

Original article (Orijinal araştırma)

Landmark-based analysis of honey bee wing variation: findings from some regions of Van, Hakkari (Türkiye) and Iran¹

Balarısı kanat varyasyonunun landmark temelli analizi: Van, Hakkari (Türkiye) ile İran'ın bazı bölgelerden elde edilen bulgular

Simanur AKÇAKAYA² 

Cengiz ERKAN³ 

Cansu Özge TOZKAR^{2*} 

Abstract

This study investigates the geometric morphometric characteristics of honey bee colonies selected from certain regions of Eastern Anatolia and Iran. Wing samples from 1738 worker bees were collected from stationary colonies in the districts of Van and Hakkari (Türkiye), as well as Iran. Shape and size differences were examined using 20 landmark points on the right forewings of the samples. Procrustes ANOVA revealed significant differences between locations and apiaries ($p<0.001$). Canonical variate analysis (CVA) and principal component analysis (PCA) showed that Gevaş and Iran samples differentiated from other groups. Discriminant function analysis showed significant differences among all locations ($p<0.0001$). Substantial differences were observed between Iran and other locations, followed by differences between Gevaş and the other locations. The distribution pattern of Hakkari samples being closer to Iranian samples rather than Van samples emerged as an intriguing finding in the study. Deformation grid analysis highlighted specific landmark points contributing to these differences. The results indicate that the geometric morphometric differences in the region have been preserved, while also pointing to the potential hybridization effects caused by migratory beekeeping practices and queen bee trade. This study provides critical baseline data for understanding the morphological variation of honey bees in the region and highlights the importance of conserving locally adapted honey bee populations.

Keywords: Eastern Anatolia, geometric morphometrics, honey bee diversity, landmark analysis, wing variation

Öz

Bu çalışma, Doğu Anadolu ve İran'ın belirli bölgelerinden seçilmiş bal arısı kolonilerinin geometrik morfometrik özelliklerini incelemektedir. Van ve Hakkari (Türkiye) illeri ile İran'daki sabit arıcılık kolonilerinden toplam 1738 işçi arının kanat örnekleri toplanmıştır. Örneklerin sağ ön kanatlarında 20 landmark noktası kullanılarak şekil ve boyut farkları incelenmiştir. Procrustes ANOVA, lokasyonlar ve arılıklar arasında önemli farklar olduğunu ortaya koymuştur ($p<0.001$). Kanonik değişken analizi (CVA) ve temel bileşen analizi (PCA), Gevaş ve İran örneklerinin diğer gruplardan farklılaştığını göstermiştir. Ayırıcı fonksiyon analizi, tüm lokasyonlar arasında önemli farklar olduğunu göstermiştir ($p<0.0001$). İran ve diğer lokasyonlar arasında belirgin farklar ortaya çıkarken bunu Gevaş ve diğer lokasyonlar arasındaki farklar izlemiştir. Hakkari örneklerinin Van örneklerinden ziyade İran örneklerine daha yakın bir dağılım paterni göstermesi araştırmada ilginç bir bulgu olarak ortaya çıkmıştır. Deformasyon ızgarası analizi, bu farklara katkıda bulunan belirli landmark noktalarını vurgulamıştır. Sonuçlar, bölgedeki geometrik morfometrik farklılıkların korunduğunu ortaya koymakla birlikte, göçer arıcılık faaliyetleri ve ana arı ticaretinin yol açabileceği melezleşme etkilerine de işaret etmektedir. Bu araştırma, bölgedeki bal arılarının morfolojik varyasyonunu anlamak için kritik temel veriler sağlamaktadır ve bölgeye uyum sağlamış yerel bal arısı popülasyonlarının korunmasının önemini vurgulamaktadır.

Anahtar sözcükler: Doğu Anadolu, geometrik morfometri, balarısı çeşitliliği, landmark analizi, kanat varyasyonu

¹ This study was part of the MSc thesis of the first author at the Institute of Natural and Applied Sciences, Van Yüzüncü Yıl University.

² Van Yüzüncü Yıl University, Faculty of Agriculture, Department of Agricultural Biotechnology, 65090, Van, Türkiye

³ Van Yüzüncü Yıl University, Faculty of Agriculture, Department of Animal Science, 65090, Van, Türkiye

* Corresponding author (Sorumlu yazar) e-mail: ozgetozkar@yyu.edu.tr

Received (Alınış): 01.10.2024

Accepted (Kabul ediliş): 29.03.2025

Published Online (Çevrimiçi Yayın Tarihi): 05.04.2025

Introduction

The Western honey bee, *Apis mellifera* L., 1758 (Hymenoptera: Apidae) is vital for ensuring environmental, agricultural, and economic sustainability. They greatly benefit the ecosystem through their contribution to the pollination of flowering plants and agricultural crops. Additionally, they support plant diversity and preserve biodiversity by pollinating wild plants for wildlife. Honey bees contribute to the global economy by providing natural products such as pollen, royal jelly, propolis, honey, and beeswax to beekeeping, agriculture, and related industries.

As a result of the adaptation of *A. mellifera* to different regions worldwide, various geographic subspecies have emerged. Each subspecies possesses morphological and physiological characteristics specific to its geographical region and exhibits genetic variations. It is noted that the Anatolian geography, which encompasses various climate types, has been influential in the evolutionary process of honey bees in Türkiye (Kence, 2006). Thus, Anatolia is one of the important bee gene centers. Honey bees in Türkiye are categorized under the species *A. mellifera*. *Apis mellifera carnica* Pollmann, 1879 is found in the Thrace region, *Apis mellifera meda* Skorikov, 1929 in the Southeastern Anatolia region, *Apis mellifera syriaca* Skorikov, 1929 in a small area in Hatay-Antakya, *Apis mellifera caucasica* Gorbachev, 1916 from Samsun to Northeast Anatolia, and *Apis mellifera anatoliaca* Maa, 1953 in the Aegean, Central Anatolia, Mediterranean, and western and central parts of the Black Sea regions. Iranian bees were first described by Skorikov in 1929 as *A. mellifera meda*. Although the distribution of this subspecies was initially reported as the Caspian Sea and Northern Iran, subsequent studies have shown that it extends to Syria and Northeastern Türkiye. The *A. mellifera meda* subspecies is widely distributed, and the presence of six geographically distinct local *A. mellifera meda* populations has been reported (Ruttner, 1988).

Quantitative morphometric analyses are frequently used in the delineation of many species. The method, which is based on the direct measurement of individual characteristics, has generally yielded successful results (Schwarzfeld & Sperling, 2014). Many researchers have conducted studies using morphological traits such as the wing and body sizes of honey bees, as well as the lengths of legs, tongues, and hairs. Through these studies, groups can be formed, and comparisons can be made using quantitative traits (Taşkıran et al., 2017). The honey bee subspecies distributed in Türkiye and the Middle East exhibit distinct morphological characteristics. For instance, one of the most common subspecies in Türkiye, *A. mellifera anatoliaca*, is characterized by a broad abdomen, short wings, and yellow-colored bodies with orange-brown rings (Ruttner, 1988). Found in northeastern Anatolia, *A. mellifera caucasica* is notable for its long proboscis (up to 7.2 mm) and dark chitinous body covered with gray hairs (Ruttner, 1988; Kandemir et al., 2000). *A. mellifera syriaca*, distributed in southeastern Anatolia, particularly in Hatay, can be identified by its slender body, short hairs, yellow abdominal segments, and a bright yellow scutellum (Ruttner, 1988; Kandemir et al., 2000). These morphological differences influence the ecological adaptations, nectar collection capacities, and colony management traits of the subspecies, providing a critical foundation for genetic and biogeographic studies (Ruttner, 1988; Franck et al., 2000).

