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ORIGINAL RESEARCH ARTICLE

Analysis of morphological variations of three *Adlerius* (Diptera: Psychodidae) species collected in two cutaneous leishmaniasis endemic foci of Turkey

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Abstract
Leishmaniasis, transmitted to human by sand flies, is a neglected vector-borne diseases and has two clinical forms: cutaneous (CL) and visceral leishmaniasis (VL) in Turkey. Subgenus of *Adlerius* are known as probable vectors of both forms of leishmaniasis in Turkey. Examined three species belonging to *Adlerius* are difficult to distinguish morphologically and have similar taxonomic characters. The aim of this study was to analyse and identify the possible morphological variations, of wild-caught sand fly specimens belonging to *Adlerius* subgenus. Six and seven morphological characters for female and male specimens were used, respectively. ANOVA, principal component analysis (PCA) and discriminant function analysis (DFA) test were performed to determine the significant morphometric variables of the three species. Univariate and multivariate analyses of 13 morphometric variables via ANOVA, DFA and PCA showed that eight (six for females, two for males) morphometric variables had an accuracy of 100% for discriminating three *Adlerius* subgenus specimens. The analyses revealed significant differences on several morphological structures of *Adlerius* specimens. These results suggested that morphological and morphometrical features can be used to discriminate three *Adlerius* species, in which one of them is proven vector *Leishmania* (*L.*) *tropica* in two foci of Turkey.

Keywords Sand flies · *Adlerius* · Morphology · Identification · Turkey

Introduction

The leishmaniases are a group of diseases caused by *Leishmania* Ross, 1903 (Kinetoplastida: Trypanosomatida) species, which obligate intracellular protozoan parasites of humans and animals and lead to a wide spectrum of clinical manifestations (Haleem and Wilson 2009). There are four species of *Leishmania* (*L. tropica*, *L. infantum*, *L. donovani* and *L. major*) causing CL and VL in Turkey (Ozbilgin et al. 2017).

Sand flies belong to the subfamily Phlebotominae which is one of the six Psychodidae subfamilies with more than 900 species described worldwide and around 24 species of *Phlebotomus* genus have been proven or suspected vectors of leishmaniasis in the Old World. *Adlerius* subgenus is believed to have derived from Central Asia where biodiversity is very high. This subgenus has been represented with five species including *P. halepensis*, *P. balcanicus*, *P. simici*, *P. brevis* and *P. kyrenia* in Turkey and has been apparently transmitted *Leishmania* parasites into various Eurasian countries (Kavur et al. 2018; Erisoz et al. 2019).

The previous studies conducted in the field and in the laboratory conditions have determined the vector competence of *Adlerius* species. They also reported that infected *Adlerius* females can transmit *L. major*, *L. tropica*, *L. infantum* and *L. donovani* (Volf and Myskova 2007; WHO 2010; Giorgobiani et al. 2012). Especially, *P. halepensis* is considered as probable vector species of *L. tropica*, caused CL,

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in a Central Anatolian Foci in Turkey (Sadlova et al. 2003; Kavur et al. 2018; Erisoz et al. 2019).

Detailed morphometric examinations and multivariate analyses of morphometric characters have been used to resolve taxonomic problems in Phlebotominae sand flies, especially in identifying the sister or closely related species (Khalid et al. 2010). Previously, the systematic studies of some medically significant arthropods such as *Aedes* (Andrew and Bar 2013), *Anopheles* (Chatpiyaphat et al. 2020), *Culex* (Börstler et al. 2014; Lorenz et al. 2017), mites (Sungvornyothin et al. 2019) and sand flies (Absavaran et al. 2019) have been studied by detailed morphological methods. These are important for indistinguishable species such as *Adlerius*'s females and males.

The males of species belonging to *Adlerius* have been identifying according to their external genitalia structures, whereas the females are determined internal structures such as pharynx and spermatheca, and these species usually have similar morphological characters. The analyses performing to determine the morphometric discriminations are an important tool for certain identification of the species especially for the females of the subgenus *Adlerius* (Zahraei-Ramazani et al. 2015).

In this study, we aimed to compare selected morphological characters of three species belonging to *Adlerius* subgenus and to determine the variations in these characters among individuals and identify in detailed the species using the statistical analyses.

Materials and methods

Study area

Field studies were carried out in the endemic areas for CL caused by *L. tropica* in Afyonkarahisar and Nigde provinces. Afyonkarahisar province has a basin of 14.570 km^2 in area and is located in the eastern part of Ege Region of Turkey (altitude 1.034 m, latitude 38° 45–20.88-N, longitude 30° 32–30.48-E). Nigde province has a basin of 7.795 km^2 in area and is located in Central Anatolia, Turkey (altitude 1.218 m, latitude 37° 53–34.09-N, longitude 34° 41–14.95-E) (Fig. 1).

