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Diversity and Geographical Distribution of Sand Flies *Phlebotomus papatasi* (Diptera: Phlebotominae) by using Geometric Morphometric Technique from two Iraqi Provinces

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Abstract:

The variation in wing morphological features was investigated using geometric morphometric technique of the Sand Fly from two Iraqi provinces Babylon and Diyala. We distributed eleven landmarks on the wings of Sand Fly species. By using the centroid size and shape together, all species were clearly distinguished. It is clear from these results that the wing analysis is an essential method for future geometric morphometry studies to distinguish the species of Sand Flies in Iraq.

Key words: Diptera, Geometric Morphometric, Sand flies, Phlebotominae.

Introduction:

Sand flies (Phlebotominae) is the main vector of Leishmaniasis diseases, There are about 700 known species of the Sand Flies, but only a few act as vectors for these diseases, especially , of the Zoometric diseases. There are twenty-four species of *Lutzomyia* which exist in the modern world known as uncertain and potential vectors of the disease, while there are about twenty species of Phlebotomies in the old world (1).

The Geometric Morphometric technique is used to diagnose the shape and structure of the Geometric Morphometric wing, and its a simple method to quantitative studies comparisons , as well as it is statistics, and mathematics methods to study analysis of the shape. The geometric scale of the virtual form of wings technique is used to find a variation in the form of insect wing communities population. Because this method is modern and sophisticated and is likely to be curious about most of the readers, but it is very useful to see how populations of insects match belonging to one species and thus facilitate the sterile insect technique that is applied (2,3). In America, nearly 30% of Sand Fly females are difficult to distinguish from one another; because these species belong to groups that have a common close relative. Therefore, the identification at species-level is difficult or even impossible. Consequently, new tools have been used to minimize the identification of species by using geometric morphometric of the ten landmarks in head of females of the genus *Psychodopygus* (4). Depending to the few studies which have been conducted to distinguish the species of Sand Flies in Iraq, we distributed eleven landmarks on the wings of Sand Fly species.

Material and Methods:

Collection of the specimen

The specimens were collected from different regions of two Iraqi provinces : Diyala province from baquba, and Babylon province Mahaweel district to identify the different variations in the shape and size of wings and the distinction among these populations by using the geometric technique which depended on Landmark characteristics (5); the method was followed in the preparation of glass slides of the wings(6); 16 males were isolated in containers and left without feed until they died and dried. After drying, the left wing of each sample was removed by precise forceps while maintaining the wing of the break;then placed between two glass slides and connect the edge of the slide with a paper tape with

the pattern information (date and place) written on one end of the slide. After the preparation of the glass slides, a digital microscope was attached to a calculator with a 1.3 megapixel digital camera and a camera with ultraviolet. After the UV imaging process is completed, images are saved for each region in special files until the analysis results.

Data collection and analysis

The wings of collected species were photographed, the CLIC program (collecting landmark for and characterization) was used to collect the data of each species separately, using specialized for morphometric analysis that was described by (7,8) . The program is available and feely downloaded from the website http: //www.mpl .ird.fr / Morphometrics. The CLIC program is composed of package of units and briefly summarized in Table 1.

Table 1. CLIC program

Program unit	Function
COO(collection of coordinates)	Collecting landmark on picture with short related information
TET (table espacios tabulaciones)	Helps to prepare a simple data base associated with the format .txt
MOG (morfometria geomtrica)	Use and visualize the data that coordinates in the format.txt
COV (mancova)	Estimate the difference between the centroid size
VAR (variation and variance)	Performs t-test comparisons of means and F test comparisons of variances for centroid size
ASI (asymmetry of shape and size)	Use ANOVA test to assessing the symmetry of shape and size

The distance between each pair of landmarks was computed by TET unit, were converted and data which were found in files with the extensions (coord .txt) of the species in same provinces were compared to file with extensions (format .txt) , and then integrated it with that of the same species in the other provinces , in order to determine the compatibility and variability of the species .

