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Original article (Orijinal araştırma)

Wing shape analysis on some species of *Terellia serratulae* (L., 1758) group (Diptera: Tephritidae) based on geometric morphometric analysis

Bazı *Terellia serratulae* (L., 1758) grup (Diptera: Tephritidae) türlerinde geometrik morfometrik analiz temelli kanat şekil analizi

Mehmet YARAN [1*] **Ayça ÖZKAN KOCA[2]** **Murat KÜTÜK[3]**

Abstract

Tephritidae (fruit flies) is one of the most important Diptera families and includes more than 200 pest species. Some species in this family have a high level of similarity and are difficult to distinguish morphologically. In this study, landmark-based geometric morphometric analysis using wing images was performed on three members of the *Terellia* (*sensu stricto*) *serratulae* group in order to distinguish *Terellia fuscicornis* (Loew, 1844), *Terellia nigripalpis* Hendel, 1927, and *Terellia serratulae* (L., 1758). Specimens of the *T. fuscicornis*, *T. nigripalpis* and *T. serratulae* used in the study were collected from three provinces (İzmir, Kahramanmaraş and Adıyaman) of Turkey between 2016 and 2018. The geometric morphometric analysis of the wings, using fifteen landmarks, indicated significant differences in the wing shapes of each species, separating them successfully into distinct groups. CVA (canonical variate analysis) results based on the wing shapes strongly support the existence of taxonomically three different species. The reidentification accuracies were high, and wing shape discriminated three species of *Terellia* with over 87% accuracy. Finally, we concluded that landmark-based geometric morphometric analysis could be a powerful tool to identify *Terellia* spp.

Keywords: Geometric morphometric, Tephritidae, *Terellia*, Turkey

Öz

Tephritidae (meyve sinekleri) 200'den fazla zararlı türü içeren en önemli sinek familyalarından bir tanesidir. Bu familyadaki bazı türler yüksek seviyede benzerlik içerir ve morfolojik olarak ayrımları zordur. Bu çalışmada, *Terellia fuscicornis* (Loew, 1844), *Terellia nigripalpis* Hendel, 1927 ve *Terellia serratulae* (L., 1758) türlerini ayırt etmek için, kanat resimleri kullanılarak landmark tabanlı geometrik morfometrik analizi, *Terellia* (*sensu stricto*) *serratulae* grubunun üç üyesi üzerine uygulandı. Çalışmada kullanılan *T. fuscicornis*, *T. nigripalpis* ve *T. serratulae* bireyleri Türkiye'nin üç ilinden (İzmir, Kahramanmaraş ve Adıyaman) 2016 ve 2018 yılları arasında toplanmıştır. On beş landmark kullanılarak uygulanan geometrik morfometrik analiz, her bir türün kanat şekillerinde önemli farklılıklar olduğunu göstermiş ve türleri başarılı bir şekilde farklı gruplara ayırmıştır. Kanat şekillerine dayalı CVA (kanonikal varyete analizi) sonuçları, taksonomik olarak üç farklı türün varlığını güçlü bir şekilde desteklemektedir. Kanat şekli, *Terelllia*'nın üç türünü 87% üzerinde doğrulukla ayırt etmiş ve tekrar teşhislerin doğrulamaları yüksek bulunmuştur. Son olarak, landmark temelli geometrik morfometrik analizin *Terellia* türlerini tanımlamak için güçlü bir araç olabileceği sonucuna varılmıştır.

Anahtar sözcükler: Geometrik morfometrik, Tephritidae, *Terellia*, Türkiye

[1] Gaziantep University, Islahiye Vocational School, Department of Medical Services and Techniques, 27800, İslahiye, Gaziantep, Turkey
[2] Maltepe University, Faculty of Fine Arts, Department of Gastronomy and Culinary Arts, 34850, Maltepe, Istanbul, Turkey
[3] Gaziantep University, Science and Art Faculty, Department of Biology, 27470, Şehitkamil, Gaziantep, Turkey
* Corresponding author (Sorumlu yazar) e-mail: yaran@gantep.edu.tr
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Introduction

The fruit fly family, Tephritidae, is one of the largest family of the Diptera and includes about 492 genera and 4,716 species (Pape et al., 2011). The genus *Terellia* Robineau-Desvoidy, 1830 includes approximately 60 species, which are widely dispersed throughout the Palearctic region (Korneyev & Merz, 1996; Norrbom et al., 1999; Korneyev, 2003, 2006; Kütük, 2009; Kütük et al., 2011; Korneyev et al., 2013; Zarghani et al., 2017; Yaran et al., 2018).

