A Geometric Morphometric Approach to the Quantitative Analysis of Worker Bee Legs in Diyala Governorate

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Abstract: Geometric morphometric measurement was processed for the adult's worker bees including social bees Apis mellifera and Apis florea and the solitary bee Lasioglossum aegyptiellum, it plays a crucial role in ecosystem balance through Symbiotic association, there are Symbiotic relationship between plant and bee. this collection of specimens were taken from different areas in Diyala Governorate bees, The aim of this study was to detect the morphological characteristics variance in the legs of the bees and identify the shape and size variance and extent convergences among species A. mellifera, A. florea and L.aegyptiellum. specimens were collected with nets from the flowers of plants that exist in the gardens and the farms,in this study the hind right legs were selected to be the part of the Bee's body in geometric morphometric approach .8 landmarks were identified in this study and placed on constant and apparent compositional points a joints on insect's leg at the loops contacting areas and the ends of different fragments a quantitative morphological variation is critical as result of impact of genetic factors polygenic traits. the analysis of PCA, Procrustes superimposition and CS were scaled. the results of this study showed that mean of centroid size of the hind right leg were1130.01,822.78 and 526.19 for the species A. florea, A. mellifera and L. aegyptiellum respectively.When comparing A. florea with A.mellifera (f =4.73, P=0.0 and T=9.26, P =5.03 and AD=307.24) and comparing A.florea with L.aegyptiellum (f=3.98 ,P=0.01and T=17.9, P=0.0 and AD=603.83) and comparing A.mellifera with L.aegyptiellum (f=1.18, P=0.75 and T=14.47, P=1.57and AD=296.59).this study demonstrate that Worker Bee Legs are different and it might to be a Supplementary tool to analysis genetic variance.

1 INTRODUCTION

The bee group can be recognized among other insects like wasps and ants by the hair that approximately covers the whole body and occasionally be dense and thin, and the record numbers of bees can reach about 20000 species within order (Hymenoptera) [1]. The native range of the honeybee (Apis mellifera) includes Europe, Africa, and southwestern Asia [2]. They are excellent pollinators of various economic crops and forest plants, and they have wide foraging range [3]. Honeybees are crucial pollinator that play a vital role in maintain ecological balance, pollination by bees improve the production and quality of crops [4], [5]. The bee Apis florea is important species used for crop and other plants pollination worldwide [6]. The honeybee (Apis mellifera Linnaeus 1758) exhibits significant variability in its natural range, including at least 33 different subspecies (geographic races) 1-4. It is native to Europe, Africa, and southwestern Asia [7], [8] The essential economic importance of bee focused on honey production, wax and royal jelly as well as the role what play to pollinate for flowering plants therefor making a incredible model of Symbiosis is called benefit exchange [9]. Lasioglossum aegyptellum is one of the most species distributing within family Halictidae that include more than 1800 species and there totally different in shapes and seize [10]. Pollination a bedrock ecological process supporting supreme blossoming plant communities, is essential to human existence, with over 500 cultivated plant species depend on insect pollinators. Solitary bees lasioglossum aegyptellum are crucial suppliers to this service, necessitating specialized foraging, nesting, and habitat resources [11]. Pollinators are required for producing 15–30% of the human foodstuff source, and peasants rely on managed honeybees through the world to provide these services [12], [13]. Morphological analyses were made with both traditional morphometric and geometric morphometric techniques [14].

Geometric morphometric is a method that offers a mathematical description of biological forms based on geometric definitions of their size and shape, enabling the discrimination between species [15]. Morphological measurement is one of the most basic examines in taxonomy. Descriptive analysis of size and shape variation is a essential technique for organismal biology studies and has enhanced significantly in the last few years [16]. The identification of honeybee subspecies using geometric morphometric has confirmed to be a valuable and more influential technique than classical morphometry [17]. Morphometric is defined as the measurement of biological shape, shape variation, and covariation of shape correlated with biotic or abiotic factors [18]. The identification of bee using Geometric Morphometric has proven to be advantageous and most effective method [17]. Leg length is an affective mechanical properties, while wings are the most commonly measured, adding legs to the research strengthen the results. Analysis combining it provides a complete morphometric picture.

2 MATERIALS AND METHODS

2.1 Sampling

The sample of study A. mellifera, A. florea and L. aegyptellum was collected randomly from different area of diyala Governorate during the winter from 1\11\2025 to 21\12\2025 Through its availability on the flower of basial and chrysanthemumat at temperature 22º in farmers and gardens and some of them are taken from their hives in the trees by using a hand made net hunting, the specimen of the study were fixed using clips and stored in plastic tubes after they killed by freezing [19] ,and then were transported to Natural History Museum at university of Baghdad, in order to recognize and identify their specific species using taxonomic keys and morphological characteristics.

