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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

New Morphometric Approach to Discriminate Honey Bee (*Apis mellifera* L.) Populations in Türkiye

Türkiye'de Bal Arısı (*Apis mellifera* L.) Populasyonlarının Ayırt Edilmesi İçin Yeni Morfometrik Yaklaşım

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Abstract

Today, 29 subspecies have been defined, each of which is adapted to a certain set of environmental characteristics, spreading all over the world except Antarctica. Many morphological and morphometric features have been used to classify honey bees from the past to the present. It has been reported that features such as length, angle and indices coming from the front wings are very efficient for classification. In recent studies, various programs have been developed and automatic classification has been attempted through the images of bee wings. This study aimed to determine the naturally occurring honey bee biodiversity in Turkiye by measuring 7 areas (A1, A2, A3, A4, A5, A6, A7) on the right front wing. For this purpose, a total of 3392 worker bee samples were collected from 143 colonies in 19 provinces of Turkiye. The photographs of the prepared preparations were taken at 1X magnification with the BAB camera system connected to the BAB STR45 stereozoom microscope. The measurements of 7 areas on the right front wings of honey bee populations distributed in Turkiye were made automatically in the BAB Bs200ProP program. Colony averages of the raw data of the area measurements of each province were taken and the results were evaluated with Discriminant Function Analysis (DFA) in the SPSS.15 package program. Multivariate analysis of variance (MANOVA) was applied to separate the groups to determine the variation within and between groups. As a result of this study, the minimum total area was seen in Van at 4.51 and the maximum total area was seen in Ardahan at 5.76. The average size of the measured areas decreased from the north-east to the south of Turkiye. Area measurements on the forewing were found to be a marker for distinguishing Anatolian (*A. m. anatoliaca*) and Caucasian (*A. m. caucasica*) honey bees.

Keywords: Türkiye, Anatolian honey bee (*A. m. anatoliaca*), Caucasican honeybee (*A. m. caucasica*), Front wing, Area

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Öz

Günümüzde Antartika kıtası hariç dünyanın her yerinde yayılış gösteren, her biri belirli bir çevresel özelliklere adapte olmuş 29 alt tür tanımlanmıştır. Geçmişten günümüze kadar bal arılarını sınıflandırmak için birçok morfolojik ve morfometrik özellikler kullanılmıştır. Yapılan çalışmalarda ön kanatlardan gelen uzunluk, açı ve indeksler gibi özelliklerin sınıflandırma için çok etkili olduğu bildirilmiştir. Son yıllarda yapılan çalışmalarda çeşitli programlar geliştirilmiş, arı kanatlarının görüntüleri aracılığıyla otomatik sınıflandırma yapılmaktadır. Bu çalışmada sağ ön kanatta yer alan 7 alan (A1, A2, A3, A4, A5, A6, A7) ölçülerek Türkiye'de doğal olarak bulunan bal arısı biyoçeşitliliğinin belirlenmesine çalışılmıştır. Bu amaçla Türkiye'nin 19 ilindeki 143 koloniden toplam 3392 işçi arı örneği toplanmıştır. Hazırlanan preparatların fotoğrafları BAB STR45 stereozoom mikroskobuna bağlı BAB kamera sistemiyle 1X büyütmede çekilmiştir. Türkiye'de yayılış gösteren bal arısı populasyonlarının sağ ön kanatları üzerindeki 7 alanın ölçümleri BAB Bs200ProP programında otomatik olarak yapılmıştır. Her ile ait alan ölçümlerinin ham verilerinin koloni ortalamaları alınarak sonuçlar SPSS.15 paket programında Diskriminant Fonksiyon Analizi (DFA) ile değerlendirilmiştir. Grup içi ve gruplar arası varyasyonun belirlenmesinde grupları ayırmada çok değişkenli varyans analizi (MANOVA) uygulanmıştır. Bu çalışmanın sonucunda minimum toplam alan 4.51 ile Van'da görülürken maksimum toplam alan 5.76 ile Ardahan'da görülmüştür. Ölçülen alanların ortalama büyüklüğü Türkiye'nin kuzey doğusundan güneyine doğru azalmıştır. Ön kanattaki alan ölçümlerinin Anadolu (*A. m. anatoliaca*) ve Kafkas (*A. m. caucasica*) bal arılarını ayırt etmede kullanılabilecek bir marker olduğu görülmüştür.