Sand fly specimens

The sand flies were caught using 113 CDC light traps (73 in Afyonkarahisar and 40 in Nigde) during the active season of sand flies, in two different periods for both provinces, August 2009 and September 2010. For the collection of sand flies, CDC Light Traps (John W. Hock Comp.) were set up 1–1.5 m above the ground before sunset and collected the

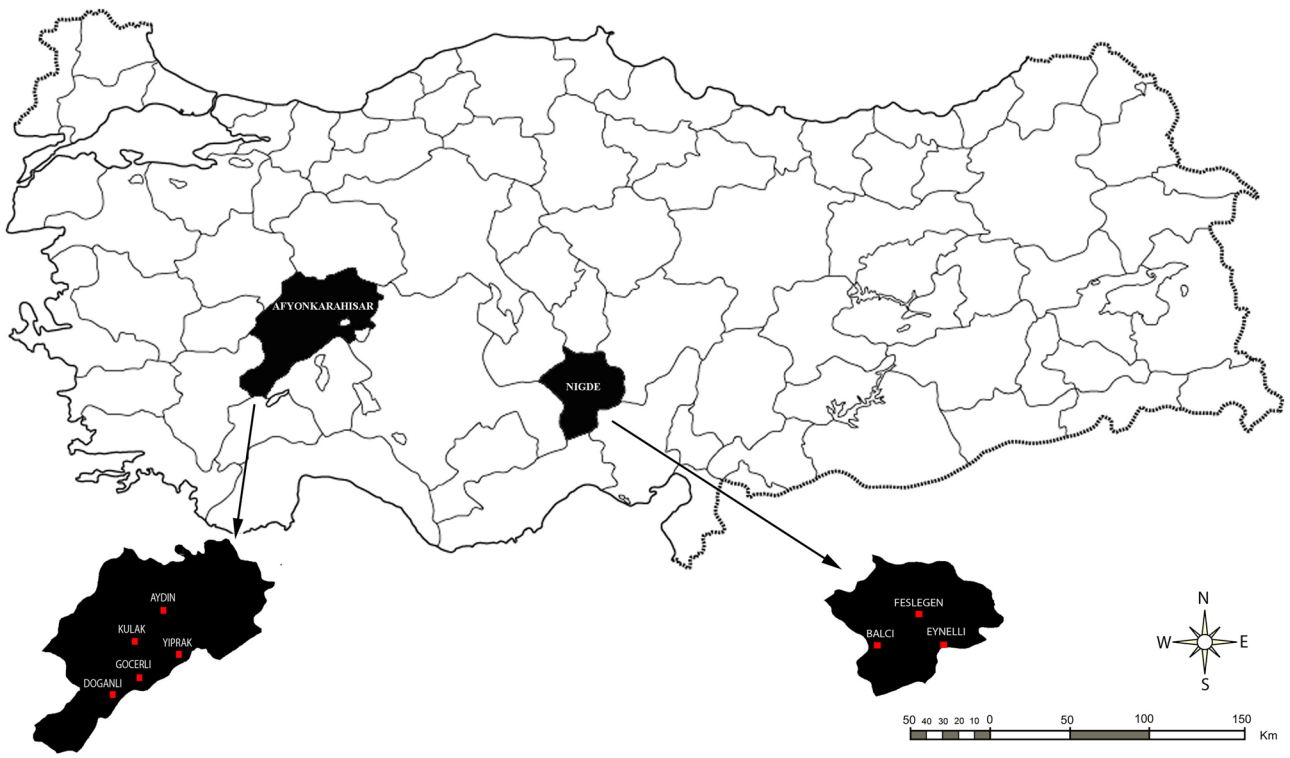


Fig. 1 Turkey map showing the locations of two provinces, Afyonkarahisar and Nigde

next morning before the sunrise. The trap locations were animal barns and houses made by brick, mud, wood and/or stone. Collected specimens were stored in 70% ethanol for transferring to the laboratory and later morphological species identification using several written identification keys (Theodor 1958; Lewis 1982; Killick-Kendrick 1990). The systematically important morphological characters of specimens were measured by direct microscopy technique at 40× magnification. We used the Olympus CX31 binocular microscope and Olympus DP20 imaging system calibrated by micrometric slide, which allows it to be connected to a computer, to take photos of preparations. After the species identification, the specimens belonging to *Adlerius* subgenus were separated for morphometrical analyses.

We could not perform species identification by molecular techniques because of emphasizing the importance of morphological differences between species of sand fly in identification.

A total of 281 (193 *P. halepensis*, 51 *P. balcanicus* and 37 *P. simici*) female specimens and 325 (253 *P. halepensis*, 51 *P. balcanicus* and 21 *P. simici*) male specimens were included in the study for morphometric analyses (Table 1).

Table 1 The number of three *Phlebotomus* specimens included in the study

Species	*n*		Total	District-Province
	Female	Male		
P. halepensis	37	55	92	AFYONKARAHISAR
	51	50	101	
	17	13	30	
	2	6	8	
	1	3	4	
	17	36	53	NIGDE
	40	68	108	
	28	22	50	
P. balcanicus	35	49	84	AFYONKARAHISAR
	6	2	8	
	1	0	1	
	1	0	1	
	0	0	0	
	2	0	2	NIGDE
	4	0	4	
	2	0	2	
P. simici	1	1	2	AFYONKARAHISAR
	10	4	14	
	2	3	5	
	0	0	0	
	0	0	0	
	13	4	17	NIGDE
	9	8	17	
	2	1	3	
Total	281	325	606	

Morphometric analysis

For female *Adlerius* specimens, six characters were determined for our morphometrical study: (i) length of 3rd antennal segment (A3), (ii) total length of 4th and 5th antennal segment (A4 + A5), (iii) ratio of length of 3rd antennal segment to total length of 4th and 5th antennal segment (A3/A4 + A5), (iv) length of pharynx (PHX), (v) length of epipharynx (EPI) and (vi) ratio of 3rd antennal segment and length of epipharynx (A3/EPI).

