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Morphometric Geometry a tool for Quantitative Genetic Comparison between females of some medical and Forensic Insect Species in Iraq

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Abstract : The study was conducted using geometric morphometric analysis on the right wings of adult females of some forensic insects, including *Chrysomya megacephala* and *Sarcophaga carnaria*, as well as medical insects, including *Musca domestica*. The aim of this study was to analyze the morphological differences, diagnostic features, and comparisons between medical and forensic insects in Diyala Province based on right wing morphology and wing centroid size (MCS), considering the medical and forensic significance of C. *megacephala*, S. *carnaria*, and M. *domestica* due to their close association with human life and the transmission of numerous pathogens. 45 right wings had utilized 15 wings from every species—and 19 landmarks had recognized on every single wing to assess alterations in shape and size in this study. Substantial changes had **noticed** in the right wing centroid size and wing form amongst female scientific and medical insects. The outcomes showed that the mean right wing centroid size is 1576.52 for C. *megacephala*, 1734.74 for S. *carnaria*, and 1191.35 for M. domestica. Statistical assessment by the F-test and t-test shown pure and important alterations. Particularly, after matching C. *megacephala* and S. *carnaria*, the results were F = 1.75 (P = 0.31), T = 5.36 (P = 1.05), and AD = 158.22. In the comparison between C. *megacephala* and M. *domestica*, the readings were F = 1.43 (P = 0.51), T = 12.54 (P = 5.22), and AD = 385.16. Finally, comparing S. *carnaria* with M. *domestica* yielded F = 1.22 (P = 0.71), T = 20.46 (P = 0.00), and AD = 543.38. Likewise, the analysis OF ANOVA for the right wing shape among female forensic and medical insects, with C. *megacephala*, S. *carnaria*, and M. *domestica*, exposed changes in morphological diversity (F = 0.63, P = 0.54). In the same way, investigation of the size of wing showed differences among the studied species (F = 0.16, P = 1.00). The analysis Linear regression strengthened the dependability of geometric morphometrics (GM) as an active device for species categorization and the recognition of genetic alterations. The statistical results Proved or showed strong quantitative hereditary variances in the shape-size relationship of the wings among the three fly species. The first canonical axis (Y1) displayed a strong link between shape and size ($r = 0.759$, $r^2 = 0.576$), while the second canonical axis (Y2) showed a relatively weaker association. These results emphasize the morphometric diversity among species and affirm the possible of the landmark-based geometric morphometric method as a powerful way for identifying and cataloging insect species depend on phenotypic characteristics.

Keywords: *Geometry, Centroid Size, Medical insect, Forensic insect*

1- INTRODUCTION :