Anahtar Kelimeler: Türkiye, Anadolu bal arısı (*A. m. anatoliaca*), Kafkas bal arısı (*A. m. caucasica*), Ön kanat, Alan

1. Introduction

Honey bee diversity of the World is represented by the described 29 honey bee subspecies. Each of these is adapted to environmental conditions, also their fitness and adaption abilities enable them to spread all over the world except for the Antarctic region (Rahimi et al., 2018). Anatolia (Türkiye) has an extraordinary morphological diversification and evolutional patterns for honeybees. Ruttner (1988) classified honeybee races in this region as *A. m. anatoliaca*, *A. m. caucasica*, *A. m. meda*, and *A. m. syriaca* on the "O" branch. *A. m. caucasica* distributes along the eastern Black Sea coast, *A. m. meda* in the southeast, and *A. m. syriaca* in the south, near the border with Syria. *A. m. anatoliaca* occurs throughout the rest of Türkiye including Thrace. mtDNA studies showed that other subspecies have been found in the European part of Türkiye, Thrace, maybe *A. m. carnica* which belongs to the branch "C" of Ruttner's classification (Smith et al., 1997; Bodur et al., 2004, 2007; Kandemir et al., 2000, 2005). Kandemir et al. (2006a) reported the existence of a fourth new mtDNA lineage of *Apis mellifera* near the Syrian border of Türkiye (Franck et al., 2000). mtDNA analyses showed that *A. m. anatoliaca* and *A. m. caucasica* are closely related to the Eastern or "C" mitochondrial lineage (Smith et al., 1997; Palmer et al., 2000 Kandemir et al., 2006a; Özdil et al., 2009a, b; Özdil et. al., 2022; Bir and Kekeçoğlu, 2023) as do *A. m. carnica* and *A. m. ligustica*. But Ruttner's approach indicates *A. m. caucasica* and *A. m. anatoliaca* belong to a separate oriental group.

The first scientific studies describing honeybees were made according to standard morphometric methods. Many different morphological characters such as the length of the wings, tongue and other mouth apparatus, the length and width ratios of the plates of the tergite and leg segments, and the wing vein indexes were used (Bodenheimer, 1941; Settar, 1983; Ruttner, 1988; Güler and Kaftanoğlu, 1999a, b, c; Kandemir et al., 2000; Güler, 2000; Güler et al., 2002; Güler and Bek, 2002; Kandemir et al., 2005; Kekeçoğlu et al., 2007; Güler and Toy, 2008; Kekeçoğlu et al., 2009; Kekeçoğlu and Soysal, 2010; Güler et al., 2013; Koca and Kandemir, 2013; Çakmak et al., 2014). In the later studies, in addition to these morphometric characters, it has been tried to distinguish honey bees by using measurements such as the angle and index of the wings. Many studies have proven that front wing features are suitable characters to classify honey bees.