For male *Adlerius* specimens, seven characters were measured; (i) length of coxite (CX), (ii) length of first style (S1), (iii) length of second (S2) style, (iv) length of genital filament (GF) and (v) length of genital pump (GP), (vi) ratio of genital filament and genital pump (GF/GP) and (vii) number of coxite hair (CXH). Morphometric measurements were done using calibrated ocular micrometer mounted both on the ocular lens and 10× and 40× objective lenses according to the size of the measured character in millimetres (mm).

Statistical analysis

Univariate statistical analysis of 13 characters (six in female, seven in male) was performed. Furthermore, normality of the data and homogeneity of variance were checked for Kolmogorov–Smirnov test and Levene's test, respectively (Tabacnick and Fidell 2013).

ANOVA test was used to find the statistically significant differences of the measured characters between *P. halepensis*, *P. balcanicus* and *P. simici*. The significant level of statistical analysis was $P < 0.05$ for all measures of morphometric characters.

In addition, multivariate analyses were carried out for measured morphometric characters. Discriminant function analysis (DFA) including Box's M test and principal component analysis (PCA) was used to determine the most important morphometric variables for discrimination of three species of *Adlerius* subgenus and to define the variabilities of 13 characters in collected specimens, respectively.

All statistical analyses of the morphometric data were done using PASW and PAST 2.17 softwares.

Results

After the dissection and identification of all specimens, 606 of them were belonging to subgenus *Adlerius* [73.59% *Phlebotomus* (*P.*) *halepensis*, 16.83% *P. balcanicus* and 9.57% *P. simici*] and included in the study for morphological analysis.

Univariate analysis of morphological characters of specimens

The ANOVA test results revealed that statistically significant differences between the morphological characters of *P. halepensis*, *P. balcanicus* and *P. simici* female and *P. halepensis* and *P. balcanicus* male specimens ($P \leq 0.05$).

Regarding female specimens, there were significant differences in all morphometric characters except A3/A4 + A5 (the measurements of *P. balcanicus* were found similar to the other two species) and A3/EPI (it was determined that the measurements of *P. balcanicus* and *P. simici* were similar). Additionally, the results of the Kolmogorov–Smirnov test for normality of data and Levene's test for the homogeneity of variances applied to all parameters of specimens were not significant (Table 2) ($P > 0.05$).

Regarding male specimens, significant differences were detected in all characters except GF (the measurements of *P. balcanicus* and *P. simici* were found similar) and CXH (similarity was observed between the values of *P. balcanicus* and *P. halepensis*) measurements. Kolmogorov–Smirnov test for normality of data and Levene's test for the homogeneity of variance applied to each character was not significant for male specimens ($P > 0.05$) expect for CXH (Table 2).

Multivariate analysis of morphological characters of specimen

Box's M test for homogeneity of covariance matrices was determined significant ($M = 0.420.282$, $P < 0.001$ for females; $M = 377.393$, $P < 0.001$ for males). Five informative variables (A3, A4 + A5, A3/A4 + A5, PHX and A3/EPI) (Fig. 2) were determined for the significant separation of the females of three species, while two out of the seven measured morphometric characters (Fig. 2), CXH and GF/GP, were selected the most informative variables by DFA for male specimens (Table 3). Two significant discriminant functions were retained by the stepwise procedure having the highest ratio of between-group to within-group sum of squares [(function 1: Wilk's lambda = 0.189, $\chi^2 = 459.120$, $df = 12$, $P < 0.001$), (function 2: Wilk's lambda = 0.909, $\chi^2 = 26.347$, $df = 5$, $P < 0.001$)] and males [(function 1: Wilk's lambda = 0.022, $\chi^2 = 1219.576$, $df = 14$, $P < 0.001$), (function 2: Wilk's lambda = 0.237, $\chi^2 = 458.906$, $df = 6$, $P < 0.001$)]. Table 3 displays the coefficients of standardized and unstandardized canonical discriminant weights and discriminant loadings of each of these morphometric characters of female and male specimens of three species.

In addition, the canonical discriminant function evaluated at female group means was 1.159 (function 1) and − 0.100 (function 2) for *P. halepensis*, − 1.159 (function 1) and

Table 2 Measurements and ratios of thirteen morphometric characters of female and male specimens of *Adlerius*