After finishing of integration of the data of species many operation including (Translation , Scaling and Rotation) have been carried out by MOG unit on coordinate landmark which therefore lead to determine the Centroid size of the wing.

In this study, centroid size was used for comparing between the species which represented the sum of square of distance between the center and every landmark put on the wing (9). Therefore, statistical significance was estimated of the difference between the centroid size of the wing done by COV unit (ManCova), which is a Multivariate analysis of Covariance and estimated it by comparing the data that were computed in file extension with (CS.txt).

Asymmetry analysis by ASI unite (Asymmetry of shape and size) was used to provided statistical assessment of asymmetry in shape and size of wing between the common species in provinces and the data would be found as output table in file extend with (CS-ASI-INFO.txt)

Results and Discussion:

Eleven landmarks were selected on the wing in each individual (Fig. 1, 2). After the matching between the wing of Diyala and Babylon provinces by using Geometric analysis over the MOG unit and as showed in Fig. 3 and 4. Which show the mean coordinate of the landmark of the two provinces that there matching except in landmark 8 , 9 and 11 . The reason for variation in wing shape and size of *Culex quinquefasciatus* the mismatch of all individuals in the three populations may be due to different environmental conditions of temperature and relative humidity and the amount of rain in the areas from where the insects were collected (10).

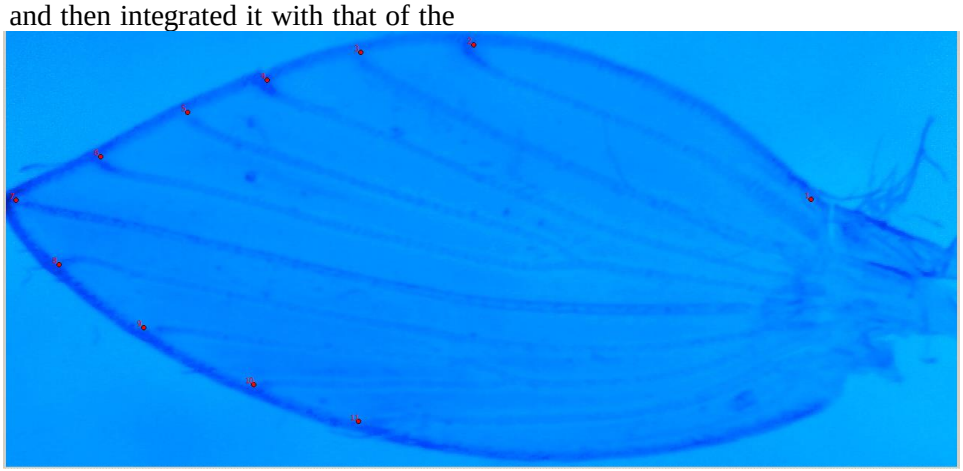

Figure 1. Eleven landmarks on the Sand flies (Phlebotominae) of left wing from Babylon.

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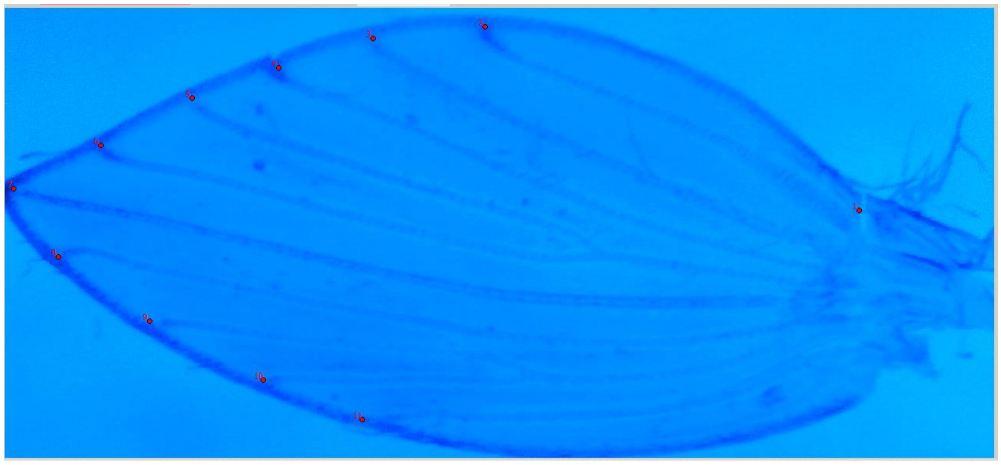