Wing shape is a key aspect of an insect's phenotype, closely linked to a critical feature as flight properties. Consequently, the study of geomorphometry or shape of wings, whether of honey bees or other winged insects, can have major impacts across different taxonomic levels for detecting geographical variations, population structures, shape differences, or taxonomic classification. Geomorphometry is a method that utilizes cartesian coordinates of landmark points instead of linear, angular, and proportional calculations in the quantitative interpretation of morphometric data. Geometric morphometry uses landmark points, which are adjusted through shifting, resizing, and orienting to remove size-related effects. Once aligned, the reference point arrangements vary only in shape and can be assessed reliably and cost-effectively using multivariate statistical methods for a large number of samples in a short time. With the advancement of geometric morphometric methods, researchers have begun to conduct classification studies of honey bee

subspecies using geomorphometric analyses of wing shapes (Kekeçoğlu et al., 2007; Tofilski, 2008; Francoy et al., 2009; Kandemir et al., 2011).

This study aimed to interpret honey bee samples collected from stationary colonies in Van, Hakkari, and Iran using geomorphometric wing analyses to reveal the similarities and differences between existing honey bee colonies. The results obtained from the parameters evaluated in this study serve as preliminary findings that will support future, more comprehensive phylogenetic studies representing the entire Eastern and Southeastern Anatolia region. Although our country is quite rich in honey bee genetic resources, unfortunately, these resources cannot be used effectively and protected. Another aim of this study was to contribute to making our honey bee genetic resources more accessible and conserved by providing geomorphometric diversity data.

Materials and Methods

Materials

The honey bee samples used in this study were obtained from stationary honey bee colonies in the regions of Van (Gevaş, Çatak, Özalp, Başkale), Hakkari (Otluca, Akçalı, Kanatlı), and Iran (Urmia-Bavan-Bardehzi-Gundikemele) between 2021 and 2022 (Table 1).

The locations from which honey bee samples were gathered, along with the number of samples collected and analyzed from each district, are shown in Table 1. A total of 1750 worker bee samples were collected from 24 apiaries, 69 hives.

The sampling process was conducted on worker bees inside the hive. Samples were individually collected using forceps and placed into sample beakers. Subsequently, the honey bees were anesthetized with ether, transferred to falcon tubes, and 96% ethyl alcohol was added. The falcon tubes were tightly sealed and stored at +4°C for geometric morphometric analyses.

From each hive, the right forewings of 30 worker bees were collected, resulting in an initial total of 2070 samples from 69 hives. During quality control, wings with deformities, damage, or inconsistent landmark identification were excluded to ensure reliable geometric morphometric measurements. Additional exclusions were made during analysis using MorphoJ software due to misaligned or statistically unsuitable TPS files. After these rigorous quality control steps, the final dataset comprised 1750 worker bee samples, of which 1738 high-quality samples were analyzed for geometric morphometric characteristics.

During the preparation stage for geometric morphometric analysis, only the right forewings of the bees were placed on microscope slides, while the other wings were separated but not used in the study. To prevent folding, alcohol was added to each wing. The right forewings were then fixed on microscope slides and labeled. During the digitization, processing, and editing of forewing images, wings fixed between slides for the landmark method were adjusted to the desired size using a Leica M165C stereomicroscope (Leica Microsystems, Wetzlar, Germany) at 20× magnification. High-resolution images were then captured using a Leica DFC450 digital camera (Leica Microsystems, Wetzlar, Germany), which has a resolution of 5 megapixels. The images were saved in JPEG format at 300 dpi. The wing photographs were labeled based on their locations and stored in separate folders on the computer.

Table 1. Study locations and beekeeping data across different altitudes in Eastern Türkiye and Iran

Country	Province	District	Village	Number of Beekeepers	Number of Hives	Coordinates	Altitude (meters)	Field dates
Türkiye	Van	Gevaş (n=622)	Merkez	1	3	38.298°N 43.106°E	1.750	July 2021
			Göründü	1	3	38.345°N 42.918°E		
			Değirmitaş	3	12	38.344°N 42.850°E		
			Ortamahalle	1	3	38.291°N 43.110°E		
		Çatak (n=188)	Dalbastı	1	4	37.904°N 42.942°E	1.500	October 2021
			Büyükağaç	2	7	37.860°N 43.097°E		
			Kaçit	2	6	37.930°N 42.989°E		
			Atlıhan	1	3	37.919°N 42.821°E		
		Başkale (n=451)	Aşağı Darıca	1	3	37.768°N 44.168°E	2.320	November 2022
			Yukarı Darıca	1	3	37.754°N 44.178°E		
			Çaldıran	1	3	37.790°N 44.125°E		
		Özalp (n=133)	Dönerdere	1	3	38.737°N 44.123°E	1.994	November 2022
			Yukarı Tulgalı	1	3	38.772°N 44.260°E		
Türkiye	Hakkari	Merkez (n=154)	Otluca	1	3	37.601°N 43.693°E	1.756	November 2022
			Akçalı	1	3	37.711°N 43.992°E		
			Kanatlı	1	3	37.716°N 44.026°E		
İran	Ürmiye	Merkez (n=190)	Merkez	1	1	37.555°N 45.083°E	1.330	November 2022
			Bavan	1	1	37.546°N 44.762°E		
			Gundikemele	1	1	37.494°N 44.793°E		
			Bardehzi	1	1	37.494°N 44.793°E		

Methods

Marking the landmark points

The image set was transformed into a TPS format utilizing specialized morphometric software 'TPS utility'. During the landmark digitization procedure, TPS data structures were generated through the application of tpsUtil32 v. 1.78 (Rohlf, 2019) software (Figure 1).

The tpsDig2 v. 2.31 (Rohlf, 2018) software was utilized to obtain the wing coordinates. Following Bookstein's criteria for landmark identification, a set of twenty specific points on the right wings were precisely mapped and recorded digitally (Bookstein, 1990). This process was repeated for all samples, and TPS files were created for statistical analyses.

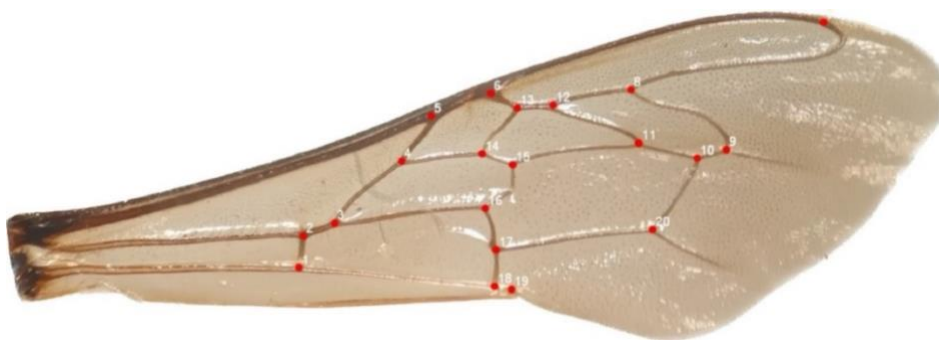


Figure 1. Landmark locations on the right forewing used in the study.