Morphometric studies of *Apis mellifera* L. to evaluate biodiversity, subspecies and intra-populational variability are very effective and convenient approaches for a long time (Ruttner et al., 1978; Ruttner, 1988; Mendes et al., 2007). Among other morphological features, wing venations patterns of honey bees were studied extensively covering angles, size of forewings and cubital index (DuPraw, 1965; Cournet and Fresnaye, 1989; Ruttner, 1987; Francoy et al., 2006). Contrary to traditional morphometrics employing size, masses and ratio of areas; relatively new geometric morphometric methods perform analyses by shape and form obtained arbitrarily of landmarks. The coordinate based geometric morphometric studies are found as the most robust approach accessible for the statistical analysis of shape (Rohlf, 2000a, b). Moreover, a comparison of both methods revealed unsuccessful and inadequate results using the classical method and encouraged applying new arrangements such as geometric morphometrics (Cavalcanti et al., 1999). In order to save time and energy, programs have been developed for image analysis on the computer. The basis of these programs is to transfer the image to the computer and transfer it to the automatic identification system with the determined points. Accordingly, more practical and easy automatic identification systems, such as TpsDig (Bookstein, 1991), ABIS (Schroder et al., 2002), DrawWing (Tofilski, 2004), DAWINO (Dicriminant Analysis With Numerical Outputs) (Uzunov et al., 2009), BAB Bs200ProP (Kambur and Kekeçoğlu, 2018; Kekeçoğlu 2018), DeepWings (Rodrigues et al., 2022), have been researched to classify honey bees through images of bees (Bookstein, 1991; Roth et al., 1999; Tofilski, 2004; Miguel et. al., 2011; Rodrigues et al., 2022).

The aim of the present work was to determine the morphometric variation of honey bees distributed in Türkiye, using the alternative image analysis method, which is area-based morphometric analysis. Area based geometric morphometric method is a relatively new technique that has generated valuable results in many fields of morphometry (Oettle et al., 2005; Nolte and Sheets 2005; Mendes et al., 2007; Kimmerle et al., 2008; Ogıhara et. al., 2008; Hayes et al., 2007; Francoy et al., 2009a,b). An application of the area-based morphometric method on Turkish honey bees is the first time in the present study.

2. Materials and Methods

A total of 3392 individuals from 143 colonies in 19 locations (Van, Hakkari, Tekirdağ, Kırklareli, Edirne, Zonguldak, Sakarya, Düzce, Artvin, Trabzon, Ordu, Isparta, Muğla, Gaziantep, Hatay, Kilis, Kahramanmaraş, Ardahan, Kars) of the seven regions of Türkiye were studied. Sampling was carried out mostly from, locally managed nonmigratory and requeening colonies in apiaries and 5-10 honey bee colonies were randomly chosen from per apiary, and all colonies were described by means of 15 worker bees. Preparation of sample collections for the microscope was done according to the method of Kekeçoğlu et al., (2020). The study material consisted of the right front wing of each worker bee. The photographs of slides were monitored at 1X magnification with the BAB camera system using stereozoom microscope. In this study, the measurements of 7 areas (A1, A2, A3, A4, A5, A6 and A7) on the right front wings were made in the BAB Bs200ProP program (BAB Imaging systems, BAB Ltd, 2007) (*Figure 1*).

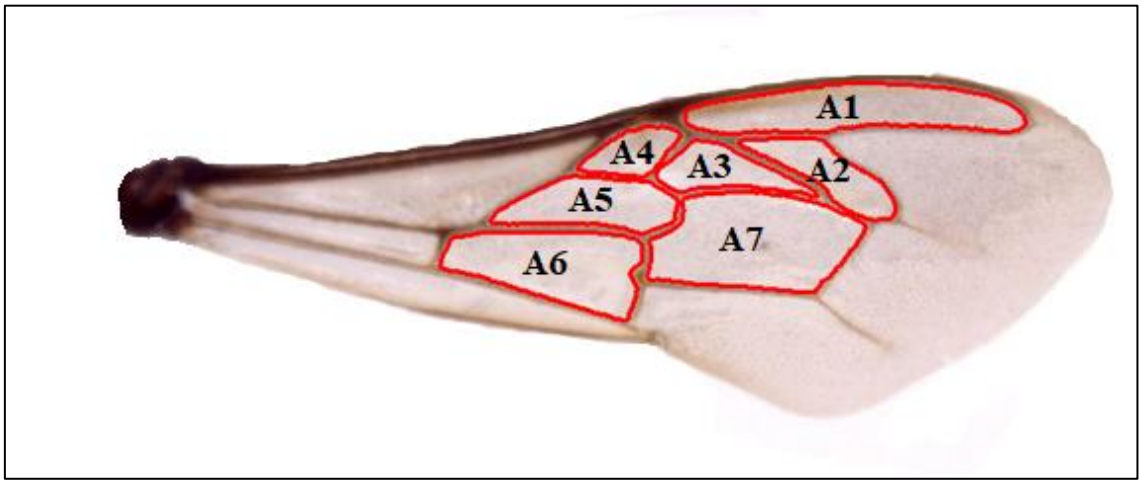

Figure 1. Forewings' areas measured on worker bees by using the BAB Bs200ProP programme