Figure 2. Eleven landmarks on the Sand flies (Phlebotominae) of left wing from Diyala.

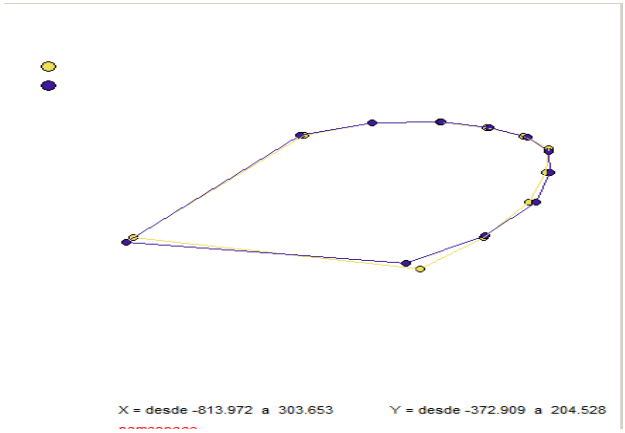

Figure 3. Mean coordinates of landmark in the left wing of Sand flies (Phlebotominae), Yellow color represents Babylon specimens and Black color represents Diyala specimens

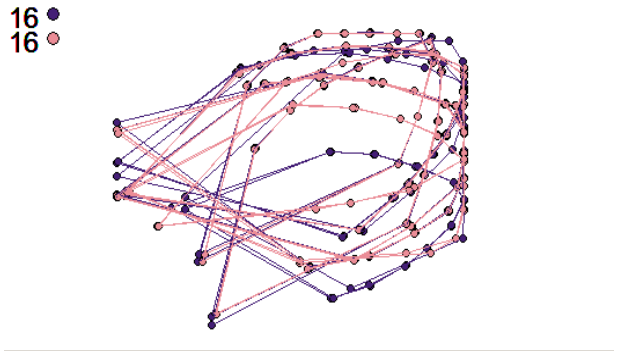

Figure 4. Using by the GPA Procrustes superimposition method, the output of consensus configuration located the 11 landmarks for each individual left wing of the two population. Black color represents Babylon specimens and Red color represents Diyala specimens.

As shown in Fig. 5 and 6, the comparative range of the centroid size of the wing demonstrate that most of the species was inside the median rang of the centroid size of each provinces as it was signified with the blue bar below the box.

Also, we found variations in the centroid size of the wing between the two provinces by studying the discriminative analysis (Fig.7) with mean centroid size of Babylon provinces reached to 1441.26 and 1429.97 for the Diyala individual. In addition (Table 2) shows the absolute difference between them was 6.56 and the variance analysis for symmetry wing. The results showed no significant difference in wing size and shape between the insects in Diyala and Babylon provinces by using ASI unit in morphometric program (Fig. 8) .The results also showed the analysis of variance for asymmetry left wing size of sand fly between Babylon and Diyala specimens(Table 3 and 4) .

