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Research Article

STUDYING THE QUANTITATIVE HETEROGENEITY BETWEEN TWO POPULATIONS OF *CULEX PIPIENS* IN TWO IRAQI PROVINCES

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ABSTRACT

The objective of this study was to investigate the quantitative heterogeneity between two populations of *Culex pipiens* (a mosquito species) and understand the underlying reasons for this variability. The study focused on two specific areas viz. Diyala and Alnajaf. To achieve this, the Geometric Morphometric Technique was employed using *C. pipiens* female wings. The principal components of the wings were analyzed, the central size of each female wing was calculated, and samples from both populations were compared using discriminant analysis based on wing size. The statistical analysis, including T-tests, revealed no statistically significant differences in the central wing size between the two studied areas. Additionally, an ANOVA analysis indicated that there were no significant differences in wing shape and size among the female insects from Diyala and Alnajaf. In summary, the study found that the central wing size and wing characteristics of female *C. pipiens* mosquitoes were similar across the Diyala and Alnajaf regions.

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INTRODUCTION

The animal kingdom is a diverse and vast world that encompasses many classes and species. One of these species is the class of insects known as Insecta. What concern us within this broad category are medical insects, their importance, and their direct and indirect relationships with human diseases, either as carriers or causes of them. The science that deals with the study of this class is called medical entomology (Scott et al., 2009).

To obtain the necessary protein for egg laying, female mosquitoes attack warm-blooded animals (Abu Al-Hab, 1982). These attacks by female mosquitoes cause significant inconvenience and serious damage, including blood loss, itching, allergies, and transmission of pathogens. Mosquitoes are among the most common insects encountered by humans and may sometimes require treatment. Carbon dioxide emitted through breathing and sweating attracts mosquitoes (Russol et al., 2011).

Symptoms or pathological reactions resulting from a mosquito bite include a pink rash around the affected area, accompanied by itching and pain. Mosquito saliva contains substances or chemical components that cause these symptoms. It has been found that the salivary glands secrete antigenic substances, agglutinins, and anticoagulants. Furthermore, mosquito saliva prevents the growth of chicken embryos and causes their death upon injection. When feeding, a mosquito's mouthparts reach capillary blood vessels, leading to punctures in multiple areas that slow or stop blood flow, resulting in exudation and bleeding in the form of small hemorrhagic spots, particularly around the eyes in children (Abul-Hab, 1978).

Mosquitoes have spread across Iraq from the far north to the south, with 16 species of *Anopheles* and 18 species of *Culex* being recorded (Abul-Hab, 1980). They are primarily concentrated in the central and southern regions. Various methods are available for diagnosing mosquitoes, including Molecular Genetic Identification and diagnosis based on the geometric scale of adult wing shape or structure (Haneen et al., 2018). The success of this crucial technique requires extensive studies, including the examination of genetic variation among populations belonging to the same species but in different geographical areas, to reveal the genetic compatibility between breeds.

The focus has been on the quantitative genetic aspect, which depends on wing shape and size using the geometric morphometric technique to determine the apparent shape of the insect's wing and ascertain heterogeneity among *Culex pipiens* mosquito populations (Abd and Ahmed, 2017). Comparisons between species from different communities can be made using the central size of the wing, which estimates total size based on arranged data. The central size of the wing is calculated by taking the square root of the sum of the squares of the distances between the center of the polygon and each landmark on the wing (Salcedo et al., 2019).

The geometric wing scale technique is widely regarded as one of the modern and accurate taxonomic methods for insect species (Tofilski, 2008; Francoy et al., 2009; Bloch et al., 2010). This study aims to investigate quantitative genetic variation between two populations of *Culex pipiens* mosquitoes found in two Iraqi Provinces, Diyala and Alnajaf.

MATERIALS AND METHODS

The present study was conducted at the Environmental Health Laboratories, Department of Environmental Health, College of Environmental Sciences, Al-Qasim University, Babylon. Thirty-two left wings from female *Culex* mosquitoes were selected from various regions in Diyala and AlNajaf, Iraq. The aim was to examine the quantitative genetic variation between the two populations of *C. pipiens* mosquitoes using the Geometric Morphometric Technique.