2.2 Specimen Preparation

Three species of bee worker were used in this study: A.mellifera, A. florea and l.aegyptiellum from different areas of Diyala Governorate and the number of complete specimen was 45 specimen, 15 specimen for each species, with the purpose of conducting geometric morphometric (GM) measurement using landmarks that right hind legs were removed using a dissection scalpel and forceps with great care so as not to harm the removed part of the insect's body and the specimen were taken and placed on a slide is placed Others were attached to it with adhesive tape to secure the specimen and prevent it from slipping or moving. [20].

The specimen was numbered and the name of the sample and information were fixed on it at the end of the slide. 15 right hand, digital microscope was used for scanning the slides by connecting it with a computer and getting a image for the legs of the bees.

2.3 Morphometric Measurement

The Morphometric analysis Was used in this study and this method based on landmarks through determining symmetric points placed on the leg, data were collecting from every image by using program Collecting Landmarks for Identification and Characterization which analysis the geometric Morphometric, the total number of the landmarks in this study are 8 (Fig. 1a, b) by the unit Collection of Co Ordinates(COO), and this includes comparing shape and size, after that send to the unit Tabla Espacios Tabulacions (TET)in the program and this is special to allegation for the data of models with each other for the comparing among the three species of bees to know the variance and symmetry, and then send to the unit Morphometria Geometrica (MOG) which responsible for translation ,scaling and Rotation, when these three procedures are complete, the Centroid size was obtained for every leg ,and the Partial Warp ,Relative Warp and variance, in this study were comparing among the three species according to Centroid size to the right hind leg, the Centroid size it's a equivalent value counts from square root of Spaces square between the center of the polygon and every landmark what is placed on any part of the body [21].

2.4 Statistical Analysis

Principal components analysis (PCA) was applied to reduce the number of variables and transforming to original uncorrelated main components. The components which represent highest proportion value (Eigenvalues-1) and explain the biggest proportion from total variance.the graphical representation were characterization to illuminate scattering of specimen and the convergence and variation.and for identify the factors what explaining the correlations among variables, Factor analysis were applied by appropriate extraction with Axes rotation proceeding to enhance the interpretability factors. for distinguishing among species and classifying specimens were utilized. in addition to valuing classification accuracy and to test significant difference among group means Analysis of Variance (ANOVA) was used to compare the values between measured specimen, the analysis (one-way-ANOVA) was applied when a single factor was measured, as result to approve the significant difference among means to determine at level of ($p < 0.05$), this analysis helped to identify how the different factors to evaluate the effect on measured characters and support the results obtained from multivariable analysis.

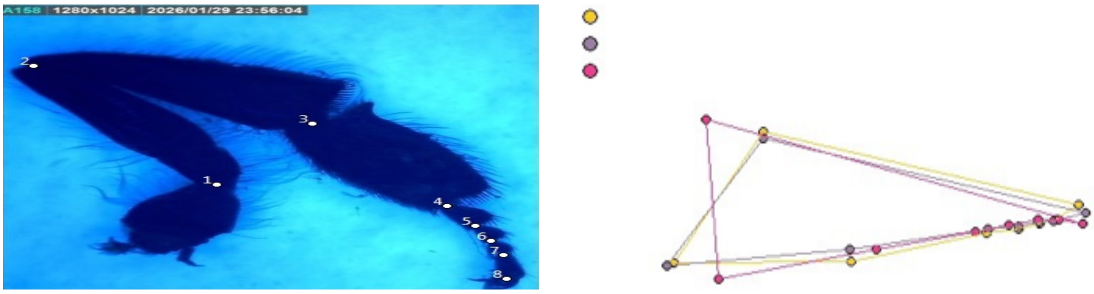
Linear regression analysis was conducted to examine the distribution of specimen along the line and to study the Allometric relationship, comprehend the association between the size and the shape of the leg besides can predicate the variable value based on another. Additionally, the canonical variables analysis (CVA) was applied to capitalize on separation among groups to improve between-group variance relative to within-group variance.