Tephritid flies are almost all phytophagous and include numerous pests of fruit and vegetable crops (Zamani & Khaghaninia, 2016). They include a number of important pest species groups that cannot be adequately identified by morphological or molecular characters (Schutze et al., 2012; Cann et al., 2015) Many species of fruit flies do not attack economically important crops and exploit the flower heads of Asteraceae plants; these are useful in the biocontrol of weeds (White & Elson-Harris, 1992; Headrick & Goeden, 1998; Zamani & Khaghaninia, 2016).

Korneyev (1985) reviewed and recognized the genus *Terellia* as having several species groups, based on similarity of structure of the male terminalia, particularly in respect to the glans of the phallus. The genus *Terellia* contains *serratulae* and *ruficauda* groups, also the Nearctic *Terellia occidentalis* (Snow, 1894) and *Terellia palposa* (Loew, 1862). All of these species have long, semi-tubular sclerites of the acrophallus and the paired flaps inside the glans sparsely covered with blunt spines as synapomorphic characters (Korneyev, 1999). According to Korneyev (1985), *serratulae* group includes seven species. These are: *Terellia serratulae* (L., 1758), *Terellia longicauda* (Meigen, 1838), *Terellia fuscicornis* (Loew, 1844), *Terellia syllibi* (Rondani, 1870), *Terellia nigripalpis* Hendel., 1927, *Terellia latigenalis* Hering, 1942, *Terellia sabroskyi* Freidberg, 1982. White (1989) revised *Terellia virens* (Loew, 1846) species group and synonymized *T. syllibi* as a junior synonym of *T. virens.* Except for *T. latigenalis,* the remaining five species of the serratulae group are widespread in Turkey (Kütük & Yaran, 2011).

The species *T. fuscicornis*, *T. nigripalpis,* and *T. serratulae* have a high level of morphological similarities, and are widespread in Turkey. However, the host plant preferences of these species are different and diverse. The artichoke fruit fly, *T. fuscicornis* is a non-frugivorous species that infest the flower heads of artichokes, *Cynara scolymus* L., 1753 and *C. syriaca* Boiss., 1846 (Asteraceae) (Freidberg & Kugler, 1989). It also infests the flower heads of milk thistle, *Silybum marianum* L., 1753 (Asteraceae) (Knio et al., 2002). In this work, we collected specimens of *T. fuscicornis* from *C. scolymus*. According to Hendel (1927), *T. nigripalpis* infests *Cirsium vulgare* (Savi) Ten, 1835 (Asteraceae), but in this study we obtained *T. nigripalpis* specimens from *Centaurea iberica* Trev. ex Sprengel*,* 1826 (Asteraceae) which is a new host plant for *T. nigripalpis*. Another species *T. serratulae* infests three genera of thistles: *Carduus* L., *Cirsium* Mill. and *Picnomon* Adans (Asteraceae) (Knio et al., 2002). In this study, we collected specimens of *T. serratulae* from *Picnomon acarna* L., 1753. Although *T. nigripalpis* and *T. serratulae* share *C. vulgare* as same host plant, however, the specimens collected from different host plants in this study.

Morphometry is an important method used to identify and determine speciation in insects, including fruit flies, due to its low cost and ease of applicability. In order to distinguish similar and related species, standard morphometric approaches have been used for many years and distinctive morphological characters facilitated studies of taxonomists. Over the last 15 years, geometric morphometric approaches dealing with strictly numerical multivariate analysis of morphological structures, especially the landmark method, have been actively applied to insect taxonomy, like species identification and determination of speciation levels (Wu et al., 2009). However, wings are often preferred in geometric morphometric studies on insects due to their two-dimensional distinctive venation structure, translucent and relatively solid structure.