To prepare the samples, the methods outlined by Sangvorn and Nopphaun (2011) were followed for preparing glass slides of the wings from 32 randomly selected females from Diyala and Najaf. Each wing was placed between two glass slides, and the edges were secured with paper adhesive tape, which also contained information about the specimen. After completing the preparation of the glass slides, they were photographed using a digital microscope (Figure 1) connected to a calculator with a digital camera. The camera had a magnification power of 1.3 megapixels and was equipped with UV rays. The images of each study site were saved for analysis.

Data collection

The data for each image were collected separately using the Collecting Landmarks for Identification and Characterization program, which is designed for engineering analyses of wings and is available at http://www.mpl.ird.fr/Morphometrics (Bookstein, 1991). In this study, 16 landmarks of the second type were utilized, which correspond to the ends of the longitudinal veins of the wing. Numbered points were placed between these intersections using the Collection of Coordinates unit within the program. These points formed connections between the 11 landmarks per wing, creating polygonal shapes essential for various analyses. The study included comparisons of wing size and shape among the samples to elucidate heterogeneity within *Culex* mosquito populations.

Samples from different areas were also analyzed to understand variance within the same population. After merging the model's data, they were transferred to the MOG unit (Morfometría Geométrica in Spanish). This unit facilitated multiple processes on landmark coordinates, including translation, scaling, and rotation. These processes allowed for the determination of central size, partial warp, relative warp, and shape variants per wing. The TET unit computed the distances between each pair of landmarks, converting the data into files with the extension (coord.txt) for each species in the same provinces. These files were then compared with a file in the .txt format, integrating data from the same species in other provinces to assess compatibility and variability.

Upon completing data integration for species, further operations such as translation, scaling, and rotation were performed by the MOG unit on landmark coordinates to determine the centroid size of the wing. Centroid size, which represents the sum of squares of distances between the center and every landmark on the wing, was used to compare species. Statistical significance of centroid size differences among wings was estimated using the COV unit (ManCova), a Multivariate analysis of Covariance, and assessed by comparing data computed in files with the extension (CS.txt).

Additionally, asymmetry analysis was conducted using the ASI unit (Asymmetry of Shape and Size) to provide a statistical assessment of asymmetry in wing shape and size between common species in provinces. The results of this analysis were presented in an output table found in a file with the extension (CS-ASI-INFO.txt) (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) in Spanish	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
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

Central volume

To compare mosquito populations, the central size of the wing was determined by calculating the square root of the sum of the squares of the distances between the center of the polygon and each of the features placed on the wing. Differences in wing shape were identified using Discriminant Analysis (Caro-Riano et al., 2009).

Software

The placement and collection of landmarks were obtained from the Collection of Coordinates unit, while the data, including the central size of wings, partial warp, relative warp, and principal component analysis data, were obtained from the MOG unit. All the data for Discriminate Analysis were obtained from the PAD unit (Spanish: Permutaciones, Análisis Discriminante). The analysis of variance data for the central size of the wing was obtained from the COV unit, and the data for the analysis of symmetry for the shape and size of the wing were obtained from the ASI unit. All these units are part of the program for collecting landmarks on wings for identification and characterization, which is available at: http://www.mpl.ird.fr/Morphometrics.

Figure 1. The Digital Microscope used in the studies.

RESULTS AND DISCUSSION

The results indicated that the wings of *C. pipiens* mosquitoes from the provinces of Diyala and Alnajaf were almost identical across most of the landmarks placed on the wings (Figure 2 and 3).

This similarity in features, despite the mosquitoes being collected from different areas, is attributed to the similarity in environmental conditions (Kitthawee and Rungsri, 2011). Furthermore, the interaction between samples from Diyala and Alnajaf is clearly demonstrated in Figure 4, which presents a Principal Component Analysis (PCA) of 32 wing landmarks. The average centroid size of the samples from the provinces of AlNajaf and Diyala in Iraq were 478.9 and 429.3, respectively, the use of Principal Component Analysis (PCA) revealed an overlap between the populations of female *Culex* mosquitoes collected from the governorates of AlNajaf and Diyala, as shown in Figure 5.

