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Comparison among Populations of Mosquitoes *Culex quinquefasciatus* Say by using Geometric Morphometric Technique from Different Regions of Iraq

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Abstract:

The geometric morphometric technique was used to study the variables in the shape and size wings of different populations of mosquitoes *Culex quinquefasciatus* from different Iraqi provinces Babylon, Baghdad and Wasit. The results showed that the average of centroid size were 366, 387.5 and 407.4 Micron in Babylon, Baghdad and Kut, respectively. The statistical analysis showed that there were no significant differences in the average of centroid size of all specimens and they belong to the same species.

Keywords: Mosquito, *Culex*, Diptera, Geometric Morphometric.

Introduction:

Mosquitoes belong to the Culicidae family of Diptera that includes two important medicinal families: Anophelinae and Culicinae [1]; This family has about 3500 species of mosquitoes belonging to 34 species, including *Culex* [2]. Carl Linnaeus has used many Latin terms in describing this species, including Gnat, Midge and *Culex* [3]. 550 species have been described so far female mosquitoes attack humans and animals to obtain the blood which is necessary to form eggs. They attack all animals with warm blood causing severe discomfort and serious harm due to loss of blood, itching and allergies as well as the transfer of pathogens[4]. Carbon dioxide (CO₂) released from respiration as is as sweat is an attractive substance for mosquitoes [5] geometric morphometric technique is used to diagnose the shape and structure of the Geometric morphometric wing. It is known as a science that studies the metrical measurement of the composition or shape and measures the extent of variations in shape or structure. Geometric Morphometric is the science that refers to the quantitative analysis of a shape which is a concept of size and shape and performs this type of analysis In general on all organisms and useful in the analysis of its fossil record, the effect of variations on the shape, the differences between environmental factors and shape, when the discovery of variations in shape, the geometric

measure of the shape of the wing or installed. It is possible to be used to determine the property or characteristic of the evolution of character occurring in the evolution, functions or evolutionary relationships between organisms process. The main objective of the measure geometric shape of the wing or composition is the statistical testing of hypotheses about the factors that affect the shape of the wing [6, 7]; and the morphometric of that method is simple quantitative study comparisons form as well as it is a branch of the shape and structure as it is a branch of statistics, and also it is a branch of mathematics 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. [8, 9] used technical Geometric Morphometric Comparison study of *Dacus ciliatus* (Loew) in Iraq. It was conducted by using the same technique and it has been noted that the flies are of the same species [10]. The effect of climatic variations on the wing and size of the *Aedes aegypti* (Diptera: Culicidae) populations in Thailand was studied using the wing geometry they found a difference in the central size according to the months of the year [11]. In this study, the central size of the wing was used to compare the populations of *Culex* spp. mosquitoes and the group from different regions of Iraq.

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Materials and Methods:

Fifteen right wings were used for females of *Culex* mosquitoes, which were collected from different regions of three Iraqi provinces: Baghdad Nahrawan, district Babylon Mahaweel district, and Nu'maniyah district to study the variations in the shape and size of wings and the distinction among these populations by using the geometric technique; The method was followed in the preparation of the glass slides of the wings [12]. Fifteen females were isolated in transparent plastic containers and left without feed until they died and dried. After drying well, the right 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 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 Collecting:

After the female wings were photographed, each image was collected separately using the software that is called Collecting Landmarks for identification and characterization. This program is for engineering analysis of wings and is available on the website www.mpl.ird.fr/Morphometrics and data was collected from images. The wings are the Landmarks anatomical points placed on the wings at intersections of longitudinal veins with transversal veins or at the ends of longitudinal veins and are used for differentiation between individuals [8].