The measurement results of the worker bee samples representing each province were evaluated with Discriminant Function Analysis (DFA) in the SPSS.15 package program. Multivariate analysis of variance (MANOVA) was applied to determine the variation within and between groups. The Cross Validation Test (CVT) was performed to see the distribution of the populations among groups, and the distribution of the samples among the groups was determined.

3. Results

The morphometric variation of the honey bee population was determined for 19 different locations in Turkiye using area-based geometric morphometrics in the current study. For this reason 7 areas on the right fore wing were measured. Based on the measurements of seven area on the fore wing of worker honey bees from 19 different locations in Türkiye, it was concluded that the highest total area value was determined for Ardahan worker honeybee with 5.76, the lowest was seen in Van with 4.51.

The highest variability of A5 and A6 areas was found in honey bees from Artvin and Zonguldak. Honeybees from Artvin and Zonguldak showed the highest value (0.69 mm^2) for the A5 area and bees from Van showed the lowest value for all area. The analysis of variance revealed that the size of the wing area has significant differences between locations ($P < .05$). The size of the measured areas decreased from the north-east to the south of Türkiye.

The CVA based on the data of the seven areas of the right front wings showed that there was more overlapping among honeybee populations couldn't be identified clearly. The clusters plotted on the CVA graph are also relatively close each other, and intergaps between the groups were not shown clearly. Although Northeast and the rest part of Türkiye well separated with two axis: The population were placed mainly upper half, Trabzon, Zonguldak, Tekirdağ population mainly in lower-right-hand quandrant and small set of samples from Southeastern (Kilis) and Aegen (Muğla) in more lower-left-hand of the plot (*Figure 2).*

Tekirdağ, Trabzon, Zonguldak, Artvin, Ardahan, Sakarya and Edirne constitute a group in the phylogenetic tree drawn according to binary distances; Kırklareli, Isparta, Ordu, Düzce, Kahramanmaraş, Hakkari, Gazianatep, Kars, Muğla, Hatay and Van grouped together and a separate group close to Kilis was formed (*Figure 3*).

One-way ANOVA showed significant differences among honeybee populations. Out of the Düzce, Ordu and Kırklareli populations, generally all honeybee populations showed the expected classification schema by pairwise distance. There were significant size differences between Northeastern (Black Sea) and Southeastern (Kilis and Gaziantep). Honeybees from Central Anatolia and Eastern Anatolian region didn't differ significantly from each other.