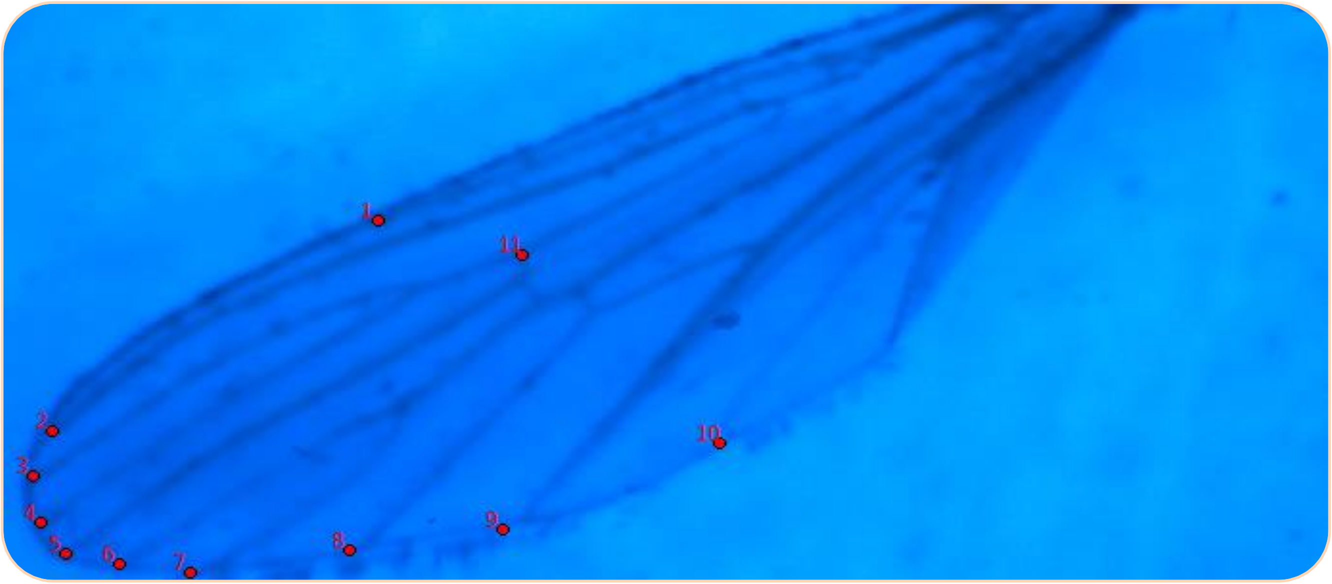

Figure 2. The left wing of C. pipiens with eleven landmarks from mosquito populations in Diyala, Iraq, used in geometric analysis.

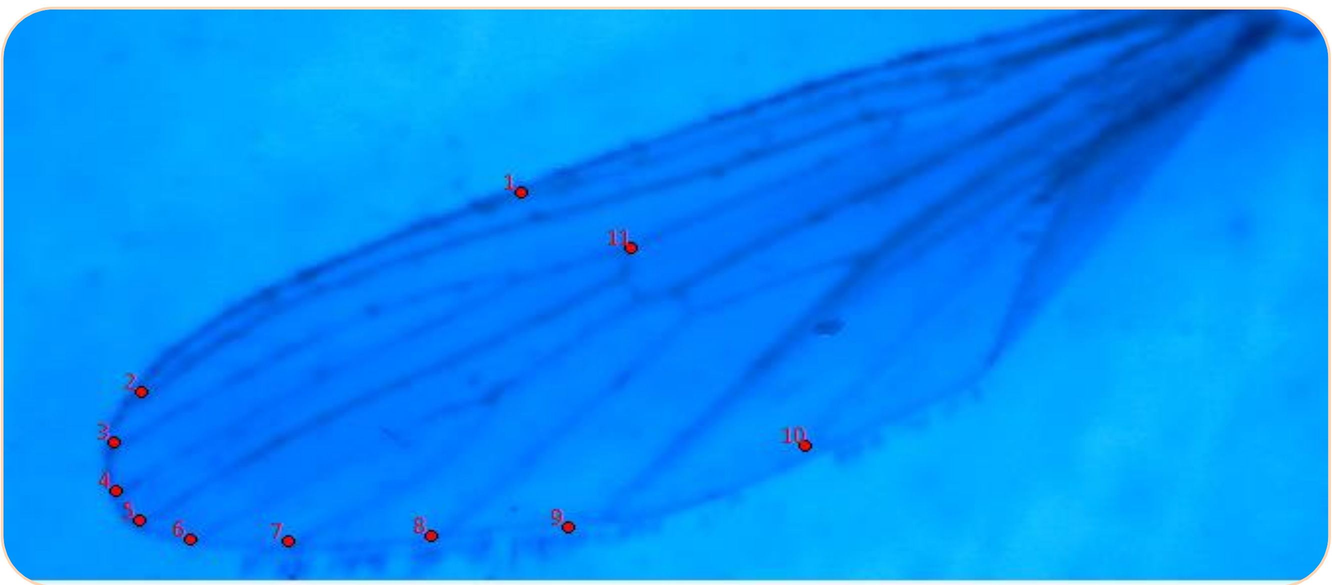

Figure 3. The left wing of *C. pipiens* with eleven landmarks from mosquito populations in AlNajaf, Iraq, used in geometric analysis.