Eleven of the first type landmark were used in this study: longitudinal veins with transversal veins; Numerous points were placed between these intersections by the COO (Collection of Coordinates) unit within the program, This unit is specially designed for coordinates. The connections between the eleven Landmarks that were placed on each wing gave us polygons which are used in analysis and included comparing the size and its shape of the wing to each sample. After completing the mapping of the Landmarks for each sample of the wing samples, the TET module was then used to integrate the sample data together to compare the populations of the *Culex* mosquitoes with the wings and determine their conformity or variability. After completing the data integration process, MOG (for the Spanish words "Morfometria Geometrica"), this unit is located within the program. Through this unit, several operations are performed on the coordinates of the Landmarks; These processes are

Translation, Scaling and Rotation. After these three operations, Centroid size, Partial Warp, relative warp and shape variables for each wing [13].

Data Analysis

Principle Component Analysis was used to detect variation within the three populations of *Culex* mosquitoes, as well as the Discriminant Analysis to detect variation within the same population group. This analysis is within the ready-made program.

Software

The put landmarks and their collection were obtained from the COO unit; Centroid Size, Partial Warp, Relative Warp and Principle. Component Analysis Data were obtained from the MOG unit and all Discriminate Analysis data was obtained from the PAD unit. The variance analysis data for the central size of the wing was obtained from the COV unit (for Spanish words "Permutaciones, Analisis Discriminante"). The symmetry analysis data for the wing shape and its volume were obtained from the ASI unit and are all within the Collecting Landmarks for Identification and Characterization program. This program is available at: <http://www.mpl.ird.fr/Morphometrics>.

Results and Discussion:

In this study, the Geometric Morphometric of Wing system was used to compare the populations of the total *Culex* mosquitoes from different regions of Iraq. This system is mainly based on the Landmark coordinates of the coordinates of longitudinal veins with the transverse veins of the wing, calculating the central size of each centroid size wing, and the isometric amount [8]. The fig. 1, 2 and 3 explain the coordinates of the eleven Landmarks, landmarks on the *Culex quinquefasciatus* right wing of Babylon, Baghdad and Wasit respectively. Figure 4 and 5 shows the average coordinates of 11 Landmark from 15, the green color of the province of Babylon and the red color represents the coordinates of the 11 landmarks of Baghdad and the Blue color of the Wasit region. When conducting the process of matching the wings using the geometry system of the shape of the wing through the MOG unit, as shown in the form that the wings of the samples are almost identical in most of the landmarks placed on the wings and the cause of the mismatch of some of the landmarks due to different environmental conditions [14]. Figures 6 and 7 show the variation in the central size of the wing according to the collection regions. Each box represents the median group distributed between spring (10, 90) and spring (25, 75). The central size of the wing was 366,387.5 and 407.4 μ m for the population groups of Babylon, Baghdad and Wasit provinces respectively table 1. The F and T test were used to compare the three populations in

the central size of the right wing. There were no significant differences between Babylon and Baghdad specimens. $P = 0.54$, $T = 0.62$ and A.D (Absolute differences) was 7.59. The total value of $P = 0.05$ and the absolute difference was 41.44. There were no significant differences between the insects of these regions in the mean size of the right wing. When comparing the female mosquitoes collected from Baghdad with that group of Wasit was $P = 0.5$. There is no significant difference in the mean size of the right wing between Baghdad and Wasit insects. This means that the females of the *Culex* mosquitoes, the total of the Babylon, Baghdad and Wasit, are the same species from fig. 8 shows that the differential analysis of the female population of mosquitoes; the total number of wings used in the analysis is 45 wings. The variation in the shape and size of the wing of specimens that was collected from Babylon less than the variation in the specimens of Baghdad and Wasit province while they were the variations in the specimens of the province of Baghdad less than the variances of the specimens that were collected from Wasit province table 2. The results show that the distance between Mahalanobis distances between the central size of the female insect wings of Babylon and the central size of the female insect wings in Baghdad population was 4.14 microns. The distance of the Mahalanobis between the central size of the insect wings of Babylon and the central size of the female wings of Wasit is 4.78 microns. The distance Mahalanobis between the central size of the wings of female insects of Baghdad and the central size of the wings of female Wasit specimens was equal to 3.15 micron. The reason for variation in wing shape and size and 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 three areas from where the insects were collected. This study is the first of its kind in Iraq for mosquitoes using the geometrical Morphometric. However, there were many countries studies on the use of the geometric Morphometric of the wing or its composition to study the variation of insect populations in this field; such as the study by [15], which included a comparison of honey bee population groups of three different regions, Italy, Carniolan and Africa depending on the geometry of wing shape or composition. They found a difference in wing size, size and central size of the wing among the three communities. These differences are due to the different environmental conditions in the three regions such as rainfall and relative humidity. In addition, the variation in shape and size of the *Phlebotomus papatasi* (Diptera: Psychodidae) and the group from the north and south of the Atlas Mountains in Morocco was studied by [16]. They found a variation in the shape and size of the wing between the two populations of the insect. They studied the genetic variation in the phenotypic mosquito *Culex quinquefasciatus* group from different geographical regions of Brazil, Argentina, using the geometric measure of the shape of the wing technology or a combination, the researchers found a mismatch Dominated ordinated defined between the populations of this insect and also found a significant difference in the size of the central wing of this insect depending on the collection area, and interpreted this variation between the three populations to different environmental conditions in these areas [17].