Morphometric parameters	*P. halepensis*	*P. balcanicus*	*P. simici*	*F* value	*P* value
Females					
Length of 3rd antennal segment (A3)	0.367 ± 0.249 (0.230–0.430)a	0.316 ± 0.019 (0.263–0.350)b	0.259 ± 0.025 (0.215–0.330)c	352.447	< 0.001
Length of antennal segments 4 and 5 (A4 + A5)	0.285 ± 0.205 (0.220–0.420)a	0.259 ± 0.015 (0.217–0.320)b	0.233 ± 0.018 (0.200–0.280)c	125.646	< 0.001
Ratio of A3/A4 + A5	1.293 ± 0.098 (0.55–1.73)a	1.222 ± 0.056 (1.090–1.360)ab	1.111 ± 0.070 (1.020–1.400)b	69.604	< 0.001
Length of pharynx (PHX)	0.246 ± 0.023 (0.200–0.480)a	0.235 ± 0.016 (0.189–0.280)b	0.209 ± 0.219 (0.180–0.270)c	45.365	< 0.001
Length of epipharynx (EPI)	0.416 ± 0.027 (0.300–0.480)a	0.384 ± 0.027 (0.310–0.470)b	0.332 ± 0.027 (0.290–0.430)c	152.191	< 0.001
Ratio of A3/EPI	0.886 ± 0.075 (0.62–1.23)a	0.827 ± 0.073 (0.66–1.05)b	0.783 ± 0.059 (0.650–0.920)b	37.361	< 0.001
Males					
Length of coxite (CX)	0.387 ± 0.020 (0.327–0.449)a	0.437 ± 0.225 (0.377–0.481)b	0.308 ± 0.165 (0.264–0.350)c	17.439	< 0.001
Length of 1st style (S1)	0.198 ± 0.009 (0.167–0.223)a	0.200 ± 0.008 (0.188–0.220)b	0.148 ± 0.008 (0.133–0.160)c	23.139	< 0.001
Length of 2nd style (S2)	0.192 ± 0.009 (0.166–0.220)a	0.196 ± 0.008 (0.178–0.219)b	0.142 ± 0.008 (0.124–0.160)c	22.935	< 0.001
Length of genital filaments (GF)	1.36 ± 0.105 (1.000–1.600)a	0.978 ± 0.126 (0.826–1.500)b	0.867 ± 0.090 (0.680–1.000)b	20.845	< 0.001
Length of genital pump (GP)	0.135 ± 0.008 (0.110–0.160)a	0.122 ± 0.008 (0.107–0.141)b	0.103 ± 0.008 (0.090–0.120)c	16.449	< 0.001
Ratio of GF/GP	10.069 ± 0.786 (7.812–12.500) a	7.981 ± 0.846 (6.257–10.714)b	8.370 ± 0.798 (7.000–10.000)c	9.499	< 0.001
Number of coxite hairs (CXH)	39.030 ± 7.996 (23.000–102.000)a	103.780 ± 11.705 (75.000–142.000)a	28.05 ± 2.872 (21.000–32.000) b	6.250	< 0.001

Data were expressed as mean ± standard deviation (SD) and minimum (min) and maximum (max)

*F and P values are based on ANOVA test. Variables in the same row with different lowercase letters are significantly different (grey colour) from one another (at $P \leq 0.05$) as revealed by one-way ANOVA followed by Tukey HSD test



Fig. 2 Morphometric features of **a** *P. simici* female (10×); **a** length of pharynx (PHX), **b** length of epipharynx (EPI), **c** length of 3rd antennal segment (A3), **d** length of 4th antennal segment (A4), **e** length of 5th antennal segment (A5). **b** *P. halepensis* male (10×); **a** genital filaments (GF), **b** genital pump (GP), **c** styles (S1 and S2), **d** coxite (CX), **e** coxite hairs (CXH)

0.642 (function 2) for *P. balcanicus*, − 4.447 (function 1) and − 0.366 (function 2) for *P. simici*, indicating that the discriminant scores for *P. simici* were far smaller than that of *P. halepensis* and *P. balcanicus*. The means of male group are 1.404 (function 1) and 0.514 (function 2) for *P. halepensis*, − 7.230 (function 1) and 0.244 (function 2) for *P. balcanicus*, 0.648 (function 1) and − 0.678 (function 2) for *P. simici*, implying that the discriminant scores for *P. halepensis* were greater than *P. balcanicus* and *P. simici*. The goodness-of-fit of these models in classifying the female and male specimens were analysed using the eigenvalues of 3.811 and 9.854, respectively. In females, canonical correlations for two functions were determined as 0.890 and 0.302, and their variances were 97.4% and 2.6%, respectively. The canonical correlations for male specimens were 0.953 and 0.873 with 75.4% and 24.6% variance, respectively. Total variations between species of *Adlerius* have been found to be better explained by functions with a high canonical correlation.

Figures 3 and 4 show the histogram of the discriminant individual scores of female and male specimens, respectively. The histograms displaying the discriminant scores

Table 3 Canonical discriminant coefficients and loadings for discriminant functions 1 and 2

Morphometric characters	Function 1			Function 2		
	Unstandardized	Standardized	Loadings	Unstandardized	Standardized	Loadings
Females						
Length of 3rd antennal segment (A3)	− 78.285	− 1.887	0.814*	− 227.071	− 5.474	− 0.310
Length of antennal segments 4 and 5 (A4 + A5)	80.592	1.573	0.536*	− 0.477	− 0.009	0.128
Ratio of A3/A4 + A5	18.672	1.659	0.362*	4.616	0.410	0.058
Length of pharynx (PHX)	− 8.856	− 0.196	0.290*	16.569	0.367	0.229
Ratio of A3/EPI	20.237	1.491	0.262*	75.989	5.598	− .0262
Constant	− 56.480	-	-	− 70.649	-	-
Males						
Number of coxite hairs (CXH)	− 0.100	− 0.849	− 0.893*	0.024	0.200	0.270
Ratio of GF/GP	1.713	1.365	0.295*	5.888	4.693	0.262
Length of 1st style (S1)	5.442	0.051	− 0.048	25.646	0.240	0.741*
Length of 2nd style (S2)	− 3.778	− 0.036	− 0.063	37.324	0.352	0.735*
Length of genital filaments (GF)	− 9.363	− 1.011	0.391	− 42.185	− 4.553	0.586*
Length of coxite (CX)	− 9.086	− 0.186	− 0.300	1.299	0.027	0.563*
Length of genital pump (GP)	133.124	1.119	0.159	461.523	3.880	0.498*
Constant	− 14.036	-	-	− 77.565	-	-