Flies affiliation to the families *Calliphoridae* and *Sarcophagidae* are regarded insects of noteworthy forensic and medical importance, on account of their widespread delivery in surroundings occupied by humans and animals that offer appropriate circumstances for implementation their life cycles 【1】. The "medical insects" term debates to arthropods used in the medicine field, with certain kinds, such as *Musca domestica*, playing a vigorous role in human and animal life due to their function as mechanical directions for frequent pathogenic sicknesses. Outside their medical relevance, particular insects, remarkably C. *megacephala* and S. *carnaria*, have obtained recognition for their scientific importance. These kinds are usually the first organisms to occupy or invade a corpse, where they nourish and place eggs and larvae shortly after death. Their immature phases are closely associated with decay and are serious in valuing the postmortem interval (PMI), both minimum and maximum【2】.The insects integration into criminal investigations has brought about the appearance of forensic entomology, a present division of entomology that stresses the role of insects in solving forensic cases【3】. Forensic entomology specifically includes the study of insects and arthropods found on rotting remains, such as flies and beetles, to facilitate in criminal and civil investigations. In particular flies are essential as a result of their expected colonization patterns, allowing forensic specialists to evaluate the PMI depend on larval improvement steps. The basis of forensic entomology exists in the precise identification of insect kinds and the comprehensive pursuing of their life cycles【4】.A central initial step in forensic inquiries is the speedy and precise identification of fly kinds to assess case resolution and to improve suitable approaches for controlling insect pests that act as disease vectors【5】【6】. Thus, there is a persistent necessity for efficient, cost-effective, and prompt identification ways. Among the most **eminent** taxonomic **instruments** for distinguishing insect kinds is geometric morphometric test of wing morphology. This method has confirmed extremely effective for entomologists in associating closely related or overlapping kinds. The geometric morphometric (GM) attitude, depend on quantitative evaluations of wing shape and size, works as a recent diagnostic tool, offering precise measurements of morphological differences【7】. Studies had showed that the geometric analysis of wing morphology effectively recognizes diversity among distinct inhabitants, thus representing an advanced way in the field of measurable genetics. The basic strength of this way resides in its ability to use comprehensive data on wing shape and size by recording particular landmarks on defined points across the wing of insect. These landmarks permit for a detailed test of shape and size variations across kinds【8】. This method is especially respected in studying complex insect groups such as those within the order Diptera【9】. Traditionally, Comstock (1893) was the first to use wing venation patterns in traditional insect cataloguing【10】Based on this basis, recent geometric morphometrics includes placing exact landmarks at vein intersections and along the wing margins that are then digitized reliably for comparative analysis【11】. Through these landmarks, scholars or scientists can calculate wing centroid sizes and analyze interspecific differences within the same family【12】, The present research goals to utilize a landmark-based geometric morphometric way to efficiently distinguish between females of three fly kinds of *C. megacephala* ***S.*** *carnaria*, *M. domestica* forensic and medical importance.

2- MATERIALS AND WORKING METHODS

2-1 - Sample Collection and Identification:

Models *of C. megacephala, S. carnaria*, and M. *domestica* had collected from various sites across Diyala Governorate via fly traps baited with poisoned animal droppings to improve collection efficiency. Succeeding group, the specimens had conserved in plastic boxes with the adding of naphthalene to avoid decay and **maintain** their morphological **characteristics**. Consequently, the models had transported to the Natural History Museum at the University of Baghdad, as they had recognized and ordered based on specialized taxonomic keys and diagnostic morphological features.

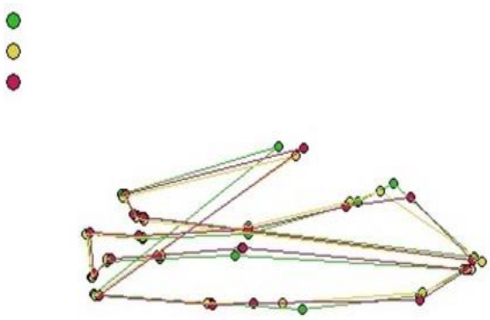
2-2- Sample Preparation and Landmark Setting

In our study, an entire of 45 right wings from female kinds of S. *carnaria* M. *domestica,* and C. *megacephala*—15 wings per kind—had examined to study the quantitative genetic disparity in shape and size of wing amongst the three kinds utilizing geometric morphometric (GM) methods. The wing models preparation succeeded the methodology described for [13], ensuring standardized and dependable specimen handling. Every sample of right wing had wisely examined internally via reasonable medical forceps to decrease injury and conserve morphological integrity. Every wing had placed between two slides of glass, with the ends decisively secured utlize G2100 adhesive tape. Stickers representative the kinds name, genus, and model number were attached to every slide for precise documentation. Upon implementation the slide preparations, the wings had taken pictures of via digital microscope linked to computer. The longitudinal intersections and cross wing veins had then recognized and marked, with every landmark numbered accordingly (Figure 1). Specialized statistical tests, containing centroid size computations and assessments of shape and size difference, had subsequently carried out to define the morphological differentiation degree between the medically and forensically important kinds.