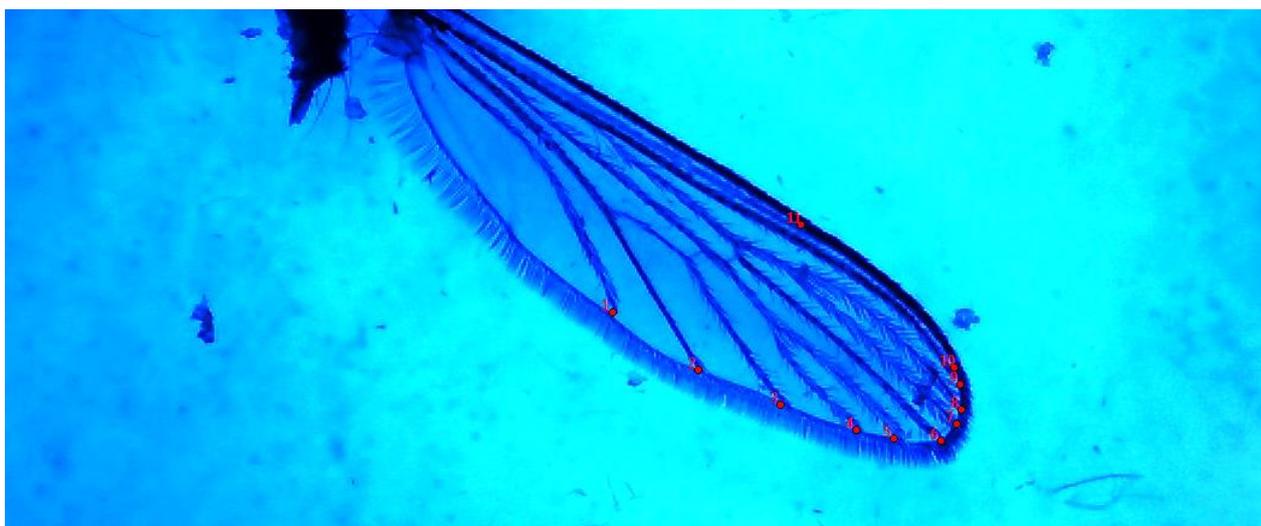


Figure1. landmarks on the right wing of *Culex quinquefasciatus* (specimen from population of Babylon province)

