial cell and discal cell were shown. It could be inferred from that wing speed in the highest altitudes is generally greater than the lower altitude, so specimens, who adopt the high altitudes, are bigger than the other specimens. Also, the differences depending on the climatic conditions were observed in the posterior cubital cells of the wings. On the other hand, it could be observed in the shape deformation graphic that the medial cells of the wing were differentiated with wind speeds. The wing shape of the flies does not differ statistically according to climatic characteristics; however it was found that wing shapes are similar with regard to shape variability.

It could be considered that this situation observed among the individuals in different populations is associated with environmental conditions which are effective during larval development. The larvae of *T. bromius* species is completely aquatic habitats of the larvae showing development and a direct exposure to water, physical and chemical changes in the larvae may create a difference in their development. In addition, it could be taken into consideration that as the larvae are predators, the dietary differences could affect their development and create a difference in terms of shape and size in larvae' adult stages of development. For example, cadmium and lead which accumulate in water were found out to have a negative influence on populations (Altunsoy and Kılıç, 2012). The intensity of these heavy metals in the water could cause minor differences in the shape.

According to the results obtained in this study, *T.bromius* species female subjects altitude, wind and precipitation changes were discovered to be statistically significant on the wing shape, while humidity was not found to be statistically significant. Geometric morphometric, organisms changes in shape and size make it easier to define and measure. However, because these changes are occurring in phenotype is not only the environmental conditions. It must be known that genetic factors are effective on the size and shape of the body. Therefore, differences in shape and size to be considered in future studies investigating the genetic factors will ensure more reliable results.

CONCLUSION

In conclusion, this study is first implication of morphometric analysis on the horse flies, which are important mechanical vectors between wild and domestic animals and also human. Ecological and climatic factors effects distribution, seasonal activity, life cycle and morphological characters of horse flies. Consequently, as could be interpreted in this study, it brings out necessity of researches on other species of family and other countries.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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