*Largest absolute correlation between each variable and any discriminant function

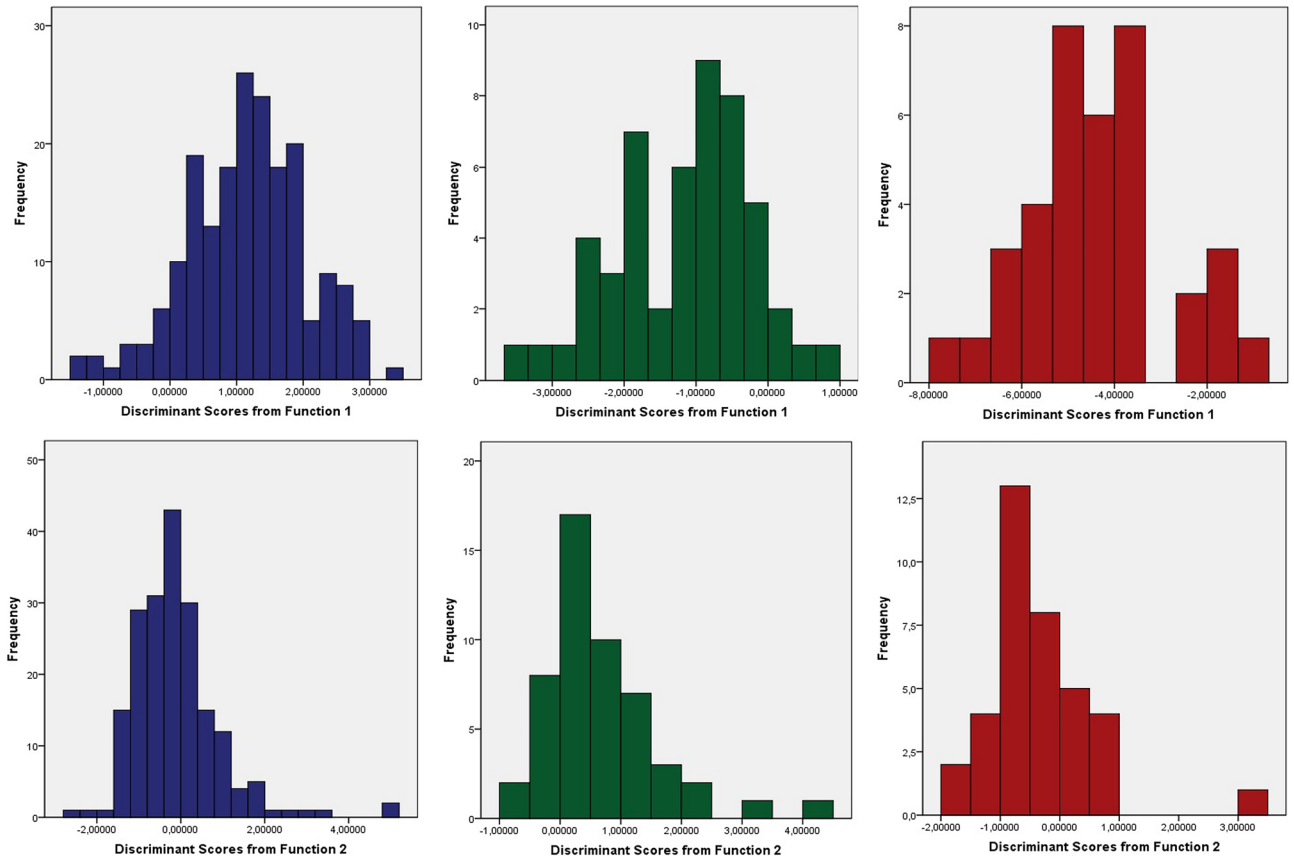


Fig. 3 Frequency distribution of discriminant scores for females of *P. halepensis* (blue bars), *P. balcanicus* (green bars) and *P. simici* (red bars)

of the function analysis revealed obvious discrimination between the aforementioned species.

PCA having two axes created by two of six characters had an eigenvalue of 1.00 and higher. The first (PC1) and second (PC2) principal component's total variance were calculated as 57.24% and 20.08%, respectively, for females of three species (*P. halepensis*, *P. balcanicus* and *P. simici*). Furthermore, similar to those in female examples, seven measured characters indicated that two axes had an eigenvalue of 1.00 and higher for males of *P. halepensis* and *P. balcanicus*. PC1 and PC2 were calculated as 49.292% and 35.662%, respectively (Table 4).

The relationship between the relative significance of the eigenvalues and the number of axis of female and male specimen of mentioned species is presented in Fig. 5.

The principal component matrix validated the measurements allowing the separation and differentiation of three species. Table 5 shows the positively and negatively correlated component to PC1 and PC2. Length of A3 and S1 was found to be the highest correlated components to PC1 in female and male specimens, respectively (loadings ≥ 0.8).