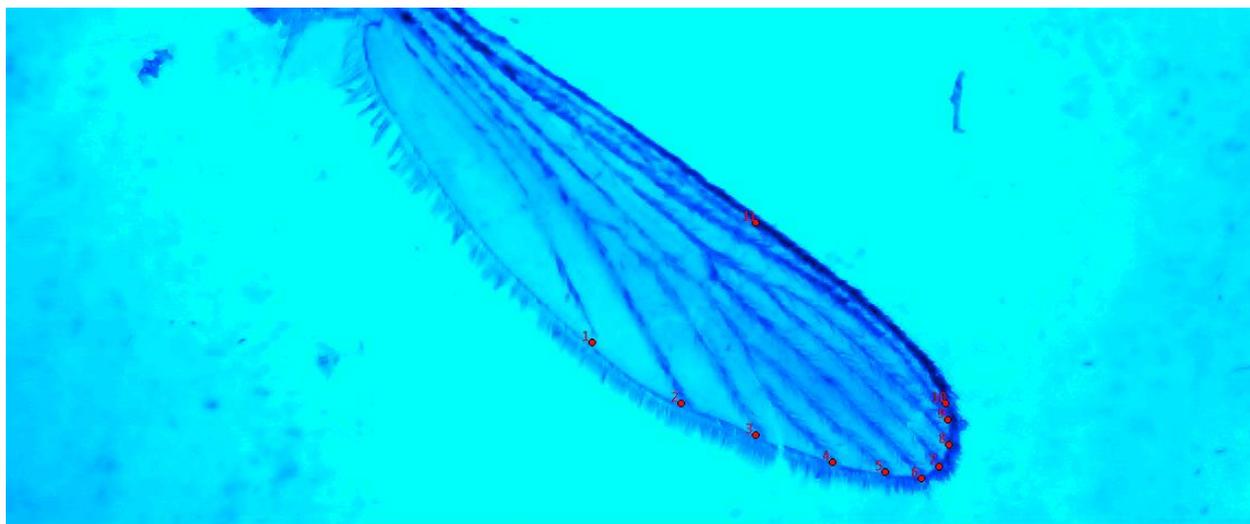


Figure 2. landmarks on the right wing of *C. quinquefasciatus* (specimen from population of Baghdad province)

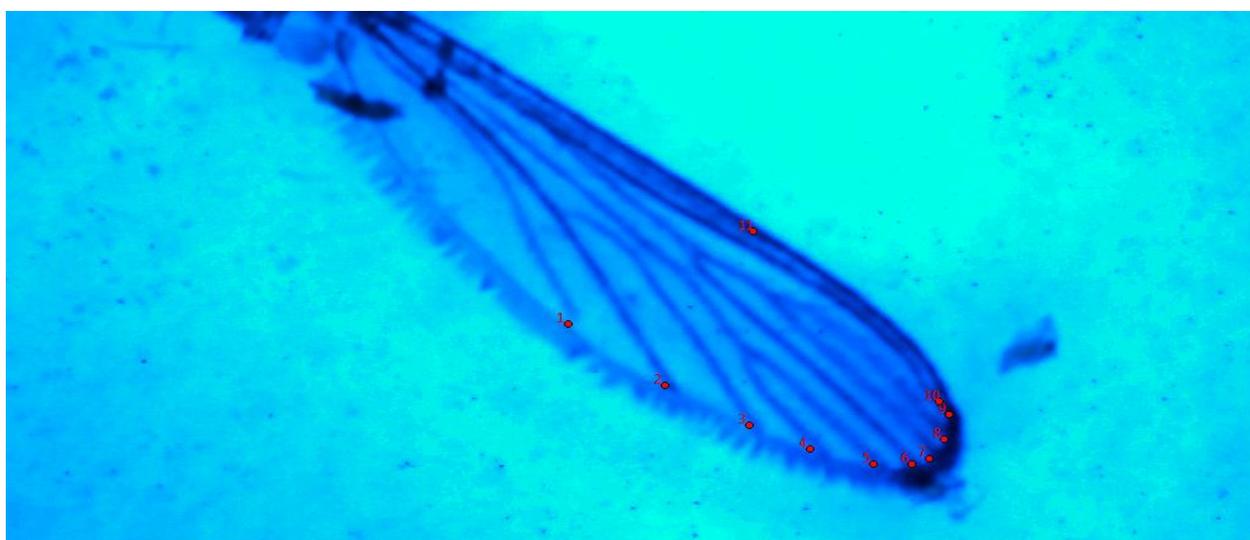
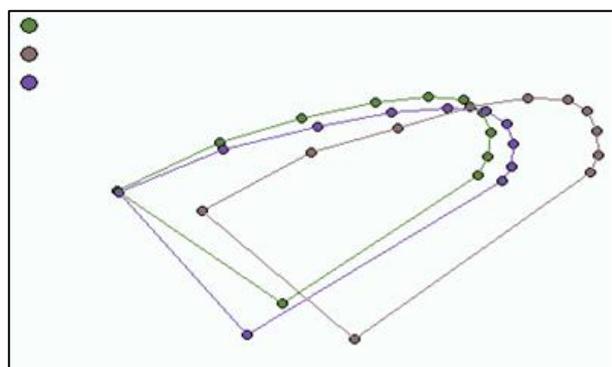
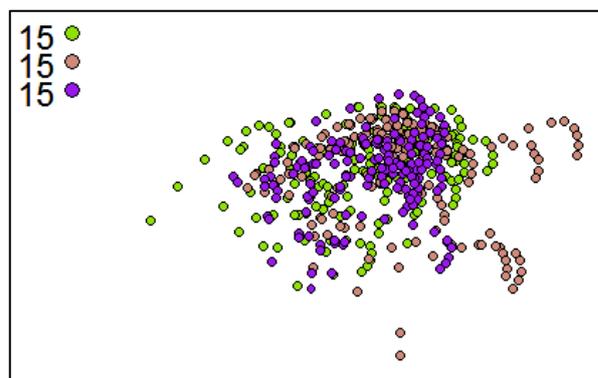