3 RESULTS AND DISCUSSION

The results of the application Geometric Morphometric approach in analyzing the shape and size variation using landmarks-based analysis and were eight accurately located on the on right hind legs among social bees A. florea, A. mellifera and solitary bee L. aegyptiellum, which gives an accurate Quantitative characterization independent of orientation, location and size, this method contributes to reveal the accurate variance which difficult to observe by traditional methods, the Statistical analysis results of Centroid Size (Table 1) were 1130.01, 822.78And 526.19 for the species A. florea, A. mellifera and L. aegyptiellum respectively. When comparing A. Florea with A. mellifera ($f = 4.73$, $P = 0.0$ and $T = 9.26$, $P = 5.03$ and $AD = 307.24$) and comparing A. florea with L. aegyptiellum ($f = 3.98$, $P = 0.01$and $T = 17.9$, $P = 0.0$ and $AD = 603.83$) and comparing A. mellifera with L. aegyptiellum ($f = 1.18$, $P = 0.75$ and $T = 14.47$, $P = 1.57$and $AD = 296.59$). leg shape in all species appear a significant difference was ($f = 0.11$, $p = 1.00$). and the leg size appear a significant difference was ($f = 0.0$, $p = 0.99$). Discriminate Analysis and linear regression analysis (Fig. 2) has showed distinctly and considerable when comparing social bees A. Florea, A. mellifera and solitary bees L. aegyptiellum it illustrates significant diverge with the species L. aegyptiellum.

The study of the distribution and variety the species of Bees provides strategically importance Going beyond mere taxonomic limitation, it can be fundamental basement to comprehend stabilizing the ecosystems and food security. Knowledge and interests with morphological characterizations is crucial to understand the accurate Taxonomic Discrimination and Structure-Function Relationship using Geometric Morphometric (Fig. 3) to distinguish the species of social bees A. Florea, A. Mellifera and solitary bee L. Aegyptiellum because of the high ability to analysis the shape (Table 2) and the size (Table 3) of the leg based on landmarks which giving a high resolution.to separation between groups are convergent genetically. Choosing the hind leg of the worker bee is non-random because it high Specialization functional importance and to gather pollen, additionally it is a tool for Taxonomical Discrimination.

The results of Statistical analysis demonstrate when using Quantitative measurement is approve that the leg centroid size ratio (MCS) (Fig. 4) was apparent variance. The result of this is compatible with the previous studies conducted by Geometric Morphometric technique based on landmarks to analysis the subspecies of Ethiopian Honeybee Apis mellifera choosing the right hind leg, the study illustrate the leg is potential part of the bee's body can used it in like these studies [22] the characteristics of the of the worker bee have a high heritability, cannot effect of temporary environments factors which make it a significant tool to reveal about the genetic origins [23], leg size and shape can used as taxonomical tool to distinguish among bee species and subspecies within a groups and between groups [24] the study about hind leg particularly In worker bee is the most specialist and complicate part morphologically, the results of this study according to Mahalanobis distances and linear regression analysis demonstrate the big variation between the species of social bees A.florea, A.mellifera and solitary bees L.aegyptiellum and that refers to the habit and the behavior of these bees additionally the sources of food and environmental factors can effect on difference in size and shape [22]. A previous study has conducted about studying the Geometric Morphometric of the leg of the stingless bees Meliponula Bocande and Meliplebeia Lendliana and the method was effective and appear a high ability to discriminate between the species [23]. This study reveals the Geometric Morphometric analysis based on landmarks has the high potency and efficiency to distinguish between A.florea, A.mellifera and L.aegyptiellum and it can convert the quantitative characterization for dimension (length and width) to complete Statistical analysis to the configuration of the measured part of the insect body.



(a) (b)

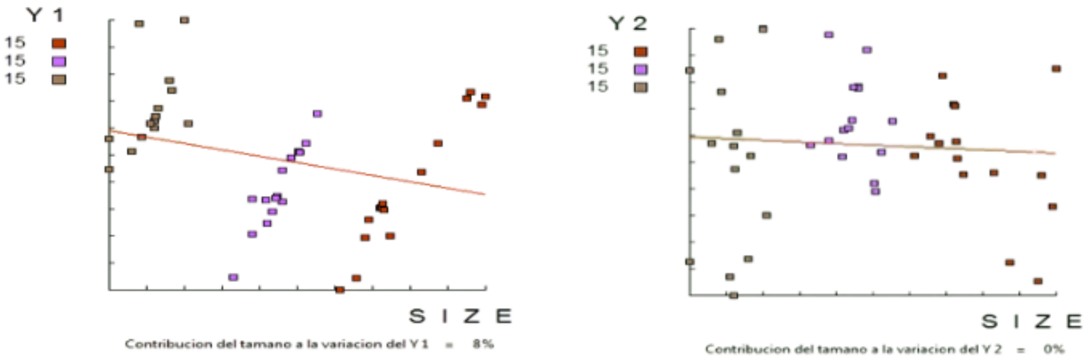
Figure 1: Landmark configuration and distribution of geometric morphometric data in bee species. a) Location of eight landmarks positioned at the segment intersections of the right hind leg of worker bees. b) Distribution of landmark configurations among *Apis florea*, *Apis mellifera*, and *Lasioglossum aegyptiellum* based on geometric morphometric analysis..

Table 1: Statistical analyses of mean of right hind legs Centroid Size variance among A. florea, A. mellifera and L. aegyptiellum (MCs= Mean Centroid size, A.d= Absolute differences, St.D= Standard Davison, Va= Variance, F= F- significant value, P= Probity, T= T - significant value).