The results showed that Mahalanobis distances between the central size of the insect wings of Babylon and the central size of the insect wings in Diyala population was 2.77 microns (Table 5). Significant differences were reported in Sand flies' (*Phlebotomus papatasi*) wing shape morphology between two study areas (North of the Atlas Mountains and South of the Atlas Mountains) in Morocco (11). The results are consistent with the study that was conducted in the south of France, they found that strong morphological variations were observed in Sand flies' (*Phlebotomus papatasi*) wings for both sexes captured in 2011 from May to October (12). In Brazil, study was conducted to distinguish five species of sand flies (Phlebotominae) in an attempt to cluster these species by studying the linear and geometric morphometric characteristics. They found that there were significant differences in several of the analyzed structures, like the ratio between the ejaculatory filament and size of the wings. After analyzing the results, they found out that that three species of *Nyssomyia* are phenetically more similar to *Migonemyia migonei* (all vectors of L. (V.)

braziliensis) than to *Bichromomyia flaviscutellata* (vector of L. (L.) amazonensis) according to the size of the centroids of the wings and their shapes. We can also find the same results the division of the genera *Nyssomyia* and *Bichromomyia* (13).

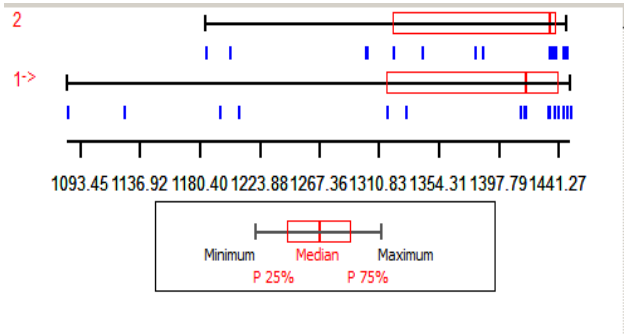

Figure 5. Variation of the centroid size of left wings for Sand flies (Phlebotominae) according to feeding. The boxes display the group median separating the 25 th and 75 th the quartiles. The wings numbers Babylon and Diyala in the figure are represented by vertical bars under the boxes. Units are pixels. P, percentile.

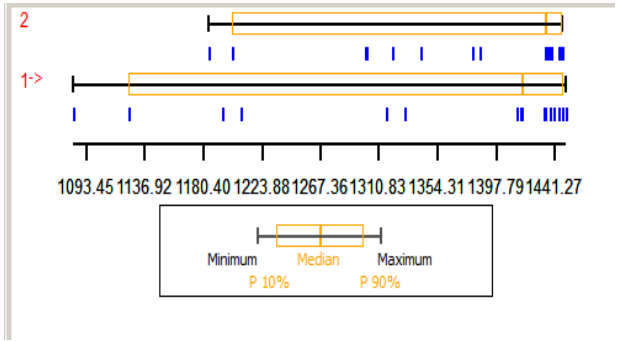

Figure 6. Variation of the centroid size of left wings for Sand flies (Phlebotominae) according to feeding. The boxes display the group median separating the 10 th and 90 th the quartiles. The wings numbers Babylon and Diyala in the figure are represented by vertical bars under the boxes. Units are pixels. P, percentile.

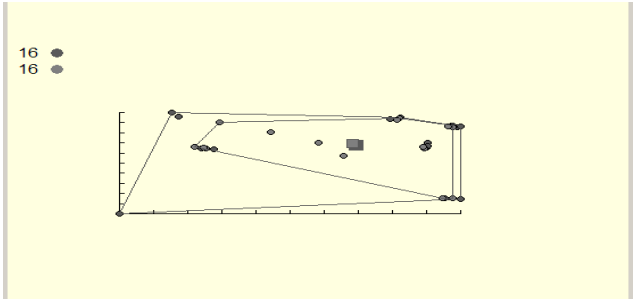

Figure 7. Scatter plot of the principle component analysis of Sand flies (Phlebotominae) specimens according to feeding based on Geometric Morphometric, Gray spots represent Babylon specimens and Black spots represents Diyala specimens, Gray square represent mean centroid size of the left wing for Babylon specimens =1441.26 and Black square represent mean centroid size of the left wing Diyala specimens =1429.97.