Figure 3. landmarks on the right wing of *C. quinquefasciatus* (specimen from population of Wasit province)



X = of - 240.251 to 151.816, Y = of -312.584 to -91.651

Figure 4. Mean coordinates of 11 landmarks for right wing of the three colonies (Green color represents Babylon, red color represents Baghdad and blue color represents Wasit populations).



X=of-1511.917 to 432.036, Y=of -551.917 to 25.083

Figure 5. Output of the consensus configuration by the GPA Procrustes superimposition method locating the 11 landmarks for each individual Right wing of the three colonies. Green color represents Babylon specimens, Red color represents Baghdad specimens and Blue color represents Wasit specimens.

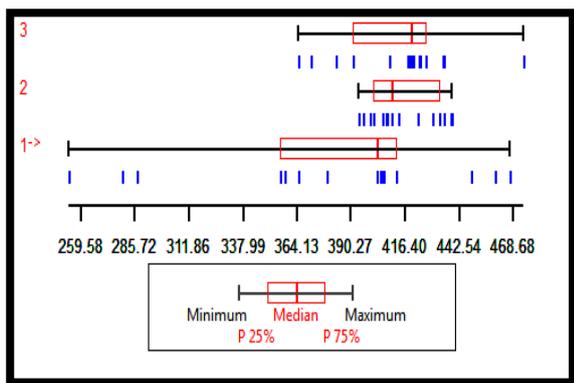


Figure 6. Variation in the centroid size of right wing in species of *Culex quinquefasciatus* according to environmental conditions. (Each box shows the group median separating the 25th and 75th the quartiles, Vertical bars under the boxes represent the wings numbers 1 ; 2 and 3 in the figure. represent Babylon ; Baghdad and Wasit specimens respectively. Units are pixels. P, percentile)

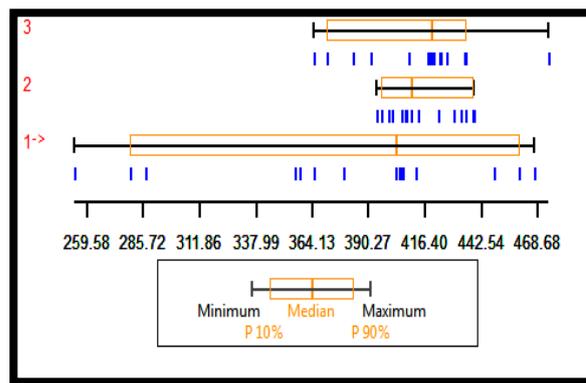


Figure 7. Variation in the centroid size of left wings according to ironmental conditions *Culex quinquefasciatus* (Each box shows the group median separating the 10th and 90th the quartiles, Vertical bars under the boxes represent the wings numbers 1 ; 2 and 3 in the figure. represent Babylon; Baghdad and Wasit specimens respectively. Units are pixels. P, percentile).