MorphoJ analysis

The TPS files containing the raw coordinates of the landmarks were analyzed using MorphoJ software version 1.06 (Klingenberg, 2011) for the purpose of extracting 'x, y' coordinates and which subsequently facilitated the derivation of the size and shape parameters. The raw coordinates were aligned by removing scale, position, and orientation differences using the Procrustes Fit Function. Procrustes Fit Analysis, Procrustes ANOVA, Principal Component Analysis (PCA), Lollipop Graph, Discriminant Function Analysis (DFA) and Canonical Variate Analysis (CVA) were conducted with Morpho J software.

Procrustes ANOVA, used to determine variations between locations and apiaries in the study, is a technique applied to identify differences of the shape and size of centroids. Centroid size is a calculation method that determines the square root of the total of squared lengths from an object's landmarks to their centroid. Canonical variate analysis (CVA) is employed to assess and explain the differences among two or more groups within a given dataset. Mahalanobis distance (MD) is a powerful parameter for measuring multivariate distance, which calculates the separation between an individual observation and a statistical distribution. Procrustes distance, on the other hand, represents the square root of the total of squared deviations in landmark configurations between datasets. This measure is typically utilized to evaluate morphological similarities or differences between objects, reflecting the average shape variation between datasets. Discriminant Function Analysis (DFA) calculates the Mahalanobis distance relative to group centroids to classify an unknown entity. Principal Component Analysis (PCA) was utilized to illustrate variations in shape across the samples. The principal elements of the landmarks were displayed using a lollipop chart. Additionally, a PCA histogram showed the percentage of observations contributing to shape variability (Klingenberg, 2011).

Results and Discussion

Results

Procrustes fit analysis (generalized Procrustes analysis)

The superimposition of the data was performed using Procrustes Fit Analysis. Out of the forewing samples, 1738 were included in the analyses by MorphoJ. Figure 2 displays a scatter diagram with the overlaid landmarks illustrating the overall morphological form of the right wing of the honey bee using twenty landmark points for the 1738 observations.

The Generalized Procrustes Analysis illustrating the scatter graph of the overlaid configuration set of 1738 right wing landmarks; blue dots indicate the average positions of the landmarks of all samples, while the small black dots denote the landmarks for individual specimens.



Figure 2. Superimposition of the landmarks on the forewing.

Procrustes Anova (one-way analysis of variance)

Separate ANOVA tables present the outputs of the Procrustes ANOVA analysis for both size and shape of centroids. The Procrustes ANOVA test, a univariate analysis of variance applied to evaluate population differences, indicated significant size and shape differences among the populations based on their locations. The differences in centroid size were also found to be significant in statistical terms ($p < 0.001$) (Table 2). Regarding centroid size, the variations among locations ($F = 149.47$) showed a higher F value than those among the apiaries ($F = 68.80$). In terms of shape, greater differences were detected among locations ($F = 38.58$) compared to those among the apiaries ($F = 17.16$) based on the F value (Table 2).

Table 2. Procrustes ANOVA results by location. Sum of squares (SS), mean squares (MS), degrees of freedom (df), Goodall's F statistic (F), and p -value (p)

Centroid size:							
Effect	SS	MS	df	<i>F</i>	<i>P</i> (param.)		
Individual	8713286.930017	1742657.386003	5	149.47	<.0001		
Residual	19902271.475878	11659.210003	1707				
Shape, Procrustes ANOVA:							
Effect	SS	MS	df	<i>F</i>	<i>p</i> (param.)	Pillai tr.	<i>p</i>
Individual	0.13805243	0.0007669580	180	38,58	<.0001	1.16	<.0001
Residual	1.22165853	0.0000198799	61452				

Canonical variate analysis (CVA)

Canonical variate analysis was used to test whether there were differences in forewing shapes according to locations and apiaries. CVA generates canonical variables (CV) by rotating and adjusting the centroids and establishes Mahalanobis distance (MD) between categories calculated from the centroids of the observations. The distributions of populations were also demonstrated by canonical variate analysis. The variation among groups was scaled by the inverse of the within-group variation. The variance percentages among the CV (canonical variate) values of the locations (Van districts, Hakkari, and Iran) are provided in Table 3. Groups determined by location and apiaries showed diversity in the discriminant analysis based on Mahalanobis and Procrustes distances according to 10,000 permutation rounds ($p < 0.0001$).

Table 3. Eigenvalues, variance, and cumulative values among CV groups formed by location

Groups	Eigen values	Variance %	Cumulative %
CV1	0.98187839	53.771	53.771
CV2	0.38370883	21.013	74.784
CV3	0.27139115	14.862	89.646
CV4	0.12405223	6.794	96.440
CV5	0.06501205	3.560	100.000

The scatter plot representing the canonical variate analysis was created to distinguish the similarities and differences in wing shapes of honey bees taken from different locations, considering the variables. According to the CVA graphs obtained from the canonical variate analysis, samples from L2 (Başkale), L3 (Çatak), L4 (Özalp) districts, and L5 (Hakkari) province were found to be intermingled, while comparing based on the Gevaş and Iran axes, the L3 (Çatak) ellipse showed more intersection with Gevaş samples, and the L2 (Başkale)-L5 (Hakkari) ellipses intersected more with L6 (Iran) samples. Although some overlaps were observed, certain samples from these four groups (L2-L3-L4-L5) clustered closely with the Gevaş (L1) and Iran (L6) groups. However, the Gevaş and Iran samples exhibited a noticeably different distribution compared to other groups (Figure 3).

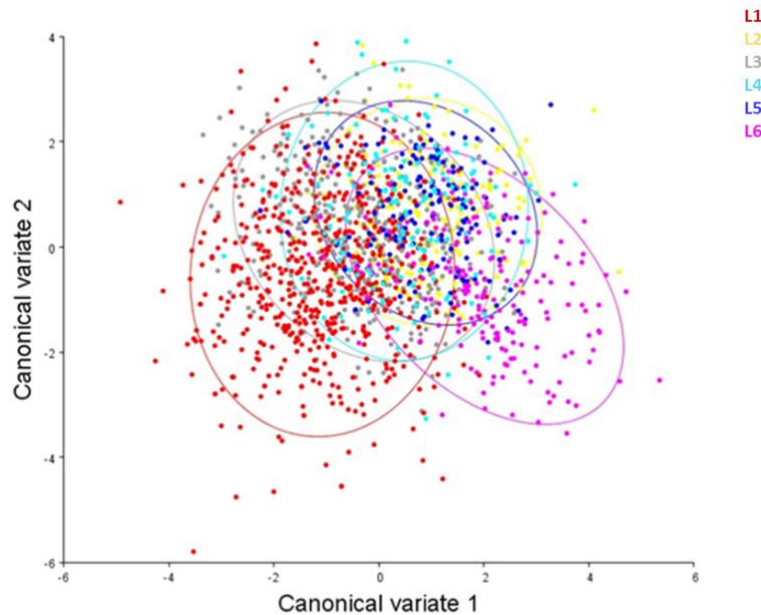


Figure 3. Scatter plot of form differences on the first two canonical variate axes, created by 20 landmarks on honey bee wing samples taken from six locations (L1-L6). L1-Gevaş is represented in red, L2-Başkale in yellow, L3-Çatak in gray, L4-Özalp in turquoise, L5-Hakkari in navy blue, and L6-Iran in pink.