Table 1. Front wing's area minimum (Min.) maximum (Max.). and average (X) values

	Colony number	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**Total**
LOCATION	**N**	**X ± Sx (Min.-Max.)**	**X ± Sx (Min.-Max.)**	**X ± Sx (Min.-Max.)**	**X ± Sx (Min.-Max.)**	**X ± Sx (Min.-Max.)**	**X ± Sx (Min.-Max.)**	**X ± Sx (Min.-Max.)**	**X ± Sx (Min.-Max.)**
		1,12±0,01	0,44±0,00	0,41±0,00	0,31±0,00	0,66±0,00	1,04±0,01	1,47±0,01	5,46±0,04
Tekirdağ	5	1,09-1,14	0,44-0,45	0,40-0,41	0,30-0,32	0,65-0,67	1,02-1,07	1,44-1,50	5,36-5,55
		0,96±0,00	0,38±0,01	0,36±0,01	0,27±0,01	0,57±0,01	0,91±0,01	1,28±0,01	4,71±0,05
Kırklareli	5	0,95-0,97	0,36-0,41	0,34-0,37	0,26-0,29	0,55-0,59	0,89-0,93	1,26-1,30	4,64-4,82
		1,07±0,02	0,42±0,01	0,39±0,01	0,28±0,01	0,65±0,01	1,01±0,01	1,40±0,01	5,21±0,09
Edirne	9	0,97-1,14	0,39-0,45	0,33-0,41	0,25-0,33	0,59-0,68	0,96-1,06	1,34-1,45	5,07-5,39
		1,15±0,01	0,47±0,01	0,44±0,01	0,32±0,01	0,69±0,01	1,08±0,02	1,55±0,02	5,70± 0,08
Zonguldak	9	1,07-1,21	0,44-0,49	0,39-0,48	0,30-0,34	0,63-0,75	1,00-1,17	1,44-1,66	5,28-6,08
		1,10±0,01	0,47±0,02	0,43±0,01	0,32±0,01	0,68±0,01	1,07±0,01	1,49±0,02	5,56± 0,08
Sakarya	5	1,06-1,13	0,44-0,52	0,41-0,44	0,30-0,34	0,66-0,72	1,03-1,08	1,45-1,55	5,39-5,74
		0,99±0,01	0,41±0,01	0,37±0,01	0,27±0,00	0,58±0,01	0,93±0,01	1,31±0,02	4,87± 0,07
Düzce	9	0,96-1,04	0,38-0,44	0,33-0,44	0,26-0,29	0,55-0,63	0,90-1,01	1,25-1,40	4,70-5,21
		1,14±0,01	0,47±0,01	0,43±0,00	0,32±0,01	0,69±0,01	1,05±0,01	1,46±0,01	5,55± 0,05
Artvin	10	1,06-1,18	0,44-0,49	0,40-0,44	0,28-0,34	0,66-0,73	0,99-1,08	1,40-1,50	5,28-5,69
		1,16±0,01	0,46±0,00	0,41±0,02	0,32±0,00	0,68±0,00	1,08±0,01	1,50±0,01	5,61± 0,04
Trabzon	9	1,14-1,19	0,45-0,48	0,39-0,44	0,31-0,34	0,66-0,70	1,06-1,12	1,46-1,57	5,50-5,79
		0,96±0,01	0,38±0,01	0,35±0,01	0,26±0,00	0,56±0,01	0,90±0,01	1,26±0,01	4,66± 0,06
Ordu	9	0,89-1,00	0,34-0,41	0,32-0,37	0,25-0,28	0,52-0,58	0,83-0,95	1,19-1,30	4,39-4,84