The results of the statistical analysis, employing T-tests and F-tests for the centroid size of the left wing from both provinces, are presented in Table 2. Additionally, no differences were observed between the mosquito wings from the two collection areas when the samples were distributed on springs 25 and 75, as well as springs 10 and 90, as depicted in Figures 6 and 7, respectively. Discriminant analysis used to compare members within a single population group indicated no significant differences between the samples from the two Iraqi provinces, as evidenced by the Mahalanobis distances between the centroid sizes of the left wing samples from Diyala and Alnajaf, which were 8.49 (Table 3). The study also included an analysis to determine the size and shape of the left wing; the findings revealed no significant differences between the samples from Diyala and Alnajaf (Tables 4 and 5).

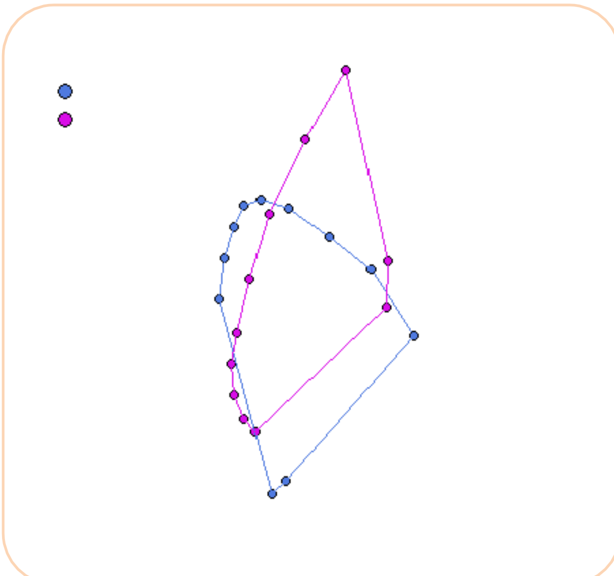

Figure 4. The rate coordinates of the landmarks for the Left wing of the population of *C. pipiens* fly, which are 11 landmarks for each wing. The green and red colors represent the population communities of Diyala and Alnajaf of Iraq respectively.

The application of Geometric Morphometrics to wing shape has emerged as a crucial tool for discerning variations among insect populations in different habitats (Simões et al., 2020; Souza et al., 2020; Oliveira-Christe et al., 2023). It was discovered by Abd et al. (2020) that variations in the wing morphological features of the Sand Fly from the Iraqi provinces of Babylon and Diyala could be attributed to the geometric morphometric technique. They also noted that environmental differences, such as temperature and rainfall, exert a phenotypic influence on mosquitoes. Morais et al. (2010) investigated the genetic variation in the phenotypic group of *C. quinquefasciatus* mosquitoes from diverse geographical regions of Brazil and Argentina. Utilizing geometric measurements of wing shape, the researchers identified a mismatch among the populations of this insect and observed a significant difference in the central wing size based on the collection area, attributing this variation to the distinct environmental conditions in these regions.