According to another CVA graph created in the canonical variate analysis, the samples from the twenty-four apiaries, regardless of location, were found to be intermingled, indicating within-group variation.

Principal component analysis (PCA) of geometric morphometric data

As another method for variation analyses based on different locations in the landmark data (20 landmarks on the forewing), principal component analysis was applied. PCA was employed to illustrate shape changes in the observations. A PCA distribution chart also displayed the percents of samples contributing to shape deviations. Eigenvalues represent the separation of different directions. The extent to which each direction explains the overall phenotypic variation in the entire data set is shown with an orthogonal bar diagram. PCA revealed 36 principal components (PCs) accounting for the total variation.

According to the diagram, the first principal component (PC1) impacted the most to shape variation with 25%, followed by the second principal component (PC2) with 12%. The first five dimensions explained 60% of the overall variation, while the first ten dimensions explained 79% (Figure 4).

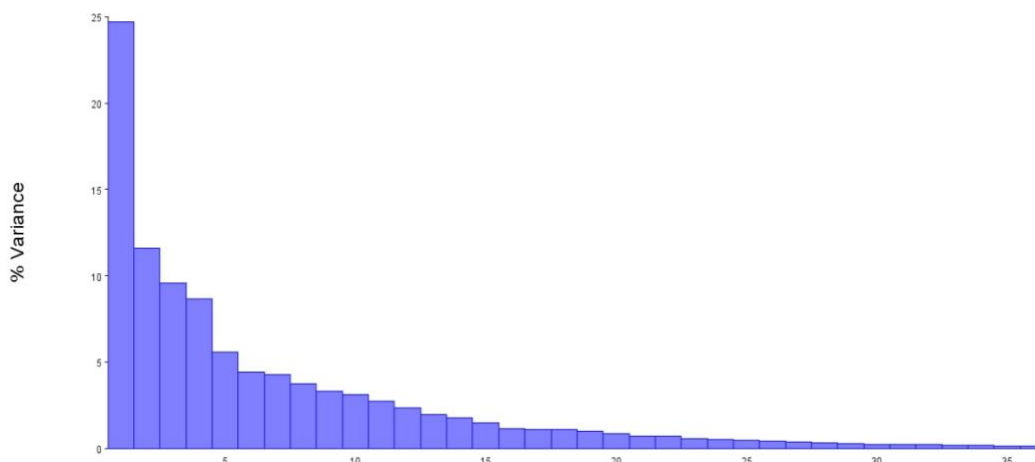


Figure 4. Variance distribution according to principal components as a result of principal component analysis.

The clusters formed in the PCA graph of the forewings of honey bee samples taken from six locations (Van districts, Hakkari, and Iran) exhibited a distribution similar to the CVA graph. Some samples from Gevaş (L1) and Iran (L6) showed a noticeable tendency to be distributed further from the center (Figure 5).

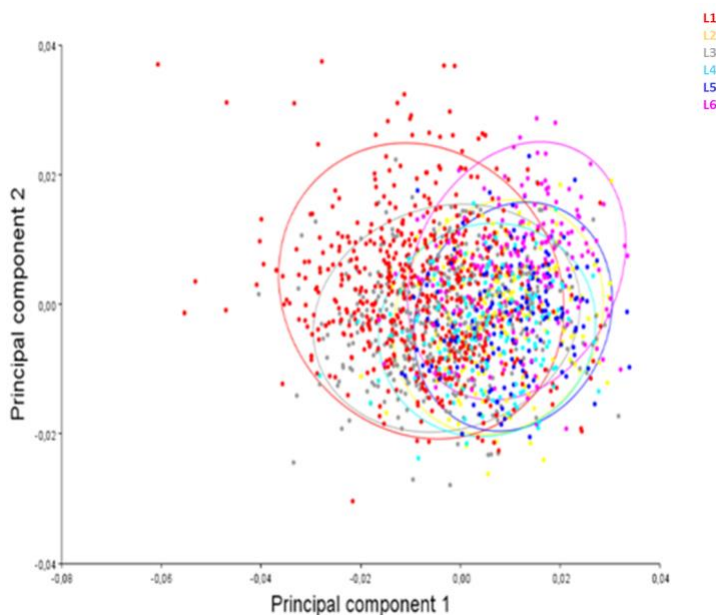
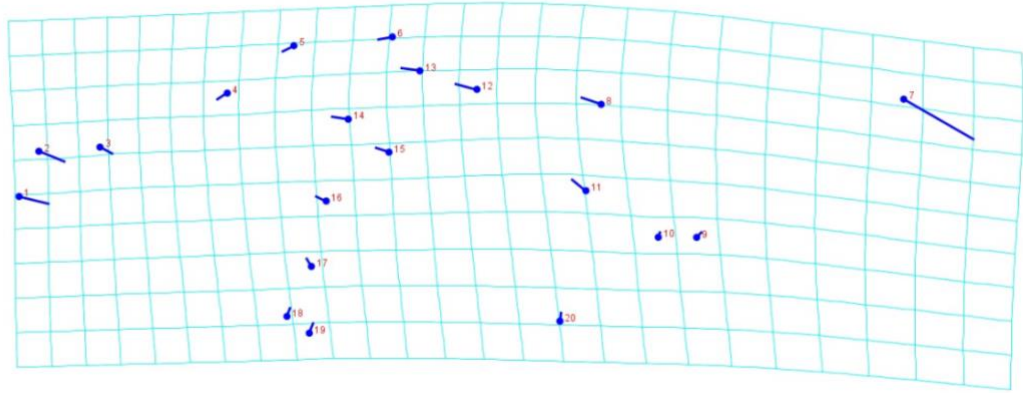


Figure 5. PCA graph of the forewings of honey bee samples taken from six locations. L1-Gevaş is represented in red, L2-Başkale in yellow, L3-Çatak in gray, L4-Özalp in turquoise, L5-Hakkari in navy blue, and L6-Iran in pink.

Lollipop glut graph

The shape changes in all forewings included in the analysis were shown in the 'lollipop glut' graph (Figure 6). The principal components of the landmarks were displayed in a lollipop diagram. The bars at the ends of the points represent the magnitude of shape change. The wing shape differences among honey

bee populations were visually analyzed using a deformation grid, which identified the landmarks where these differences were concentrated. The deformation grid defines the landmark regions that contribute most to the separation. The least change was observed at landmark points 9 and 10, while the greatest difference was seen at landmark point 7. This is followed by the magnitude of differences at landmark points 2 and 3. Variations among populations were also detected at the remaining landmark points.



PC1

Figure 6. Landmark points where the wing shape differences were concentrated among honey bee populations. Blue points represent the average landmark values.

Discriminant function analysis (DFA)

DFA selects the metrics that generates the most significant differences between data groups. An accurate classification test analyzes the mean values of these two groups in terms of Procrustes distances or Mahalanobis distances. Significant differences were found in pairwise comparisons among all locations (four districts of Van, Hakkari, and Iran) based on both Mahalanobis and Procrustes distances ($p < .0001$) (Table 4).