2-3- Data collection

Upon achievement of the wing imaging process, data from every image had separately collected via the specialized software Collecting Landmarks for Identification and Characterization, **created** for geometric tests of wing morphology. The analytical process **concentrated on** identifying morphological landmarks, described as anatomical points sited at intersections between cross veins and longitudinal, not only... but also at the termini of longitudinal veins (Figure 2). These landmarks work as vital reference points for distinguishing among inhabitants belonging to the same genus or kinds. In current study, a entire of 19 landmarks had registered for every kind, associated with the intersections of longitudinal and cross veins. Landmark coordinates had identified utilize the COO module inside the software, which is specifically created for the accurate determination of coordinate data. By linking these landmarks on every wing, polygonal configurations had generated, offering the basis for subsequent geometric tests. The tests engaged comparing the shape and size of wing of individual kinds to evaluate the extent of morphological difference among the three studied fly kinds (C. megacephala, S. carnaria, and M. domestica) in addition to detect difference within inhabitants of the same kinds. Subsequent landmark placement, the model data had collected by the TET module, which consolidates separate datasets to ease inter-population comparisons. Subsequently, data were processed through the MOG module, where three essential operations—translation, scaling, and rotation—were applied to standardize landmark configurations. These procedures enabled the calculation of centroid size, partial warps, relative warps, and shape differences for each wing. In this study, particular emphasis was placed on the centroid size of the right wing, which served as a primary metric for comparative analyses among the three species. Centroid size, is an isometric morphological measure calculated as the square root of the sum of squared distances between the centroid of the polygon formed by the selected landmarks and each individual landmark [14].

(Figure 1a)



(Figure 1b)

(Figure 1a) illustrates the coordinates of the 19 numbered landmarks positioned at the termini of the cross and longitudinal veins on the wings of female specimens. (Figure 1b) demonstrates the alignment of the coordinates of these 19 landmarks, which were placed at the intersections of longitudinal and cross veins as well as at the ends of longitudinal veins, across all 45 samples analyzed in the study. In (Figure 1a), the colors green, red, and blue represent the species *C. megacephala*, *S. carnaria*, and *M. domestica*, respectively.

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2-4- Software

Landmarks were placed on the wings based on data extracted from the COO module, a component of the specialized software Collecting Landmarks for Identification and Characterization, available at the international website (http://www.mpl.ird.morphometrics/). This software was utilized to analyze the study data and to identify morphological differences among the examined species.

3_ RESULTS AND DISCUSSION

A landmark-based geometric morphometric approach was employed to distinguish the morphological genetic differences among females of three medically and forensically important insect species: *C. megacephala*, *S. carnaria*, and *M. domestica*. Species identification was considered a critical initial step for the analysis. The study was conducted using morphological methods combined with several statistical analyses, which were categorized into analyses of size and shape variation. The variation in the mean centroid size of the wings among the three species was notably high, as presented in Table (1) The average centroid sizes recorded were 1576.52 for *C. megacephala*, 1734.74 for *S. carnaria**, and 1191.35 for **M. domestica*.

Pairwise comparisons between species revealed the following results:

- Between *C. megacephala* and *S. carnaria*: (F = 1.75, P = 0.31; t = 5.36, P = 1.05; AD = 158.22)

- Between *C. megacephala* and *M. domestica*: (F = 1.43, P = 0.51; t = 12.54, P = 5.22; AD = 385.16)

- Between *S. carnaria and M. domestica*: (F = 1.22, P = 0.71; t = 20.46, P = 0.00; AD = 543.38)

(Table 2 and Table 3) present the analysis of variance (ANOVA) comparing wing size and shape among the three species. Based on the Bonferroni test, the results indicated the presence of statistically significant differences in both wing shape and size at a probability level of $p < 0.01$. Furthermore, landmark-based morphometric analysis was utilized to assess sexual dimorphism among the three species, focusing on both size and shape dimensions. ANOVA was conducted on the symmetry of the right wing size and shape in female specimens, using the ASI module included in the specialized software. The statistical tables' interpretation shown important morphological alterations between the kinds, indicating a high and obvious alteration in the right wing morphology among females of the three kinds. The analysis of wing size for sexual dimorphism, depend on centroid size (CS), yielded an F-value of 16 with a P-value of 1.00. A distinct difference in wing shape was also observed, with an F-value of (0.63 and a P-value of 0.540).

Table 1. Comparison of the variation in centroid size of the right wing among female kinds of medically and forensically significant insects: *C. megacephala*, *S. carnaria, and M. domestica*.