In this study, we aimed to differentiate between three species (*T. fuscicornis*, *T. nigripalpis* and *T. serratulae*) in the *Terellia serratulae* group, which are distributed in Turkey, based on geometric morphometric analysis of the wings. Two species of the *serratulae* group, *T. longicauda* and *T. sabrosky,* were not included in the analysis because of insufficient available material. The main purpose of this study was to use geometric morphometric approach to measure wing size and shape for previously identified specimens of *T. fuscicornis*, *T. nigripalpis* and *T. serratulae,* and to determine: (1) whether wing size and shape are effective discriminators between species; (2) the extent of differences between these species based on wing analysis, and (3) if any of these species be suspected as conspecific based on morphometric shape data.

Materials and Methods

Sample collection and preparation

Three species of the *serratulae* group were chosen for analysis, *T. fuscicornis*, *T. nigripalpis* and *T. serratulae.* Samples of the species were collected from three locations in İzmir, Kahramanmaraş and Adıyaman Provinces, Turkey. Detailed information of sampling sites for all individuals are shown in Table 1.

A total of 120 females from the three species (40 of *T. fuscicornis,* 41 of *T. nigripalpis* and 39 of *T. serratulae*) were used in this study. The right wing was separated from each specimen and mounted on a slide using Entellan mounting medium. To obtain x and y coordinate scores from landmark, wing images were taken by a camera attached to Olympus SZX 12 microscope on 12.5x magnification for each specimen of wing, and saved as JPEG format.

Table 1. Collection sites in Turkey and host plants of three *Terellia* spp.

Species	N	Province	Coordinates, Altitude	Date	Host plant
T. fuscicornis	40 ♀♀	İzmir, Urla	38°18' N, 26°45' E, 95 m	28.06.2018	*Cynara scolymus*
T. nigripalpis	41 ♀♀	Kahramanmaraş, Çağlayancerit	37°44' N, 37°14' E, 1461 m	30.05.2016	*Centaurea iberica*
T. serratulae	39 ♀♀	Adıyaman, Besni	37°43' N, 37°49' E, 1022 m	07.05.2018	*Picnomon acarna*

***N**: number of individuals.

Statistical analysis

Fifteen homologous Type 1 landmarks (Figure 1) (Bookstein, 1991) were chosen for comparison following the method described by Schutze et al. (2012).

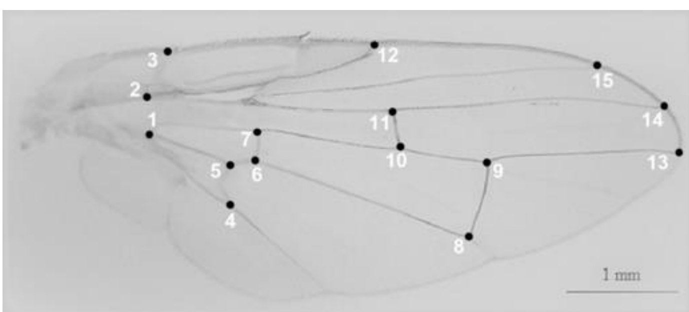

Figure 1**.** Right wing of *Terellia fuscicornis* showing each of the 15 landmarks adopted from Schutze et al. (2012).

All landmarks were digitized using the computer program tpsDig 2.12 (Rohlf, 2008) for which x, y coordinates were generated and saved as a text file (all specimens were scored by a single experimenter in order to reduce the measurement error). Thus, the geometry of shape was captured by a configuration of topographically corresponding landmarks (Marcus et al., 2000) digitized on each specimen.

Two-dimensional coordinates of the landmarks, obtained from tpsDig, were aligned using the generalized Procrustes superimposition analysis (GPA) (Rohlf & Slice, 1990; Dryden & Mardia, 1998; Rohlf, 1999). GPA removed all information of the configurations that were not related to shape, minimizing the distance between homologous landmarks by translating, rotating, and scaling all specimens. Then, shape differences in wing were tested using several statistical analyses. Size analysis was performed on the centroid size (CS) values (Bookstein, 1991), which was calculated as the square root of the summed squared distances of each landmark from the center of the landmark configuration.