Table 4. Discriminant Function Analysis results for honey bee populations from different locations. (The permutation test using the T-square statistic is equivalent to a test using the Mahalanobis distance)

Comparison	Procrustes Distance	Procrustes Distance (P)	T-Square (P)	Mahalanobis Distance	T-Square (value)	T-Square (P)
L1-L2	0.0170	<.0001	<.0001	2.4732	793.2917	<.0001
L1-L3	0.0081	<.0001	<.0001	1.5804	600.9674	<.0001
L1-L4	0.0157	<.0001	<.0001	2.3151	525.5498	<.0001
L1-L5	0.0195	<.0001	<.0001	2.3573	614.1914	<.0001
L1-L6	0.0216	<.0001	<.0001	3.0277	1231.4230	<.0001
L2-L3	0.0120	<.0001	<.0001	1.7549	459.6203	<.0001
L2-L4	0.0065	<.0001	<.0001	1.7482	282.9438	<.0001
L2-L5	0.0052	<.0001	<.0001	1.4762	270.1946	<.0001
L2-L6	0.0122	<.0001	<.0001	2.3100	678.3607	<.0001
L3-L4	0.0105	<.0001	<.0001	1.8260	390.9388	<.0001
L3-L5	0.0150	<.0001	<.0001	2.0070	529.3059	<.0001
L3-L6	0.0174	<.0001	<.0001	2.5553	1005.9193	<.0001
L4-L5	0.0077	<.0001	<.0001	1.4653	191.4848	<.0001
L4-L6	0.0145	<.0001	<.0001	2.6448	595.9205	<.0001
L5-L6	0.0135	<.0001	<.0001	2.2919	454.5249	<.0001

According to Procrustes distances, shape differences were observed among all locations, with the greatest variation between Iran (L6) and other locations. Following Iran, the variation between Gevaş (L1) and other locations was also noteworthy. The most pronounced difference was between Iran (L6) and Gevaş (L1), followed by variations between Gevaş (L1)-Hakkari (L5), Iran (L6)-Çatak (L3), and Gevaş (L1)-Başkale (L2). Mahalanobis Distance (MD) quantifies how many standard deviations a point deviates from the average of a statistical distribution. As the distance increases, the variation between a sample and a distribution becomes broader. Accordingly, the widest variation was observed between Iran (L6) and Gevaş (L1) (Table 4).

The histogram data enabling pairwise comparisons based on discriminant scores confirmed the PCA and CVA results, showing variations between Gevaş and other locations (Figure 7 a-b-c-d-e), Iran and other locations, and overlaps between L2-L3-L4-L5 locations.

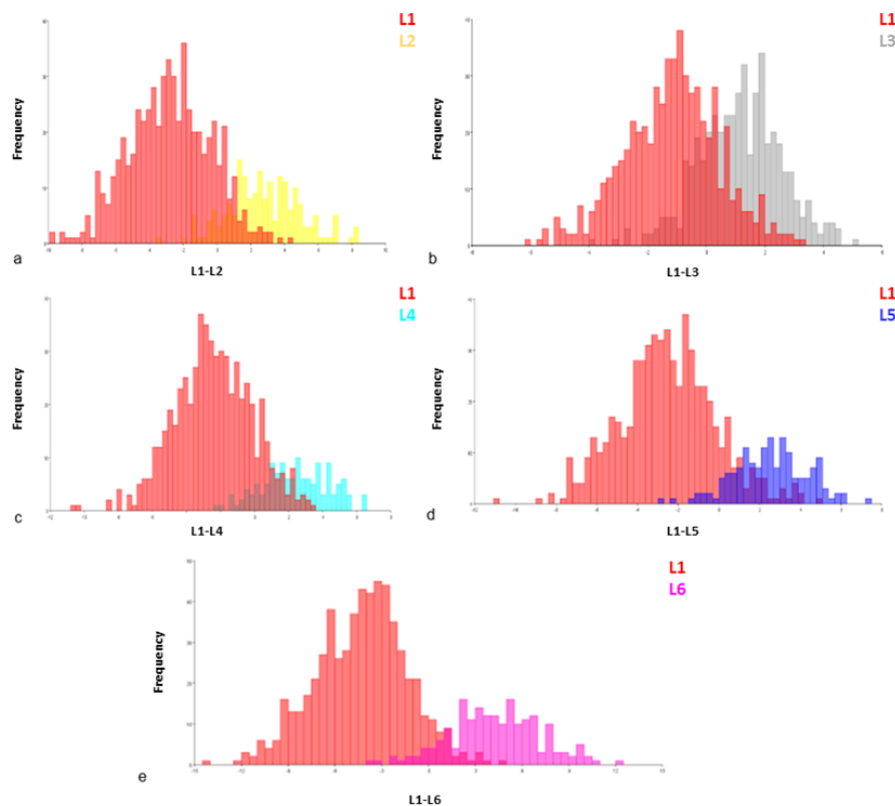


Figure 7. a) Histograms representing the measurement of discriminant scores for initial data variability among locations. a) Gevaş-L1 (red), Başkale-L2 (yellow) b) Gevaş-L1 (red), Çatak-L3 (gray) c) Gevaş-L1 (red), Özalp-L4 (turquoise) d) Gevaş-L1 (red), Hakkari-L5 (blue) e) Gevaş-L1 (red), Iran-L6 (pink).

Deformation grids were examined pairwise among locations, showing differences between landmarks 1 and 2 between L1 (Gevaş) and other locations (except L2-Çatak). Landmark 8 showed differences between L1 (Gevaş) and other locations; it was different between L6 (Iran) and other locations. The difference between L5 (Hakkari) and L1 (Gevaş) was minimal. The magnitude of the difference at landmark 8 was greatest between Gevaş and Iran. At landmark 7, samples from all locations showed differences, with the least difference between L1 (Gevaş) and L2 (Çatak), and the greatest difference between L1 (Gevaş) and L5 (Hakkari); L1 (Gevaş) and L6 (Iran). Samples from L1 (Gevaş) and L3 (Çatak) showed the most distinction from all other groups at landmark 7. Landmark 11 showed variation between L1 (Gevaş) and L2 (Başkale), L1 (Gevaş) and L6 (Iran), and between Iran and Çatak, Özalp, and Hakkari, with the largest change observed between Gevaş and Iran. Samples from L6 (Iran) showed distinct differences from other groups, particularly at landmark 8.

Discussion and Conclusion

In this study, the clusters formed in the PCA graph of the forewing samples of honey bees collected from six locations (Van Districts, Hakkari, and Iran) showed a close distribution, and the intersection areas of the axes were dense. However, some samples from Gevaş and Iran exhibited a noticeable spread outside the center. It is thought that one of the main reasons for the most prominent differences emerging in the samples collected from the Iranian side of the border is the lower intensity of colony movements (migratory beekeeping and colony sales) and queen bee sales in this region compared to Türkiye. Although the research was conducted based on stationary beekeeping conditions, colony flow occurs in the regions during nectar flow periods. This disrupts the uniformity of local genotypes due to the mating behavior of queen bees. In this study, Hakkari samples showed a distribution pattern closer to Iranian samples rather than Van samples. In Badalı's (2010) study, which included samples from different locations including Iran, samples collected from Artvin, Iran, and Hakkari formed different clusters according to PCA analysis results, while samples from Iraq and Azerbaijan formed close clusters and showed similar characteristics. Honey bee populations in Southeastern Anatolia, including Hakkari, show distinct morphological traits that separate them from other regions in Türkiye, such as Van and Iran. This clustering is supported by geometric morphometric analyses, which highlight significant deviations in wing vein junctions among different populations (Kekeçoğlu & Soysal, 2010; Kekeçoğlu et al., 2020).