Species	M.Cs.	St.d.	Va.	F	P	T	p	A.d.
1 - *Ch.me.*	1576.52	91.27	8330.29	1 *vs.* 2 1.75	1 *vs.* 2 0.31	1 *vs.* 2 5.36	1 *vs.* 2 1.05	158.22
2 - *Sa.ca.*	1734.74	69.01	4762.68	1 *vs.* 3 1.43	1 *vs.* 3 0.51	1 *vs.* 3 12.54	1 *vs.* 3 5.22	385.16
3 - *Mu.do.*	1191.35	76.26	5815.79	2 *vs.* 3 1.22	2 *vs.* 3 0.71	2 *vs.* 3 20.46	2 *vs.* 3 0.00	543.38

Table 2. Analysis of variance (ANOVA) comparing right wing shape symmetry among female specimens of medically and forensically important insects: *C. megacephala*, *S. carnaria*, and M. *domestica*.

Source	Ss.	Df.	Ms.	f.	*P-value*
Model	0.0002	5	0.000035	0.33	0.8908
Individual	0.0000	2	0.000004	0.04	0.9599
Side	0.0000	1	0.000034	0.32	0.5743
Side*i	0.0001	2	0.000065	0.63	0.5404
Residue	0.0058	36	0.000104		

Table 3. Analysis of variance (ANOVA) comparing right wing size similarity among female specimens of medically and forensically important insects: *C.megacephala*, *S. carnaria*, and M. *domestica*.

Source	Ss.	Df.	Ms.	f.	*P-value*
Model	0.0032	170	0.000019	0.19	1.0000
Individual	0.0017	68	0.000024	0.25	1.0000
Side	0.0005	34	0.000013	0.14	1.0000
Side*i	0.0011	68	0.000016	0.16	1.0000
Residue	0.1184	1224	0.000097		

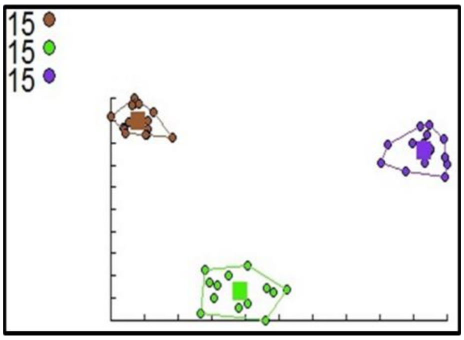

Figure3
Principal components analysis (PCA) of the variation in right wing shape among female specimens of medically and forensically important insects. In the figure, brownish, green, and blue colored points represent wing samples of C. megacephala, S. carnaria, and M. domestica, respectively. Each polygon encloses the distribution of samples for a given species, while each square denotes the mean centroid size of the right wing for each species

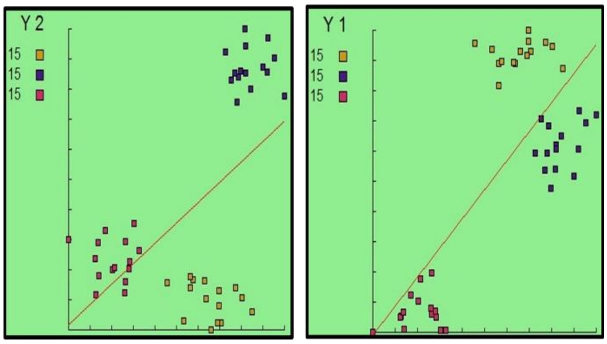

Figure 4.
Linear regression analysis between centroid size and the first principal component of right wing shape based on factor analysis among female specimens of medically and forensically important insects. In the the figure, yellow, blue, and red dots represent wing samples of C. *megacephala*, S. *carnaria*, and M. *domestica*, respectively. The yellow line depicts the regression line illustrating the relationship between centroid size and wing shape variation.

Table 4. Comparison of linear regression between centroid size and the first principal component of right wing shape among female specimens of medically and forensically important insects: C. *megacephala*, S. *carnaria*, and M. *domestica*.