In addition, PC2 had positive correlation with ratio of A3/A4 + A5 (loadings ≥ 0.45) and CXH (loadings ≥ 0.8) for male specimens. EPI (loadings ≥ 0.45), A4 + A5 (loadings ≥ 0.45) (females) and ratio of GF/GP (loadings ≥ 0.45) (males) showed a negative correlation with PC2 (Table 5; Fig. 6).

Discussion

Leishmaniasis have been mainly seen in the cutaneous form in Turkey as well as the Middle East and West Asian countries. Different or common sand fly species can be vector species of the causative agents of CL in these countries, and the identification morphologically or molecularly has importance especially to apply control measures within integrated control programs of vector-borne diseases. In particular, the statistical interpretation of the differences and similarities between species with the controversial vectorial capacity of leishmaniasis provide us with important information about the distribution of the disease.

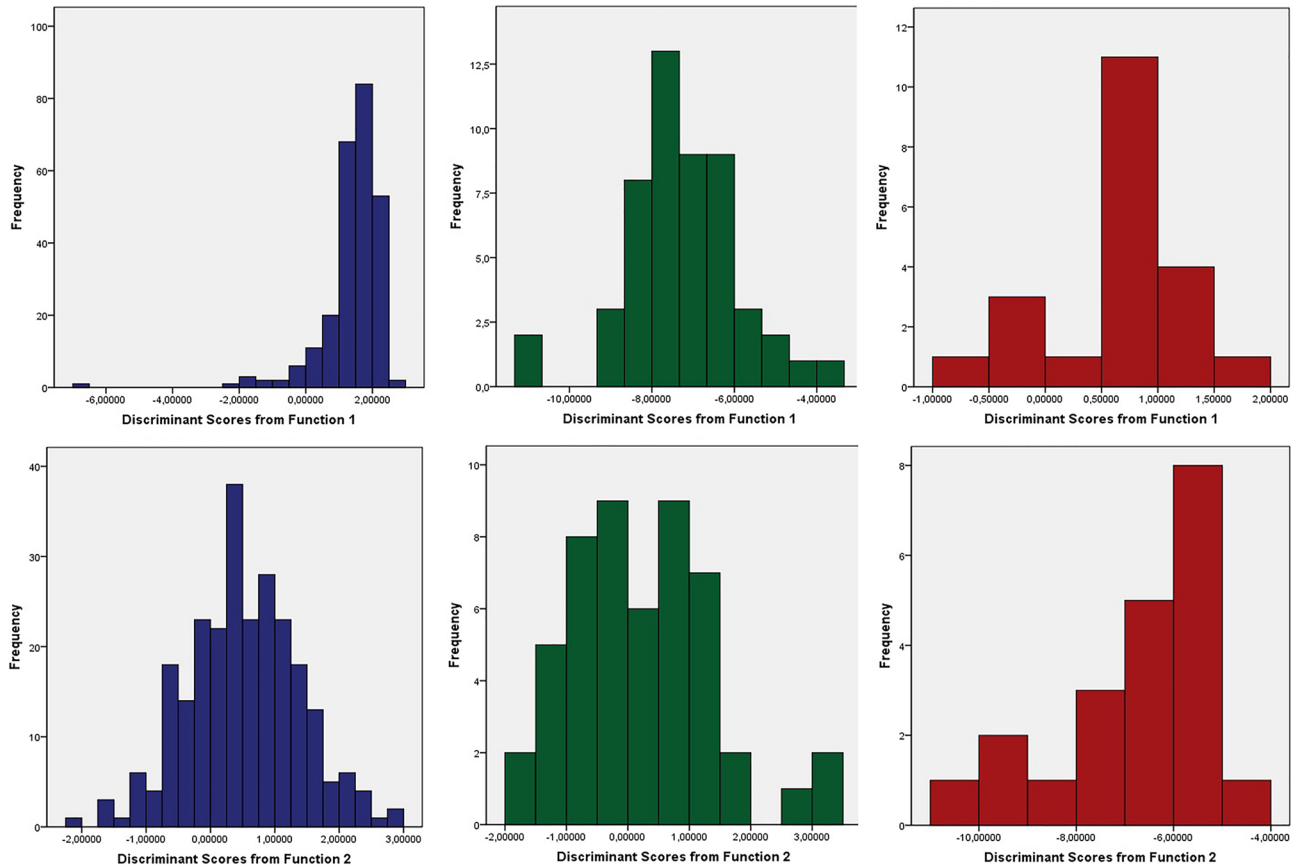


Fig. 4 Frequency distribution of discriminant scores for males of *P. halepensis* (blue bars), *P. balcanicus* (green bars) and *P. simici* (red bars)

In sand flies like *Adlerius*, subgenus identification is difficult for the researchers since the morphological characters of different species are similar and overlap among themselves. Therefore, it is important to administer statistical analysis of morphological characters that is for sand fly identification. In females of *P. halepensis*, *P. balcanicus* and *P. simici*, the length of antennal 3rd are 0.25–0.28 mm, 0.28–0.35 mm and 0.25–0.41 mm, respectively. In males of *P. halepensis*, *P. balcanicus* and *P. simici*, the hairs on coxite are 15–20, 72–123 and 24–65, respectively (Perfil'ev 1966).