In our study, statistically significant differences were found in pairwise comparisons between all locations (4 districts of Van, Hakkari, and Iran) and apiaries, based on both Mahalanobis distances and Procrustes distances. In a study conducted by Özkan Koca (2012); according to Ruttnner's (1988) classification, 3 populations in Iran (2nd Area Central and Western Iran, 3rd Area Northeastern Iran, 4th Area Subtropical areas of the Caspian Sea) clustered together using the DFA method. The differences between populations were also found to be significant according to pairwise test results. Colonies of all subspecies were distinctly separated from each other. While *A. m. meda* colonies in Eastern and Southeastern Anatolia were completely within their own groups, almost all (98.3%) of the colonies in Iran and Northern Iraq were within their own groups. A small portion (1.7%) of *A. m. meda* colonies in Iran and Northern Iraq was included in the Eastern and Southeastern Anatolia group. Morphometric analyses show distinct clustering of *A. m. meda* populations. Iranian populations form a separate cluster from Turkish populations, which include both *A. m. meda* and *A. m. caucasica* (Adl et al., 2007; Kence et al., 2009). Discriminant analysis and Mahalanobis distances reveal that Iranian, Central Anatolian, and Caucasian honey bee populations form distinct clusters, with geographical barriers likely contributing to these differences (Adl et al., 2007; Kekeçoğlu & Soysal, 2010).

According to the histogram data enabling pairwise comparisons based on discriminant scores, the greatest variation is observed between Gevaş district and other locations, and between Iran and other locations. In a morphometric analysis conducted by Özbakır & Fıratlı (2013), which is one of the studies on honey bee populations in different geographical regions using discriminant separation analysis for grouping, Syrian and Iranian honey bee samples formed different groups. Iranian honey bees were more similar to the samples from Hakkari, Van, and Şırnak, which are close to it; Syrian honey bees formed closer groups with samples from Mardin, Kilis, Hatay, and Şanlıurfa, which are neighboring it. According to Ftayeh et al. (1994), the bees in the region from Lake Van to the Mediterranean corner belong to one of the six ecotypes of Iranian honey bees (*A. m. meda*). Recent studies on the morphometric and genetic relationships of *Apis mellifera meda* populations in regions such as Eastern Anatolia, Southeastern Anatolia, Syria, and Iran reveal distinct clustering patterns influenced by geographic and ecological factors. (Adl et al., 2007; Bodur et al., 2007; Kence et al., 2009; Modaber et al., 2019). The genetic and morphometric variations among honey bee populations are significantly influenced by geographic proximity. For instance, bees from regions like Ardabil and Azarbaijan in Iran show genetic resemblance due to their geographical closeness (Rajabi-maham et al., 2018). Similarly, bees from Central Anatolia and Caucasian regions show closer genetic

relationships compared to those from Iran (Adl et al., 2007; Kekeçoğlu & Soysal, 2010). Ecological factors, such as local climate and flora, also contribute to the observed variations. The presence of distinct ecotypes in different regions of Türkiye, for example, is attributed to the diverse ecological conditions across these areas (Kandemir et al., 2000, Kekeçoğlu & Soysal, 2010).

In our study, according to Procrustes distances, it was observed that the shape differed among all locations. This difference was most pronounced between Iran and other locations. Following Iran, the variation between Gevaş and other locations was also noteworthy. According to the Mahalanobis distances, the widest variation is between Iran and Gevaş. In the study conducted by Badalı (2010), when the DFA distribution graph was examined, each group separated from each other with a high level of significance ($p < 0.001$). The two groups consisting of Iranian and Artvin samples showed different clustering from other groups, but there were few signs of separation. While Azerbaijani and Hakkari samples were close to each other, Iraqi and Hakkari samples formed completely different groups. In our study, Hakkari samples showed similarity to Başkale, Özalp, and Iranian samples. Additionally, according to the DFA results using forewing data from Badalı (2010), it was determined that the first axis explained 47.3% of the total diversity, and the second axis explained 35.1%. In our study, according to the DFA results, it was evident that the first axis explained 77.5% of the total variation, and the second axis explained 22.5%.

In this study, ANOVA showed greater variation in terms of F value for centroid size between locations compared to apiaries belonging to beekeepers. Based on the F value, more differences were detected in terms of shape between locations than between apiaries. In the study by Dolatti et al. (2013) centroid sizes of forewings in different geographical regions of Iran were compared. The results showed a significant difference in the centroid sizes of forewings ($F = 10.6$, $p = 0.000$). In a study where honey bee samples from 1987-1988 were assessed with MorphoJ software, the Procrustes ANOVA test utilized to assess population disparities revealed statistically meaningful shape variations between sites ($p < 0.0001$), but not meaningful regarding size of centroids. In the same study, the Procrustes ANOVA test with recent (2017) honey bee samples showed statistically significant differences in shape between locations ($p < 0.0001$) (Kösoğlu et al., 2021).

According to the CVA graphs obtained from canonical variate analysis, it was observed that the samples from Başkale, Özalp districts, and Hakkari province overlapped. Although there were occasional overlaps, this quartet including Çatak district clustered closely with the groups formed by the Gevaş and Iranian samples. However, it was seen that Gevaş samples and Iran samples had different distributions compared to other groups. In addition, the Çatak ellipse intersected more with Gevaş samples, while Başkale and Hakkari ellipses intersected more with Iran samples. These results were confirmed by deformation grids. The close distribution of the samples from Hakkari to the Iranian samples is interpreted as an indication of natural or artificial bee entry from Iran into Türkiye across the border. The Gevaş region stands out from the other sampled regions in terms of both the number of colonies and production techniques. Accordingly, it is assumed that there is a gene flow of the same genotype into the region. Furthermore, the observed uniformity in the district colonies suggests the possibility of selection, even under beekeeping conditions. In the study by Kösoğlu et al. (2021), where wing samples from different geographical regions were analyzed using Morpho J software, the CVA from the populations indicated that old and recent groups formed distinct clusters. While historical and new sets were located in two clusters on the graph, differences represented by populations within their own groups (old or new) were also observed. The CVA graph of the samples taken from 24 apiaries also indicated intra-group variation in our study.

The rich flora of Van province, the change of climatic characteristics over short distances, its geographical location, and beekeeping culture provide an extremely favorable environment for production activities. Therefore, it is of great importance to determine the honey bee subspecies *Apis mellifera meda*, which is accepted to exist in part of the Eastern Anatolia region based on previous research (Özdil et al., 2012), and to protect local genotypes adapted to the region. Scientific research conducted so far has

revealed that the genetic characterization of honey bee populations in Van province was related to the local race *Apis mellifera meda* (Bodur et al., 2007; Kence et al., 2009). However, there are also findings indicating heterogeneity in honey bee populations in Van province (Tunca & Kence, 2011). In this study, fixed honey bee populations from Van districts and Hakkari, along with samples from Iran, were evaluated for genetic similarity and difference levels and current population potential using geomorphometric methods. It is thought that due to migratory beekeeping, queen bee trade, and selective breeding activities, *Apis mellifera meda* has been hybridized with *Apis mellifera syriaca*, *Apis mellifera caucasica*, and *Apis mellifera anatoliaca* races from time to time in Van province and its surroundings. In the survey study conducted by Erkan & Aşkın (2001), the bee races used in migratory beekeeping activities in Van province were also evaluated. At the end of the study, 66% of the breeders used Caucasian bees, while 20% used Iranian bees. This situation confirms the theory that migratory beekeeping and queen bee sales affect the local bee gene pool in the long term. However, the use of different bee breeds may cause differentiation in the local gene pool. Nevertheless, the direction and extent of this effect have not yet been comprehensively investigated.