Wing size differences between species were analyzed through Kruskal-Wallis test and box plots using Statistica 8.0 software (Statsoft, 2007). The landmark coordinates obtained from tpsDig were used as an input in Morpheus (Slice, 2002) and MorphoJ v.1.06 (Klingenberg, 2011) softwares. These softwares were first perform a GPA to extract shape information from the data and remove differences in orientation, position and isometric size. After GPA superimposition analysis, MANOVA (multivariate analysis of variance) and pairwise analysis was performed in Morpheus to see differences in wing shape of species. The relationship between CS and shape variation was examined by multivariate regression using MorphoJ. The statistical significance of this test was estimated by permutations using 10,000 runs (Klingenberg, 2011). The coordinates of the landmarks were also analyzed using tpsRelw 1.46 (Rohlf, 2007) to perform relative warp analysis (RWA-singular value decomposition analysis), and to calculate singular values for each principal warp and the relative contribution of each landmark. The relative similarities or dissimilarities of the *Terellia* spp. were analyzed by discriminant function analysis (DFA) and canonical variate analysis (CVA) followed by cross validation test (a leave-one-out) using MorphoJ. In order to find out the intensive deformations on the wing shape and comparison wing deformation of *Terellia* spp., the wing shape differences were illustrated on deformation grids using Morpheus software. To determine the significance of differences in the wing shapes, we performed permutation tests (10,000 runs) with Mahalanobis and Procrustes distances. The UPGMA (an unweighted pair group method with arithmetic mean; Rohlf, 2004) dendrogram was constructed by using Mahalanobis distances calculated from the DFA to show the relationships among the *Terellia* spp. based on wing shape.

Results and Discussion

Size analyses

Wing centroid size significantly differed between the three *Terellia* spp. (F = 71.2, P < 0.05). *Terellia fuscicornis* had larger wing size than *T. nigripalpis* and *T. serratulae*. *Terellia serratulae* had the smallest wing sizes (Figure 2). The Kruskal-Wallis test, based on the CS data for wing (H = 90.0, P < 0.05), also demonstrated that there are significant centroid size differences between the species. The relationship between CS and wing shape variables showed a significant, but low allometric residue: 6.9 % (P < 0.0001).

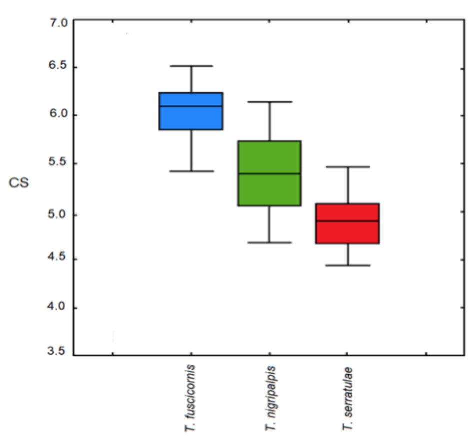

Figure 2. Size differences of three *Terellia* spp. in the wing based on geometric morphometric analysis. CS, average centroid size.

Shape analyses

Differences between the three *Terellia* spp. tested with pairwise analysis and MANOVA. For pairwise comparisons and MANOVA, individuals of these *Terellia* spp. were assigned into three species group. In the pairwise comparisons for wing shape, the differences between the species were found statistically significant (P < 0.05). In addition, all groups were found to be significantly different according to MANOVA (Wilks' λ = 0.000, p < 0.05). As a result, a significant shape differentiation was determined between the species.