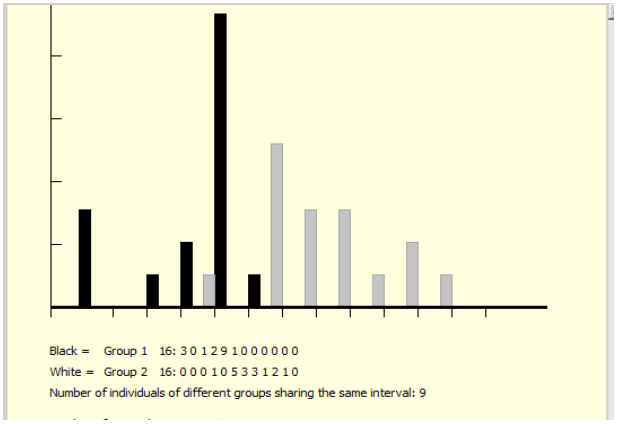

Figure 8. Discriminate analysis of two groups for Sand flies (Phlebotominae), Mahalanobis distances between centroids size were as follows: Babylon specimens to Diyala specimens = 2.77, Black color in this figure represented to Babylon specimens and Gray color represented to Diyala specimens.

Table 2. Comparison between Babylon and Diyala specimens of the centroid size of the left wing for Sand flies (Phlebotominae).

Group	M.CS	St.D	Va.	F	P	T	P	A.D
1	1441.26	120.22	14454.11	1.12	0.91	0.11	0.07	6.56
2	1429.97	127.61	16285.54					

1: Babylon , 2 : Diylaa , M.CS : Mean Centriod size , St.D : Standard Deviation , Va. : Variance , P : Probability , A.D : Absolut differences

Table 3.Analysis of variance for asymmetry left wing size of Sand flies (Phlebotominae) between Babylon and Diyala specimens .

Source	SS	Df	MS	F	Signification
Model	52422.73	3	17474.24	1.81	0.1681
Individual	36234.71	1	36234.71	3.76	0.0628
Side	0.9052	1	0.905235	0.00	0.9923
Side*i	16187.11	1	16187.11	1.68	0.2058
Residue	270178.05	28	9649.21		36

Table 4. Analysis of variance for asymmetry left wing shape of Sand flies (Phlebotominae) between Babylon and Diyala specimens .

Source	SS	Df	MS	F	Signification
Model	**0.1198**	**54**	**0.00248**	**2.74**	**0.0000**
Individual	**0.418**	**18**	**0.002321**	**2.87**	**0.0001**
Side	**0.0243**	**18**	**0.002981**	**1.67**	**0.0409**
Side*i	**0.0537**	**18**	**0.000810**	**3.68**	**0.0000**
Residue	**0.4080**	**504**			

Table 5. Mahalanobis distances between centroids size were as follows: Babylon specimens; to Diyala specimens

	B	D
B	**0.00**	
D	**2.77**	**0.00**

***B: Babylon D: Diyala**

Conclusion:

The wing analysis is an essential method for future geometric morphometry studies to distinguish the species of Sand Flies in Iraq, all species are clearly distinguished.

Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- The author has signed an animal welfare statement.
- Ethical Clearance: The project was approved by the local ethical committee in University of Baghdad.

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التنوع والتوزيع الجغرافي لذبابة الرمل (Diptera: Phlebotominae) *Phlebotomus papatasi* باستخدام المقياس الهندسي من مناطق مختلفة من العراق

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الخلاصة :

تم دراسة الاختلاف في الصفات المظهرية للأجنحة باستخدام تقنية المقياس الهندسي لمجاميع سكانية مختلفة من ذبابة الرمل من محافظتين مختلفتان هما بابل وديالى. حيث تم تحديد أحد عشر معلم موزعة على أجنحة هذه الأنواع. تم تمييز جميع الأنواع باستخدام حجم وشكل الجناح. هذه النتائج تسلط الضوء على تحليل الجناح كأداة فعالة لدراسات الاختلافات المورفولوجية لجناح ذبابة الرمزل.

الكلمات المفتاحية: Diptera، هندسي الشكل، ذباب الرمال، -Phlebotominae .