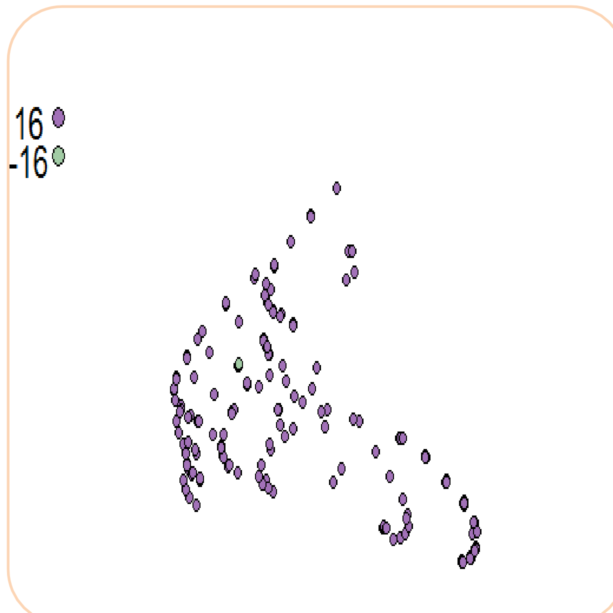

Figure 5. The diffusive figure of the distribution of *C. pipiens* specimens along the first and second Principal component analysis PCs for the shadow space coordinates derived from the coordinates of the original features of each wing, which numbered 11 landmarks (green and red represent the population communities Diyala and Alnajaf of Iraq respectively.

Table 2. Comparison between the populations of *C. pipiens* fly from Diyala and Alnajaf /Iraq in the central size for the left wings

Specimens	Mean Centroid size	Standard Deviation	Variance	Calculated F value	Probability	Calculated T value	Probability	Absolute Differences
C. pipiens (N)	478.9	49.6	0.18	1.3	0.18	5485 .91	10301	101.4
C. pipiens (D)	429.3							

N: AlNajaf D: Diyala

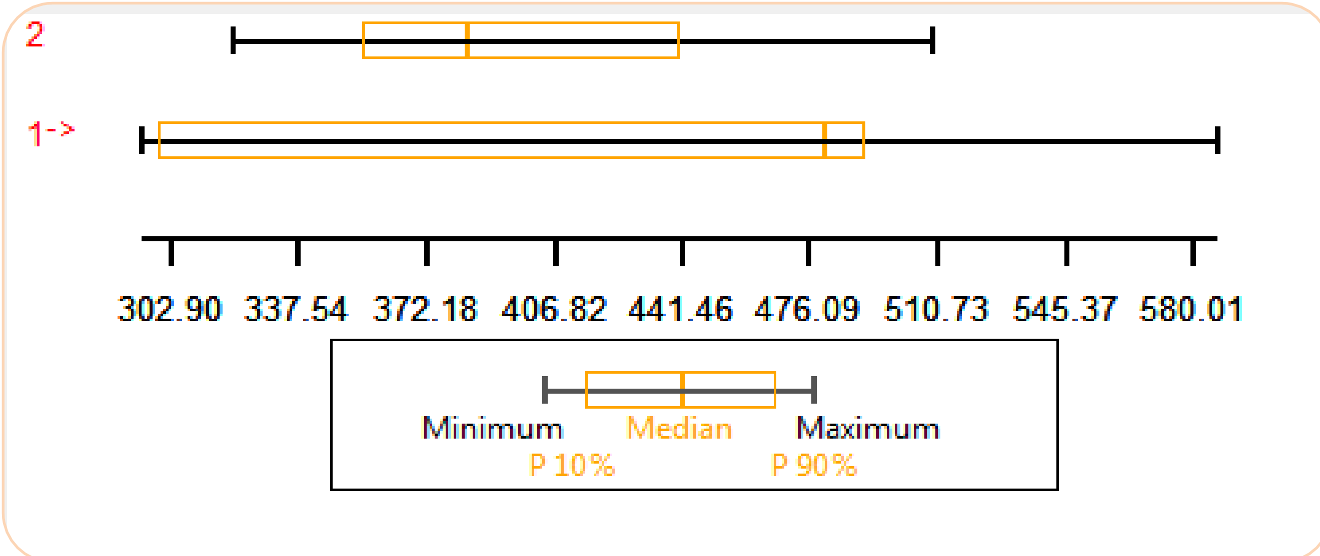

Figure 6. The Left wings of *C. pipiens* fly, the central size according to the population it belongs to. Each box in the figure represents the middle group distributed between spring 10% to spring 90%, and the blue lines under each box represents the wings, while the numbers 1 and 2 in the figure represent the population communities of Diyala and Alnajaf, Iraq respectively.