In the Procrustes ANOVA test, the shape and the centroid were estimated from total variation. Procrustes ANOVA test showed that there were statistically significant differences between these *Terellia* spp. in terms of both size and shape (P < 0.0001). The relative warps were calculated with the data obtained from wings by using an orthogonal alignment projection method. According to the results of RWA of wings, singular values were explained by 26 relative warps. The landmarks 5, 6 and 7 were determined as having the highest relative contributions. The landmarks 8, 9 and 15 were associated with the highest variances for aligned specimens with values of s^2 = 0.0000794, 0.0000967 and 0.0000831, respectively, whereas landmark 5 was associated with the lowest variance (s^2 = 0.0000170). In RWA, individuals of *T. fuscicornis* and individuals of *T. serratulae* were in overlapping groups, while the individuals of *T. nigripalpis* formed a non-overlapping cluster with the other species (Figure 3). For wing shape, CVA resulted in separation of the three *Terellia* spp. Shape variation between the species was explained by two axes. The first and the second axes explained 68.3% and 31.7% of the total variation, respectively. On the CVA scatter plot, three groups are clearly visible: first group included individuals of *T. fuscicornis*, the second group included individuals of *T. nigripalpis*, and the third group included individuals of *T. serratulae* (Figure 4). All pairwise permutation tests performed with Mahalanobis distances revealed that a highly significant difference in the wing shape of species (Table 2; permutation test, 10,000 runs, P < 0.0001). With Procrustes distance estimators, we also obtained significant difference in wing shapes (P < 0.0001).

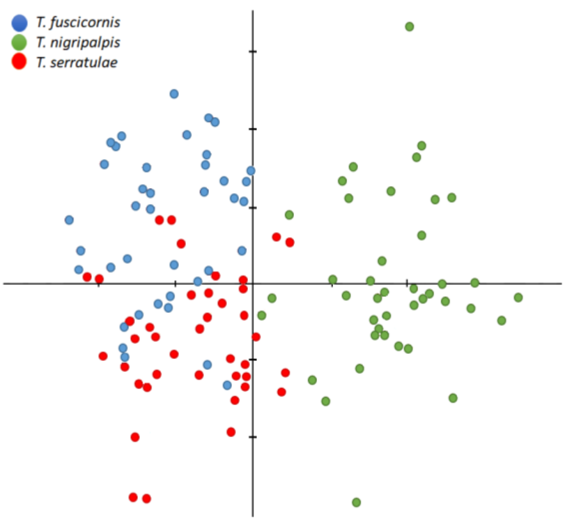

Figure 3. Two-dimensional scatter plot of relative warp analysis based on wing shape of three *Terellia* spp.

Table 2. Difference in the shape of wings of three *Terellia* spp.

Species	Mahalanobis distances			Procrustes distances		
	T. fuscicornis	*T. nigripalpis*	*T. serratulae*	*T. fuscicornis*	*T. nigripalpis*	*T. serratulae*
T. fuscicornis	-	<.0001	<.0001	-	<.0001	<.0001
T. nigripalpis	7.390	-	<.0001	0.031	-	<.0001
T. serratulae	5.760	5.764	-	0.021	0.028	-

* *P*-values above the diagonal; distances between populations below the diagonal, *P* < 0.0001 denote a significant difference.