		0,97±0,01	0,39±0,00	0,37±0,00	0,28±0,00	0,58±0,00	0,94±0,01	1,31±0,01	4,85± 0,04
Isparta	9	0,92-1,01	0,38-0,41	0,36-0,39	0,27-0,29	0,56-0,60	0,90-0,98	1,25-1,37	4,72-4,92
		1,01±0,01	0,43±0,00	0,39±0,01	0,29±0,00	0,63±0,01	1,00±0,01	1,39±0,01	5,13± 0,06
Muğla	8	0,97-1,07	0,41-0,45	0,33-0,41	0,26-0,30	0,60-0,66	0,95-1,05	1,35-1,42	5.00-5,35
		1,01±0,01	0,42±0,01	0,37±0,01	0,29±0,00	0,59±0,01	0,94±0,01	1,33±0,02	4,94± 0,06
Gaziantep	9	0,97-1,07	0,40-0,46	0,34-0,40	0,28-0,29	0,54-0,62	0,90-1,01	1,27-1,46	4,76-5,29
		1,02±0,03	0,43±0,01	0,38±0,01	0,28±0,01	0,61±0,02	0,98±0,02	1,36±0,02	5,06± 0,11
Hatay	7	0,94-1,14	0,41-0,47	0,35-0,43	0,26-0,31	0,56-0,67	0,93-1,04	1,29-1,47	4,81-5,53
		1,00±0,01	0,43±0,01	0,39±0,01	0,31±0,00	0,62±0,01	1,00±0,01	1,41±0,00	5,17± 0,04
Kilis	3	0,99-1,02	0,42-0,45	0,38-0,40	0,31-0,32	0,61-0,64	0,99-1,01	1,40-1,41	5,14-5,21
		0,99±0,01	0,40±0,01	0,36±0,01	0,27±0,00	0,56±0,01	0,90±0,01	1,29±0,01	4,77± 0,06
Kahramanmaraş	8	0,95-1,06	0,37-0,43	0,33-0,38	0,26-0,29	0,51-0,60	0,85-0,94	1,24-1,37	4,54-5,08
		1,18±0,01	0,49±0,00	0,44±0,00	0,33±0,00	0,71±0,01	1,08±0,01	1,53±0,01	5,76± 0,04
Ardahan	7	1,16-1,20	0,48-0,50	0,43-0,45	0,32-0,34	0,69-0,73	1,07-1,10	1,49-1,57	5,67-5,88
		1,02±0,01	0,40±0,01	0,38±0,00	0,28±0,01	0,59±0,01	0,93±0,01	1,31±0,01	4,91± 0,05
Kars	8	0,98-1,06	0,38-0,42	0,36-0,40	0,26-0,31	0,56-0,61	0,89-0,98	1,27-1,35	4,79-5,08
		0,91±0,01	0,38±0,01	0,34±0,00	0,26±0,00	0,53±0,01	0,87±0,01	1,22±0,01	4,51± 0,05
Van	6	0,87-0,94	0,36-0,40	0,33-0,35	0,25-0,27	0,51-0,55	0,89-0,90	1,19-1,27	4,43-4,58
		0,96±0,01	0,40±0,01	0,36±0,00	0,27±0,00	0,56±0,01	0,89±0,01	1,29±0,01	4,74± 0,05
Hakkâri	8	0,92-0,99	0,37-0,43	0,34-0,39	0,26-0,28	0,52-0,59	0,86-0,92	1,24-1,31	4,54-4,85
Average	143	1,04±0,01	0,43±0,01	0,39±0,01	0,29±0,00	0,62±0,01	0,98±0,01	1,38±0,01	5,12± 0,04
		0,99-1,08	0,40-0,45	0,36-0,42	0,28-0,31	0,59-0,65	0,94-1,02	1,33-1,43	4,39-6,08