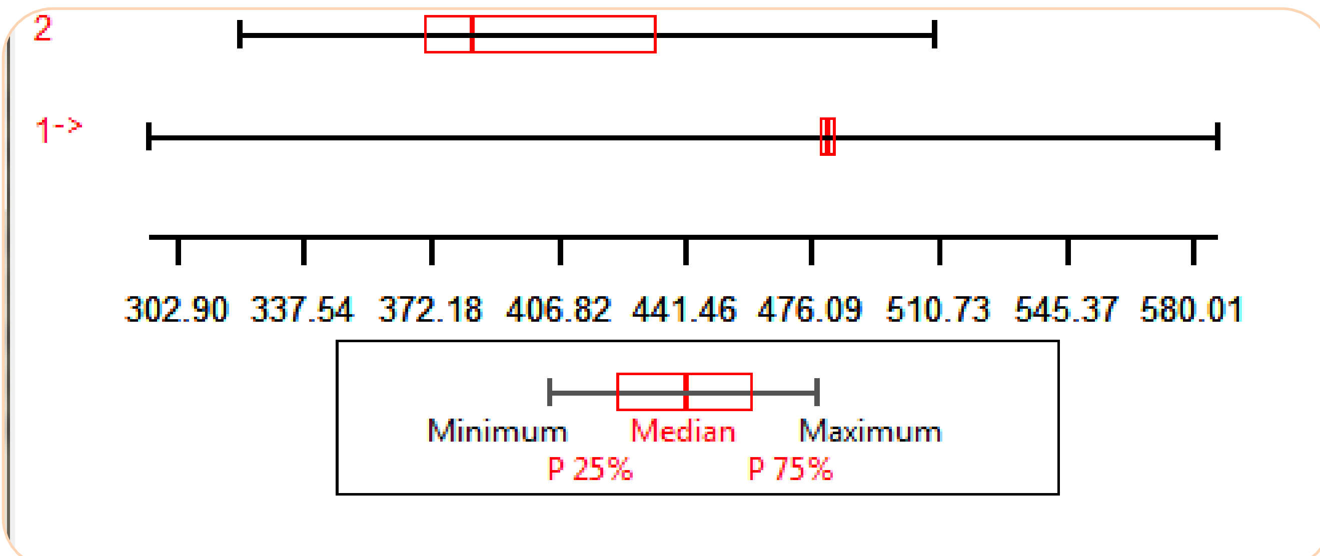

Figure 7. The Left wings of *C. pipiens* fly, the central size according to the population it belongs to, as each box in the figure represents the median group distributed between spring 25% and spring 75%, and the blue lines under each box represent the wings, while the numbers 1 and 2 in the figure, they represents the population communities Diyala and Alnajaf, Iraq respectively.

Table 3. Comparison of distances Mahalanobis Distances between population specimens of *C. pipiens* fly Diyala and Alnajaf, Iraq.

Specimens	*C. pipiens* (n)	*C. pipiens* (D)
C. pipiens (N)	0.00	0.00
C. pipiens (D)	8.49	0.00

N: Alnajaf D: Diyala

Table 4. ANOVA showing left wing Shape symmetry in populations of *C. pipiens* fly from Diyala and Alnajaf, Iraq.

Source	SS	DF	MS	F	Signification
Modal	0.0018	3	0.000588	1.21	0.3258
Individual	0.0005	1	0.000544	1.12	0.2999
Side	0.0005	1	0.000506	1.04	0.3171
Side*i	0.0007	1	0.000714	1.46	0.2363
Residue	0.0136	28	0.000487		

Table 5. ANOVA showing left wing size symmetry in populations of *C. pipiens* fly from Diyala and Alnajaf, Iraq.

Source	S S	D F	M S	F	Signification
Modal	0.0418	54	0.00077	0.72	0.9802
Individual	0.0217	18	0.00120	1.12	0.8541
Side	0.0040	18	0.00022	0.21	0.7973
Side*i	0.0160	18	0.00088	0.83	0.7820
Residue	0.5428	504	0.00107		

Several theories have been proposed to explain biological diversity as influenced by environmental variations, which are linked to the latitude and longitude of the province's location, a critical factor determining phenotypic shape differences (Owen, 2009). Further studies have shed light on the diversity, distribution, and ecology of mosquitoes in the region, contributing to the control of *Culex* spp. through the use of plant extractions and Nano-emulsions in Iraq (Kathiar et al., 2022a, b). In conclusion, the geometric morphometric technique has proven to be an essential method for distinguishing different species of *Culex* spp. in the provinces of Iraq.

AUTHORS' CONTRIBUTIONS

RAO, SAK and SAK conceived the idea, planned and designed the studies, executed the experiments, collected and analyzed the data, wrote the manuscript and edited it.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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