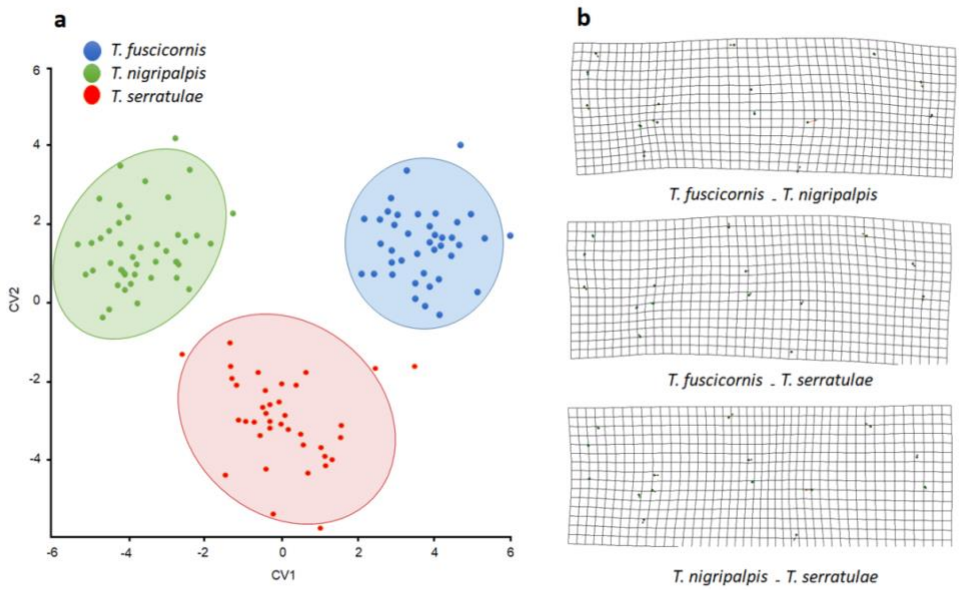

Figure 4. a) Two-dimensional scatter plot of CVA based on wing shape of three *Terellia* spp., species group are indicated by circles whose diameters represent the 95% confidence intervals around the group centroid; b) Comparison of the deformation grids for the three *Terellia* spp.

Figure 5 shows the phenetic relationships between the *Terellia* spp. based on Mahalanobis distances computed from the DFA. The phenogram resulted in two main branches. The first branch consisted of *T. fuscicornis* and *T. serratulae*; the second branch consisted of *T. nigripalpis*.

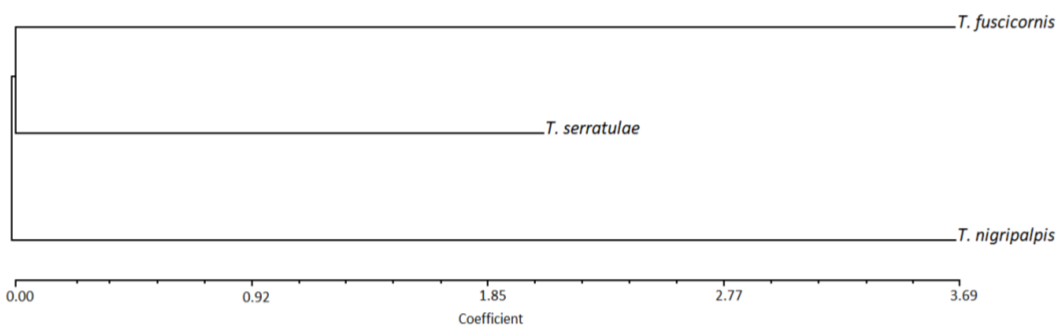

Figure 5. UPGMA phenogram showing the wing shape relationship among three *Terellia* spp. based on Mahalanobis distances.

Table 3 summarized the group assignments with respect to species, and the three *Terellia* spp. were correctly classified to their assigned groups (100%). Cross validation test based on two discriminant functions reassigned 95% of the colonies to their correct groups. The percentage of correct classifications was high for all leave-out-one cross-validated groups (*T. fuscicornis* 100%, *T. nigripalpis* 97.6% and *T. serratulae* 87.2%) (Table 4).

Table 3. Classification results of three *Terellia* spp. based on wing

Species	N	*T. fuscicornis*	*T. nigripalpis*	*T. serratulae*
T. fuscicornis	40	40 (100.0)	- (0.0)	- (0.0)
T. nigripalpis	41	- (0.0)	41 (100.0)	- (0.0)
T. serratulae	39	- (0.0)	- (0.0)	39 (100.0)

*N, number of specimens; percent classifications are in parentheses.

Table 4. Reclassification of three *Terellia* spp. based on wing

Species	N	*T. fuscicornis*	*T. nigripalpis*	*T. serratulae*
T. fuscicornis	40	40 (100.0)	- (3.4)	- (3.4)
T. nigripalpis	41	- (0.0)	40 (97.6)	1 (2.4)
T. serratulae	39	2 (5.1)	3 (7.7)	34 (87.2)

*N, number of specimens; percent classifications are in parentheses.