Table 4 The Eigen values, percent variance and cumulative variance of the axes from the principal component analysis of 13 morphometric character measurements of female and male specimens of *Adlerius*

Gender	Axis (principal component)	Initial Eigen values		
		Total	Variance (%)	Cumulative (%)
Females	1	3.435	57.244	57.244
	2	1.205	20.088	77.332
	3	0.777	12.948	90.280
	4	0.567	9.443	99.723
	5	0.013	0.210	99.933
	6	0.004	0.067	100.00
Males	1	3.450	49.292	49.292
	2	2.496	35.662	84.955
	3	0.617	8.811	93.765
	4	0.258	3.690	97.455
	5	0.124	1.772	99.227
	6	0.053	0.751	99.978
	7	0.002	0.022	100.000

In this study, we demonstrated the results of statistical analysis of the morphological differences among the three sand fly species belonging to *Adlerius* subgenus that are probable or proven vector species of *L. tropica* (Kavur et al. 2018; Giorgobiani et al. 2012). *P. halepensis* and *P. balcanicus* specimens are especially noticeable in transmitting *L. tropica* collected from two different provinces in Turkey (Kavur et al. 2018). They were used in this morphometric study because of their important vectorial capacity. The results showed that the measurements of antennal segments and their associated structures as well as mouth-parts and parts of genitalia could be used to accurately separate the females and males of *P. halepensis*, *P. balcanicus* and *P. simici*.

P. halepensis, proven vector of *L. tropica*, was found to be common species in both study areas. It is mainly distributed in all geographical regions of Turkey except for Eastern

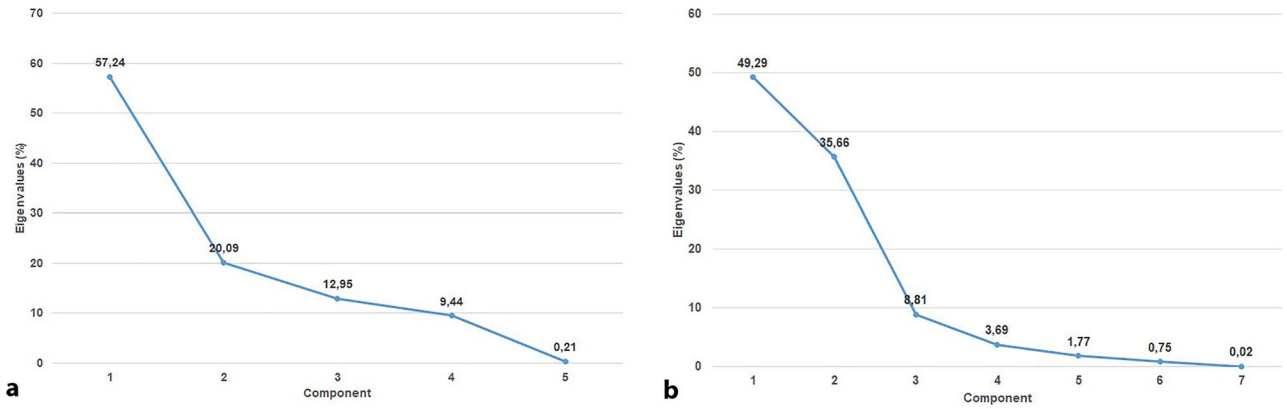


Fig. 5 A scree plot highlighting the relationship between the eigenvalues and the number of axis in a principal component analysis of 6 morphometric measurements of **a** female specimens of three species. **b** Males

Anatolia Region. It was also found as dominant species in Iran especially border of Turkey (Sanliurfa in South East Anatolian Region, Afyonkarahisar and Denizli in Eagean Region, Corum, Konya and Nigde in Central Anatolian Region, Canakkale in Marmara Region, Rize in Black Sea Region) (Ertabaklar et al. 2005; Yaman and Dik 2006; Toprak and Ozer 2007; Ozensoy et al. 2009; Tok et al. 2009; Akhoundi et al. 2012; Kavur et al. 2018; Erisoz et al. 2019).

In Turkey, to our knowledge, no statistical study on morphological measurements of the subgenus *Adlerius* has been carried out. However, species of the subgenus were grouped according to their DNA barcodes and *P. halepensis* and *P. balcanicus* were categorized in the same group (Erisoz et al. 2019). In our study, we determined noteworthy differences among that the length of A3 of female specimens, S1 lengths of male specimens and number of the coxite hairs. Moreover, there are studies focusing on morphological characters that differentiate the *Adlerius* subgenus species by geographical areas and one from Iran determined the relationship of three females of *P. comatus*, *P. balcanicus* and *P. halepensis*. Similar to our findings, the results of the aforementioned study in Iran the branches of these three species are not genetically different and they also have overlapping ranges of morphological characters (Zahraei-Ramazani et al. 2013).

In addition, morphological differences between females of *P. caucasicus* and *P. mongoliensis* species in Iran were interpreted using PCA, DFA and *t* test as we did in our study. The first function of PCA was reported to be correlated with Ascoid 4/A4 and A4 + A5 values and the second function with A4 and A3/Epipharynx values (Absavaran et al. 2019). Similarly, we determined that the characters of both female and male specimens were associated with the two functions obtained from PCA. Our results also revealed that A3 was highly positively correlated with PC1 while EPI and A4 + A5 were negatively correlated with PC2 for female specimens. Furthermore, S1 and S2 were highly positively correlated with PC1 while GF/GP value was negatively correlated with PC2 for male specimens (Table 5).