Another example of hybridization between subspecies is the analysis results of honey bee samples collected from 55 different locations in 7 different geographical regions of Türkiye, using morphometric observations as well as molecular methods. According to these results, a close relationship was found between *A. m. anatoliaca*, *A. m. meda*, and *A. m. caucasica* subspecies (Kekeçoğlu & Soysal, 2010). In the study conducted by Kence et al. (2009), samples from 7 populations of honey bees, including 5 *Apis mellifera meda* populations from Iran and 2 populations (Artvin, Hakkari) from Türkiye, were examined. Of the three groups that emerged from the morphometric analysis, the first included all Iranian populations, while the rest contained *A. m. meda* and Caucasian bees from Türkiye. Our study was conducted with honey bee samples from stationary beekeepers in Özalp, Başkale, Çatak, and Gevaş districts, as well as Hakkari and Iran, which were thought to be less affected by migratory beekeeping activities and did not purchase queen bees. The results revealed that there are still preserved geomorphometric differences as well as similarities between Van (Gevaş), Iran, and Hakkari populations. The overlapping distributions observed may result from the limited number of samples collected from certain regions (particularly Iran) and the influence of environmental and anthropogenic factors (e.g., migratory beekeeping, queen bee trade, and hybridization). Although the sampled regions are geographically distant, the distribution of honey bees and the mating behavior of queen bees reduce the effect of this distance. The data on the presence of *A. m. meda*, which shows local characteristics in honey bee populations in Van province, needs to be updated.

Morphometric methods provide great advantages when used to assess biodiversity and for taxonomic purposes. Classical and geometric morphometry are important tools for identifying and distinguishing subspecies in honey bees. While traditional morphometry is restricted to distance measurements and distance rotations, geomorphometric analyses not only encompass these measurements indirectly but also facilitate wing morphology analysis through the landmark-based technique, making it an accepted cheap, fast, and precise method for identifying honey bee races and populations. Kandemir et al. (2011) stated that the analysis of landmarks found in wing shapes was a powerful and reliable method for distinguishing honey bee subspecies. Oleksa & Tofilski (2015) showed that in particular research, morphometry was more effective than molecular markers in identifying subspecies, and morphological traits were better suited for differentiating ecotypes among honey bee races. In this study, geometric morphometric analysis method, which is more reliable and advantageous than classical morphometry for the classification and identification of *Apis mellifera* L. subspecies, was used; these analyses were performed to evaluate the effect of hybridization on fluctuating asymmetry. In some previous studies conducted with honey bee samples collected from various regions of Türkiye, the Van region was represented by a limited number of populations, and it was concluded that the samples showed similarities to *A. m. meda* based solely on classical morphometric data. In this study, honey bee samples from stationary colonies taken from Van, Hakkari, and Iran were measured and evaluated using the geometric morphometry method.

Apis mellifera, which is vital for agricultural economy, shows diversity in terms of morphological and genetic characteristics. Morphometric, geomorphometric, and molecular studies on honey bees are very useful methods for determining genetic and geographical differences. Scientific research reveals that Anatolia is a honey bee gene center and that there are five separate honey bee races and ecological forms in this region (Smith, 2002). Today, no country has such a diversity of honey bee races together. Factors such as honey bee diseases and pests, pesticides, commercial queen bee sales that have not been tested for suitability to regions, uncontrolled mating, and migratory beekeeping can reduce biodiversity in bees, and in some cases can cause serious yield losses or even colony losses. (Hristov et al., 2020). Especially the desire to increase yield causes breeders to turn to genotypes that can exhibit their characteristics in their own geographical conditions, which can lead to the deterioration of local genotypes on the one hand and the homogenization of the gene pool and the reduction of diversity on the other hand. Migratory beekeeping, which is conducted for the same purpose and gains a different dimension day by day, carries the same risks and can threaten the ecological adaptation and genetic uniqueness of local genotypes (Jara et al., 2020). For these reasons, the identification and protection of local honey bee races is extremely important. These determinations should be taken into account not only for the economic contributions of beekeeping but also for the cultural history, ecological structure, and scientific future of our country.

In this study, the similarities of the samples taken from Başkale, Çatak, and Özalp districts of Van province indicate the hybridization of honey bee colonies due to migratory beekeeping, queen bee trade, and selective breeding activities conducted in these regions. On the other hand, the analysis results can be interpreted as the honey bees in Gevaş district being less exposed to activities that would cause hybridization due to their more distant positioning between Gevaş and Iran, but it would be appropriate to examine the district samples with other studies. Expanding the sampling to cover broader areas will contribute to the identification of honey bee populations from Gevaş and other regions and aid in the conservation of local bees as genetic resources. For this purpose, the distance of these district bees from other genotypes of the country should also be evaluated. Right forewing size and shape revealed variation between different locations in this study.

It is crucial to determine population structures and evaluate regional differences, as anatomical differences influenced by agroecological factors and beekeeping activities may indicate different subspecies or ecotypes of the same species. Our current dataset includes few samples from Iran. Increasing the number of samples and expanding the geographical coverage in Iran in future studies would allow for a more comprehensive understanding of honey bee population structures. While wing morphometry is increasingly used to determine phenotypic variations between specific levels in insects, it is used for taxonomic purposes in honey bees. This study provides preliminary data for more comprehensive future research on honey bee populations in the Eastern Anatolia region. To ensure the reliable identification samples, it is recommended to establish a database that includes geometric morphometric profiles specific to this region, along with geographical and genetic markers. Environmental data can be collected to associate morphometric variations with ecological factors. Molecular analyses, such as mitochondrial DNA sequencing or microsatellite markers, can be applied to validate morphometric differentiation and assess genetic purity. Thus, it can be clearly determined to what extent the stationary honey bee gene pool in Van province is *Apis mellifera meda* and the impact of migratory beekeeping practices on stationary populations, thereby revealing the current status of honey bee biodiversity.