Multivariate identifications of the landmark-based geometric morphometric data (the shape variables) can be generated through a variety of methods (Rohlf, 1999). The thin plate spline (TPS; Bookstein, 1991) approach is another method that is a suitable way to visualize possible shape differences as smooth deformations. TPS allows mapping the deformation in shape of target region of a species group into another. When wing shape differences between the three species were illustrated by deformation grids, the deformation grids were carefully checked for wing shape, the highest deformations were seen in pairs *T. fuscicornis* and *T. nigripalpis.* The landmarks 8, 9 and 15 were associated with the three highest variances for aligned specimens on wings and these are also the points where high deformations were observed (Figure 4).

Discussion

One of the species of the *serratulae* group, *T. fuscicornis,* which occurs in many countries of the Mediterranean Basin, is closely associated with its host (Merz & Korneyev, 2004). Another species, *T. serratulae,* has wide distribution in the Palearctic region (Merz & Korneyev, 2004). However, *T. nigripalpis* only occurs in Turkey and Iran (Hendel, 1927; Görmez, 2011; Kütük & Yaran, 2011; Namin & Korneyev, 2018). These three species can be distinguished morphologically by the following characters: coloration of third segment of antenna, coloration of palpus, oviscape length and host plant (Freidberg & Kugler, 1989; Kütük & Yaran, 2011). These characters generally do not have a quantitative basis. Morphologically, the body length and wing length of male and female individuals in all three species are very similar to each other (Freidberg & Kugler, 1989; Görmez, 2011). In this study, the differences in size and wing shape of previously identified three *Terellia* spp. in Turkey were investigated by the landmark based-geometric morphometric approach. In previous studies, landmark based-geometric morphometric method was effectively applied to differentiate cryptic species [such as cryptic species of *Rhagoletis* (Yee et al., 2009), cryptic species of *Bactrocera* (Kitthawee & Rungsri, 2011; Schutze et al., 2012)], different species [such as *Bactrocera dorsalis* and *Ceratitis capitata* (Pieterse et al., 2017)] and species complex [such as *Anastrepha fraterculus* complex (Perre et al., 2014; Prezotto et al., 2019), *Ceratitis* FAR complex (Cann et al., 2015)] within the Tephritidae family. In our study, the geometric morphometric approach was applied for the first time to distinguish three species of the *serratulae* group in the genus *Terellia.* The findings show the importance of landmark based-geometric morphometric analysis in distinction of morphologically closely related species in the same taxonomic group.

Our results indicate significant wing shape and size differentiation between the three studied species of the serratulae group. The identification accuracies were complete and wing shape morphometry discriminated to three species with 100% accuracy. The higher reassignment classifications by geometric morphometric provided valuable results in clarifying morphologically closely related species. However, three *Terellia* spp. were completely separated based on the size and shape of wings. Although *T. fuscicornis,* and *T. serratulae* do not separate completely in RWA, CVA result based on the wing shape strongly support the existence of three taxonomically distinct species. All species showed intraspecific variation in RWA and CVA analyses. Individuals of all three species feed on more than one host (Freidberg & Kugler, 1989; Knio et al., 2002). Consequently, the heterogeneity in each species depends probably on host preference. Haddad et al. (2017) also investigated genetic and morphometric variations of *T. serratulae* in Lebanese populations, and emphasized that the difference in phenology between host of *T. serratulae* suggests intraspecific variation.

Insect wings are good indicators of population responses to changes that occur in their environment (Johansson et al., 2009). Thus, variation in host and population density are key factors associated with fly wing polyphenisms. Our findings clearly show that we can distinguish between *Terellia* spp. based on wing size and shape. This research is the first to study members of *Terellia* in Turkey in this way. Although the geometric morphometric approach applied in this research is a useful method, we cannot conclude that it is a sufficient method to distinguish *Terellia* spp. However, molecular tools should also be used along with size-independent characters in order to evaluate the species differentiation within the genus *Terellia.* In order to see the preference of host as environmental impact, on fly wing polyphenisms of different species new researches should be applied by using both molecular tools and size-independent characters.

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