Coefficients with higher absolute values correspond to variables with greater discrimination. Absaravan et al. (2019) showed that ratio of Ascoid 4/A4 was the most discriminating character to separate female specimens of *P. caucasicus* and *P. mongoliensis* in Iran. In our study, all of morphometric characters except for EPI determined high discriminant loading scores, selected by DFA for females. CXH and GF/GP were found to be having high discriminant loading scores for male specimens (Table 3). Although our measurements for *P. balcanicus* and *P. halepensis*'s CXH were found close to each other, the fact that the number of coxite hairs in *P. balcanicus* (90–220) was higher

Table 5 Characterisation of female and male specimens of *Adlerius* using the loadings of principal component analyses on 13 morphometric variables

Morphometric characters	Principal component	
	PC1	PC2
Females		
Length of 3rd antennal segment (A3)	0.980	-
Ratio of A3/A4 + A5	0.732	0.477
Length of epipharynx (EPI)	0.722	−0.559
Length of pharynx (PHX)	0.719	-
Length of antennal segments 4 and 5 (A4 + A5)	0.703	−0.495
Ratio of A3/EPI	0.637	0.646
Males		
Length of 1st style (S1)	0.924	-
Length of 2nd style (S2)	0.909	0.304
Length of genital filaments (GF)	0.746	0.641
Length of coxite (CX)	0.633	0.706
Length of genital pump (GP)	0.767	-
Ratio of GF/GP	0.475	−0.720
Number of coxite hairs (CXH)	-	0.923

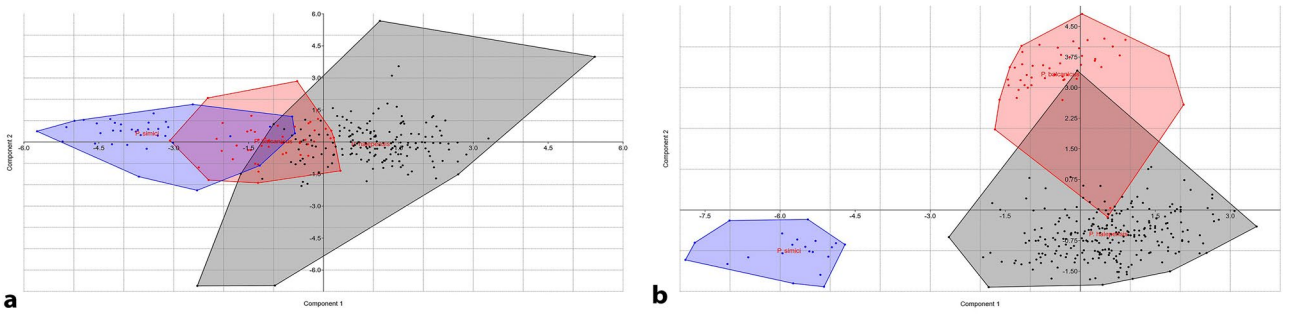


Fig. 6 Principal component analysis (PCA) of **a** female and **b** male specimens (grey areas: *P. halepensis*, red areas: *P. balcanicus*, blue areas: *P. simici*)

than that of *P. halepensis* (27–85) prominent the CXH parameter in species differentiation. GF/GP ratio was also specific for male specimens of *Adlerius* species examined (Lewis 1982). However, A3 value was found to be the most discriminating character differentiating the three mentioned species of *Adlerius* subgenus (0.230–0.430 $n=193$ for *P. halepensis*, 0.263–0.350 $n=51$ for *P. balcanicus* and 0.215–0.330 $n=37$ for *P. simici*) female specimens while CXH was found to be most discriminating character for males (23.000–102.000 $n=253$ for *P. halepensis*, 75.000–142.000 $n=51$ for *P. balcanicus* and 21.000–32.000 $n=21$ for *P. simici*) (Table 2; Fig. 6).

Environmental changes affect not only population densities and morphological size values of sand fly variations in different periods of the year but also transmission risk of the pathogens. Oguz et al. (2017) examined wing size variations of male and female *P. tobbi*. They found that specimens collected in August had the smallest wing size as confirmed by a Mann–Whitney post hoc test. In addition, *Aedes aegypti* is a known vector of several viruses including yellow fever virus, dengue virus chikungunya virus and Zika virus. Its population density is important for public health and vector control strategies. Vargas et al. (2010) showed that in natural conditions, the morphological size values of this important vector are not the only manifestation of larval habitat quality, larval competition or temperature; it critically depends also on the relative humidity during the embryo development. In our study, we did not examine the effects of environmental variables on the morphological measurements of sand flies caught from two different provinces during the summer months. But we determined only most informative variables of the three *Adlerius* sand fly species.

Conclusion

The results of our study indicate that statistical analyses based on morphological characters of vector sand flies can be used are critical to identify the species. They also support the three *Adlerius* species discrimination with measured morphometric characters. We suggest that more detailed studies be carried out for the identification of the females of five *Adlerius* species caught previously in Turkey. In addition, further studies on morphology supported with the genetic diversity of the populations of the subgenus *Adlerius* are substantially important.

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Declarations

Conflict of interest The authors declare no competing interests.

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