References

- Adl, M., H. Gençer, Ç. Firatlı & R. Bahreini, 2007. Morphometric characterization of Iranian (*Apis mellifera meda*), Central Anatolian (*Apis mellifera anatoliaca*) and Caucasian (*Apis mellifera caucasica*) honey bee populations. *Journal of Apicultural Research*, 46 (3): 225-231.
- Badalı, M. N., 2010. İran'ın Kuzeyinde Yayılış Gösteren Balarısı Popülasyonlarının Morfometrik ve Geomorfometrik Analizi. Ankara Üniversitesi Fen Bilimleri Enstitüsü, (Unpublished) Yüksek Lisans Tezi, Ankara, Türkiye, 63 s (in Turkish with abstract in English).
- Bodur, Ç., M. Yıldırım & A. Özkan, 2007. Morphometric and genetic analysis of honey bee populations in the East Anatolian region. *Turkish Journal of Zoology*, 31 (1): 41-51.
- Bookstein, A., 1990. Informetric distributions. Part I: unified overview. *Journal of the American Society for Information Science*, 41 (5): 368-375.
- Dolatti, L., J. N. Rafie & H. Khalesro, 2013. Landmark-based morphometric study in the fore and hind wings of an Iranian race of European honey bee (*Apis mellifera meda*). *Journal of Apicultural Science*, 57 (2): 187-197.
- Erkan, C. & Y. Aşkın, 2001. Van ili Bahçeşarayı İlçesi'nde arıcılığın yapısı ve arıcılık faaliyetleri. *Yüzüncü Yıl University Journal of Agricultural Sciences*, 11 (1): 19-28 (in Turkish with abstract in English).
- Franck, P., L. Garnery, M. Solignac & J. M. Cornuet, 2000. Molecular confirmation of a fourth lineage in honey bees from the Near East. *Apidologie*, 31 (2): 167-180.
- Francoy, T. M., D. Wittmann, V. Steinhage, M. Drauschke, S. Müller, D. R. Cunha & L. S. Gonçalves, 2009. Morphometric and genetic changes in a population of *Apis mellifera* after 34 years of Africanization. *Genetics and Molecular Research*, 8 (2): 709-717.
- Hristov, P., R. Shumkova, N. Palova & B. Neov, 2020. Factors associated with honey bee colony losses: A mini-review. *Veterinary Sciences*, 7 (4): 166 (1-17).
- Jara, L., C. Ruiz, R. Martín-Hernández, I. Muñoz, M. Higes, J. Serrano & P. Rúa, 2020. The effect of migratory beekeeping on the infestation rate of parasites in honey bee (*Apis mellifera*) colonies and on their genetic variability. *Microorganisms*, 9 (1): 22 (1-18).
- Kandemir, I., A. Özkan & S. Fuchs, 2011. Reevaluation of honey bee (*Apis mellifera*) microtaxonomy: A geometric morphometric approach. *Apidologie*, 42 (5): 618-627.
- Kandemir, İ., M. Kence & A. Kence, 2000. Genetic and morphometric variation in honey bee (*Apis mellifera*) populations of Turkey. *Apidologie*, 31 (3): 343-356.
- Kekeçoğlu, M. & M. Soysal, 2010. Genetic diversity of bee ecotypes in Turkey and evidence for geographical differences. *Romanian Biotechnological Letters*, 15 (6): 5646-5653.
- Kekeçoğlu, M., M. Bouga, M. İ. Soysal & P. Harizanis, 2007. Morphometrics as a tool for the study of genetic variability of honey bees. *Journal of Tekirdag Agricultural Faculty*, 4 (1): 7-15.
- Kekeçoğlu, M., M. Kambur, M. Uçak, T. Çaprazlı & S. Bir, 2020. Biodiversity of honey bees (*Apis mellifera* L.) in Turkey by geometric morphometric analysis. *Biological Diversity and Conservation*, 13 (3): 1-10.
- Kence, A., 2006. Genetic diversity of honey bees in Turkey and the importance of its conservation. *Uludağ Bee Journal*, 6 (3): 25-32.
- Kence, M., H. J. Farhoud & R. I. Tunca, 2009. Morphometric and genetic variability of honey bee (*Apis mellifera* L.) populations from northern Iran. *Journal of Apicultural Research*, 48 (4): 247-255.
- Klingenberg, C. P., 2011. MorphoJ: An integrated software package for geometric morphometrics. *Molecular Ecology Resources*, 11 (2): 353-357.
- Kösoğlu, M., R. I. Tunca, N. Özsoy & Y.T. Tuna, 2021. Determination of the wing morphology differentiation of old and recent honey bee samples from western Turkey using geometric morphometrics. *Turkish Journal of Entomology*, 45 (4): 463-474.
- Modaber, M., N. Rafie & H. Rajabi-Maham, 2019. Population genetic structure of native Iranian population of *Apis mellifera meda* based on intergenic region and COX2 gene of mtDNA. *Insectes Sociaux*, 66 (4): 413-424.
- Oleksa, A. & A. Tofilski, 2015. Wing geometric morphometrics and microsatellite analysis provide similar discrimination of honey bee subspecies. *Apidologie*, 46 (1): 49-60.

- Özbakır, G. O. & C. Fıratlı. 2013. Morphometric Classification of Honey bee Populations (*Apis mellifera* L.) Along the Southeast border of Turkey. Bulgarian Journal of Agricultural Science. 19 (6): 1396-1400.
- Özdil, F., İ. Aytekin, F. İlhan & S. Boztepe, 2012. Genetic variation in Turkish honey bees *Apis mellifera anatoliaca*, *A. m. caucasica*, *A. m. meda* (Hymenoptera: Apidae) inferred from RFLP analysis of three mtDNA regions (16S rDNA-COI-ND5). European Journal of Endocrinology, 109 (2): 161-167.
- Özkan Koca, A., 2012. Ortadoğu'da Yayılış Gösteren *Apis mellifera* L. (Hymenoptera Apidae) Alt Türlerinin Geometrik Morfometri Yöntemleriyle Analizi. Ankara Üniversitesi Fen Bilimleri Enstitüsü, (Unpublished) Doktora Tezi, Ankara, Türkiye, 167 s (in Turkish with abstract in English).
- Rajabi-Maham, H., T. Ghasemi & S. Pashaei-Rad, 2018. Genetic diversity evaluation of Persian honey bees (*Apis mellifera meda*) in North West of Iran, using microsatellite markers. Journal of Wildlife and Biodiversity, 2 (1): 37-46.
- Rohlf, F. J., 2018. TpsDig Version 2.31 Ecology & Evolution. SUNY at Stone Brook, USA.
- Rohlf, F. J., 2019. TpsUtil32 v.1.78 software: tpsUtil (Version 1.78). SUNY Stony Brook, Stony Brook Morphometrics.
- Ruttner, F., 1988. "Morphometric Analysis and Classification, 66-78". In: Biogeography and Taxonomy of Honey bees (Ed. F. Ruttner). Springer Berlin, Heidelberg, Germany, 284 pp.
- Schwarzfeld, M. D. & F. A. H. Sperling, 2014. Species delimitation using morphology, morphometrics, and molecules: definition of the *Ophion scutellaris* Thomson species group, with descriptions of six new species (Hymenoptera, Ichneumonidae). ZooKeys, 462: 59-114.
- Smith, D. D. R., 2002. Genetic diversity in Turkish honey bees. Uludag Bee Journal, 2 (3): 10-17.
- Taşkıran, Ö., N. M. Dayıoğlu & D. Kabakçı, 2017. Bal Arılarının (*Apis mellifera* L.) Sınıflandırılması ve Ekolojik Koşulların Morfolojisi Üzerine Etkisi. Arıcılık Araştırma Dergisi, 9 (2): 68-77.
- Tofilski, A., 2008. Using geometric morphometrics and standard morphometry to discriminate three honey bee subspecies. Apidologie, 39 (5): 558-563.
- Tunca, I. R. & M. Kence, 2011. Genetic diversity of honey bee (*Apis mellifera* L.: Hymenoptera: Apidae) populations in Turkey revealed by RAPD markers. African Journal of Agricultural Research, 6 (29): 6217-6225.