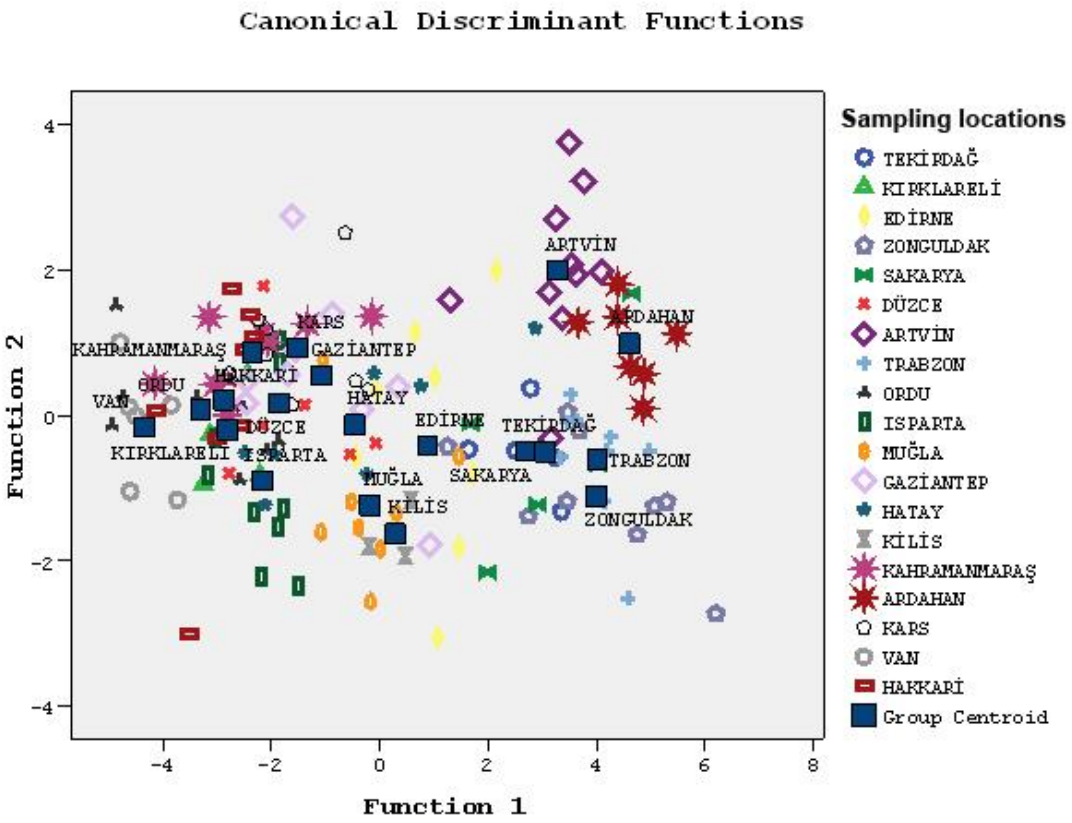

Figure 2. Two dimensional clustering in Canonical Variates Analysis of individuals data from 7 different geographic region.

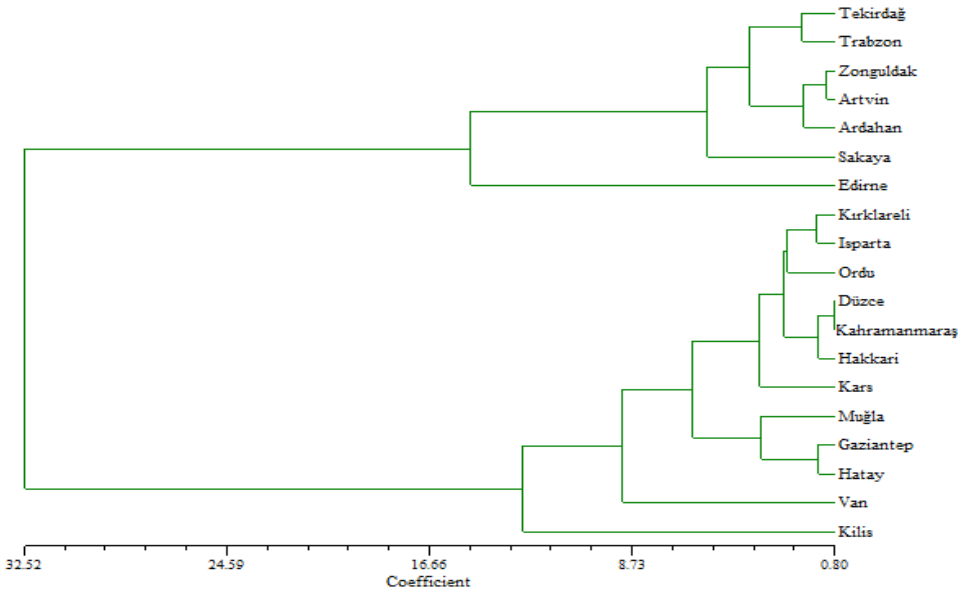

Figure 3. Phylogenetic tree according to UPGMA (Un-weighed Pair Group Method) method based on standardized Euclidean distance

4. Discussion

Morphometric techniques are more advantageous being practical, cheap and no requirements of special acknowledge that rather preferred comparison to biochemical and molecular techniques (Sheppard and Smith, 2000). Although gene expressions that are affected largely by environmental conditions cause disadvantages, statistical methods like PCA and DFA apply more than a dozen characters, and morphometric studies are founded more reliable than enzyme locus studies (Rinderer et al., 1990).