Table 1. Compared the centroid size of the Right wing for *C. quinquefasciatus* that collected from Babylon; Baghdad and Wasit provinces.

Group	M.CS.	St.D.	Va.	F	P	T	P	A.D.
B	366.0	51.4	2605,2	B-D=2.2	B-D=0.35	B-D=0.62	B-D=0.54	B-D=21.52
D	387.5	75.8	5749.1	B-K=28.2	B-K =0.76	B-K =2.11	B-K =0.05	B-K =41.44
W	407.4	9.6	92.2	D-K =62.2	D-K =7.70	D-K =0.68	D-K =0.50	D-K =19.91

M.CS: Mean centroid size, St.D: Standard Deviation, Va.: Variance, P:Probability, A.D : Absolute differences. B: Babylon, D: Baghdad, W: Wasit

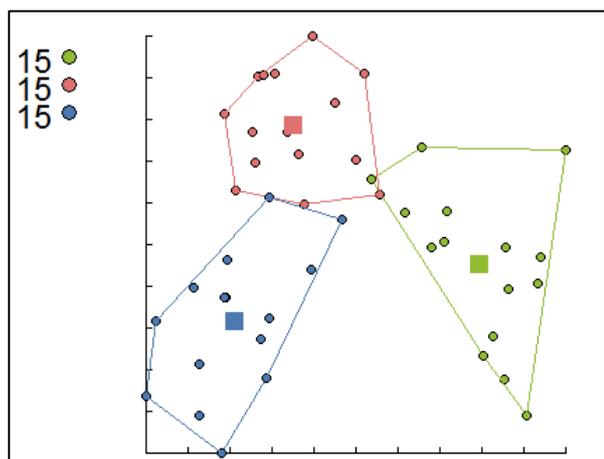


Figure 8. Scatter plot of the principle component analysis of *C. quinquefasciatus* to environmental conditions based on Geometric Morphometric (green spots represent Babylon specimens red spots represent Baghdad specimens and blue spots represents Wasit specimens, green square represent mean centroid size of the right wing for Babylon specimens = 366.0; red square represent mean centroid size of the right wing Baghdad specimens = 387.5 and blue square represent mean centroid size of the right wing for Wasit specimens = 407.4).

Table 2. Mahalanobis distances between centroids size were as follows: Babylon specimens; Baghdad specimens to Wasit specimens.

	B	D	W
B	0.00		
D	4.14	0.00	
W	4.78	3.15	0.00

• B : Babylon , D : Baghdad , W : Wasit

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المقارنة بين المجتمعات السكانية لبعوض الكيولكس *Culex quinquefasciatus* باستخدام المقياس الهندسي من مناطق مختلفة من العراق

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الخلاصة:

استخدمت تقنية المقياس Geometric Morphometric Technique لدراسة التغيرات في شكل وحجم الجناح بين المجتمعات السكانية لبعوض الكيولكس *Culex quinquefasciatus* من محافظات مختلفة من العراق وهي بابل وبغداد وواسط. بينت النتائج ان معدل الحجم المركزي centroid size كان 366.0، 387.5، 407.4 مايكرون لعينات محافظة بابل و بغداد والكوت على التوالي واطهرت نتائج التحليل الاحصائي بعدم وجود فروقات معنوية احصائية في معدل الحجم المركزي لجميع العينات وانهم يعودون لنفس النوع .

الكلمات المفتاحية: البعوض، بعوض المنزل الجنوبي، المقياس الهندسي، رتبة ثنائية الاجنحة.