Variables	R	r^2	T	Df
Y1	0.759	0.576	7.63	43
Y2	0.555	0.308	4.37	43

Y = a + bx, r: Correlation coefficient, $r^{2:}$ Determination coefficient

DISCUSSION

Figure 3 presents the results of the Principal Components Analysis (PCA) used to assess the morphometric patterns of right wing shape among female specimens of three medically and forensically important fly species: M. domestica, C. *megacephala*, and S. *carnaria*. The PCA revealed clear distinctions and significant morphological differences among the species, as shown by the distribution of individual samples within the principal components space (PC space). Each point represents a single specimen, and the separation of groups supports the hypothesis of significant quantitative genetic differentiation among the species. Figure 4 further highlights the utility of geometric morphometric analysis as an effective tool for species classification. The linear regression analysis between centroid size and right wing shape, based on factor analysis, demonstrated clear quantitative genetic variation among the females of C. *megacephala*, S. *carnaria*, and M. *domestica*. Results from Table 4 support this finding, showing evident genetic differences in the size–shape relationship across the three species. The first principal trend (Y1) revealed a strong correlation between size and shape (r = 0.759, r^2 = 0.576), while the second trend (Y2) showed a weaker association. These outcomes underscore morphometric differentiation that can be applied to precise classification and evolutionary genetic studies. They also indicate a strong genetic influence of size on wing shape, suggesting that these morphometric traits are reliable tools for genetic discrimination, useful for taxonomic and forensic purposes. The findings of this study align with previous research. One study employed wing venation morphometric analysis to differentiate 15 important species from the Calliphoridae and Sarcophagidae families, achieving a classification accuracy of 97% 【6】. Similarly, another study applied the geometric morphometric (GM) approach to distinguish between Haematobosca sanguinolenta and H. aberrans in Thailand. Using 19 landmarks and PCA alongside discriminant analysis, the research achieved an overall identification accuracy of 99.3%, demonstrating the effectiveness of GM techniques in resolving closely related species 【15】,The results of this study are also consistent with a study conducted in Thailand that aimed to evaluate the effectiveness of two different methods for identifying two species of malaria-transmitting mosquitoes, Anopheles dirus and Anopheles baimaii. The first method was genetic barcoding: the COI gene sequence was used to analyze genetic differences between the two species and distinguish each one. The results showed significant overlap in genetic values within and between species, meaning there was no clear "barcode gap," and therefore this method was ineffective in distinguishing between the two species. The second method was geometric morphometric analysis: wing shape was analyzed using 18 reference points to identify morphological differences between the two species. The results showed clear discrimination between the two species, with 92.42% of samples correctly classified using this method. This reflects that geometric morphometric analysis of wing shape and size is an effective and rapid tool for classifying sister species of malaria-transmitting mosquitoes 【16】 Sontigun conducted another study consistent with my findings, investigating the use of geometric measurements of wing shape and size for primary and secondary identification of S. carnaria. Landmark-based wing morphometric tests of alteration (AMO) had executed on 524 skin fly kinds from 12 species from Thailand. The right wing of every kinds had eliminated or taken away, places or fixed on a microscope slide, and photographed and digitized via 18 landmarks. Wing shape and size variation between genera and species was analyzed using standard AMO, whereas wing shape and size difference between genera of every kind had analyzed by discriminant function analysis. Cross-validation testing had utilize to evaluate classification dependability. The outcomes of the current study show that wing shape can be utilize to separate genera and kinds and to differentiate genera of the same kind with a high degree of reliability. Consequently, landmark-based AMO is an effective and useful additional way for differentiating S. carnaria species and genera 【17】.

CONCLUSION;

The Results Of The Study Indicate The Effectiveness Of Geometric Morphometric Analysis In Distinguishing Between Fly Species Of Medical And Forensic Importance Based On The Wing Veining System, Indicating The Presence Of Clear Significant Differences In The Studied Morphological Features. This Supports The Possibility Of Using This Modern Technique As An Accurate Classification Tool That Contributes To Medical And Forensic Studies Related To Insects, As Accurately Identifying The Insect Species Helps In Controlling Disease Vectors And Estimating The Time Of Death.

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