The distribution of Anatolian honeybee populations was first studied by Buttel Reepen (1906) in small areas of Aegean and Marmara regions. Employing morphologic characters that divided Türkiye to seven zones by Bodenheimer (1941). Maa (1953) was the first systematist to characterize and named as subgenus of *A. m. anatoliaca* by morphometrics. Adam (1983) examined honeybees in Anatolia and found similar results with Bodenheimer that 4 races with many ecotypes with rich gene sources. Many researchers reviewed through alloenzyme variations of Anatolian honeybees supported the findings of Ruttner (1988) in general (Darendelioglu and Kence, 1992; Kandemir and Kence, 1995; Güler and Kaftanoglu 1999a,b,c; Güler et al. 1999; Kandemir et al.,1995, 2000; Güler et al., 2002).

Both morphometric and alloenyzme studies resulted that five subgenus of *A. mellifera* throughout Türkiye including *A. m. anatoliaca* (Ruttner 1988; Smith et al., 1997; Palmer et. al., 2000; Kandemir et. al., 2006a), *A. m. caucasica* (Ruttner 1988; Smith et. al., 1997; Palmer et al., 2000; Kandemir et al., 2006a), *A. m. carnica* (Bodenheimer 1941; Smith et. al., 1997; Palmer et. al., 2000; Kandemir et. al., 2006a), *A. m. syriaca* (Ruttner 1988; Palmer et al., 2000; Kandemir et. al., 2006 a, b, c), *A. m. meda* (Ruttner 1988).

The area-based geometric morphometric methodology has been used as a tool to investigate the relationship of honeybees in the present study. Area-based geometric morphometrics is a relatively new technique that has generated valuable results in many fields of morphometry (Oettle et al., 2005; Nolte and Sheets 2005; Mendes et al. 2007; Kimmerle et al., 2008; Ogıhara et. al., 2008; Hayes et al., 2007; Francoy et al., 2009a, b).

In our previous traditional morphometric study (Kekeçoğlu, 2007) with the current regional population, the linear regression was found between wing size and geographical location (longitude and latitude), the present analysis enables comparisons of size independent covarians in wing area.

Canonical Variance Analysis (CVA) of shape data revealed strong two main cluster that showed dimensional reduction of variability via canonical variates analysis (CVA) can be appropriate to explore area variability within homogenous samples subspecies (Francoy et al., 2009a, b).

The average area measurements were found 5.5, 5.2, 5.3, 5.3 ve 5.3 mm^2 respectively for *A. m. carnica*, *A. m. macedonica*, *A. m. mellifera*, *A. m. ligustica* ve *A. m. caucasica* subspecies respectively (Uzunov et. al., 2009).

Francoy et al. (2006) reported A7 as 0.92 for African bees and 1.09 for Italian and Carnica bees for the A7 area. As a result of the present study, similar values were found with Tekirdağ, Ordu, Düzce, Isparta Van and Hakkari African bees; while Ardahan, Trabzon and Artvin have higher values. The remaining provinces gave similar values with Carnica and Italian bees.

The area-based geometric morphometric technique can extract subtle differences like these, which can be unexpected or difficult to extract with traditional morphometry. Kekeçolu (2007) found that the specimens from Northeastern honeybee, *A. m. caucasica* formed a strong close cluster with Anatolian honeybee, *A. m. anatoliaca* based on traditional morphometric analysis. However, in this study, in contrast to previous study, the small set of samples from Northeast Anatolia further resolved and formed a distinct cluster that may belong to Caucasican honeybee (Rutner 1988).

5. Conclusion

The valuable results from are-based morphometric were appeared for the description of differences between honey bee populations through North and south of Türkiye. The present results offered an important basis for future comparative studies between honeybee populations, from Georgia and Northeastern part of Türkiye to better clarify the origin and characteristics of Northeastern population of Türkiye.

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