Species	M.Cs.	A.d	St.d	Va.	f	p	t	p
1 - A. florea	1130.01	307.24	116.68	13614.18	1 vs. 2 4.73	1 vs. 2 0.0	1 vs. 2 9.26	1 vs. 2 5.03
2 - A. mellifera	822.78	603.83	116.68	13614.18	1 vs. 3 3.98	1 vs. 3 0.01	1 vs. 3 17.9	1 vs. 3 0.0
3 L. aegyptiellum	526.19	296.59	53.64	2877.43	2 vs. 3 1.18	2 vs. 3 0.75	2 vs. 3 14.47	2 vs. 3 1.57

Table 2: Statistical analysis of right hind leg shape among species A.florea, A.mellifera and L.aegyptiellum using Model II oneway ANOVA (Arnqvist and Märtensson).

Source	Ss.	df.	Ms.	f.	P-value
Model	0.0303	60	0.000506	0.24	1.0000
Individual	0.0183	24	0.000761	0. 36	0.9981
Side	0.0063	12	0.000528	0.25	0.9985
Side*i	0.0057	24	0.000239	0.11	1.0000
Residue	0.9191	432	0.002128		



(a) (b)

Figure 2: Linear regression analysis of Y1 (Factor I) and Y2 (Factor II) showing the relationship between centroid size and the first principal component of right hind leg shape in worker bees of *A. florea*, *A. mellifera*, and *L. aegyptiellum*. Different colors represent bee species (red, yellow, and brown, respectively), while the yellow line indicates the linear regression model. The vertical axis represents the principal component score, and the horizontal axis represents centroid size.

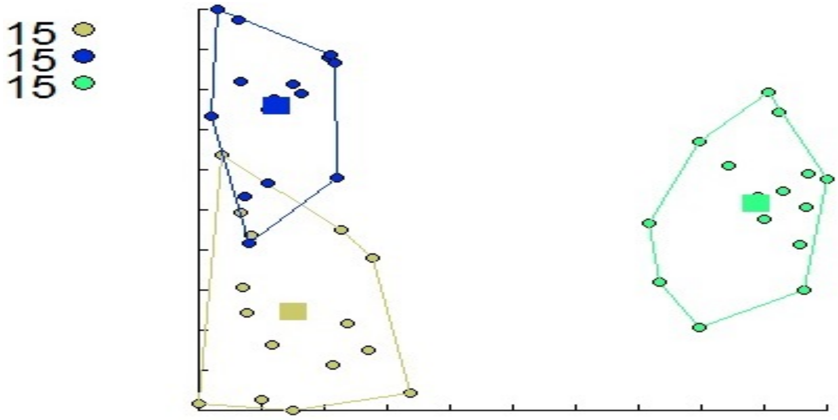
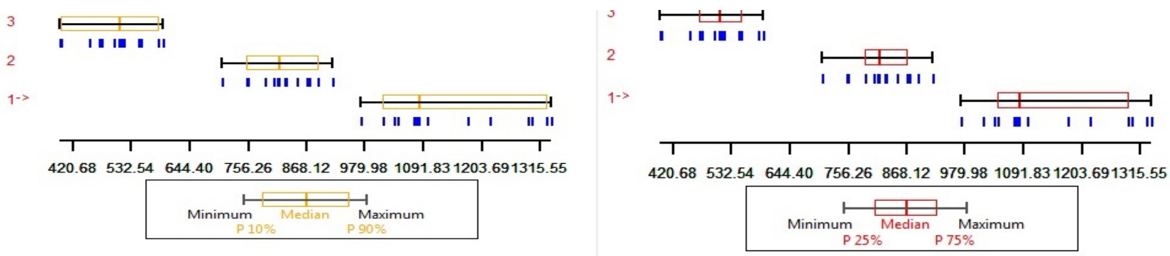


Figure 3: Discriminant analysis showing the shape variation of the right hind legs of worker bees of *A. florea*, *A. mellifera*, and *L. aegyptiellum*. Species are represented by different colors (yellow, blue, and green, respectively). The plot displays two canonical axes: the vertical axis represents CV1 and the horizontal axis represents CV2.



(a) (b)

Figure 4: Box plot analysis of centroid size variation in the right hind legs of adult worker bees of *A. florea*, *A. mellifera*, and *L. aegyptiellum*. a) Distribution of centroid size values among the three bee species, represented by numbers 1, 2, and 3, respectively. The box plots illustrate the median, minimum and maximum values, and interquartile ranges. b) The interquartile range (25–75%) is represented by the boxes, while the blue scattered lines indicate individual specimen measurements.

Table 3: Statistical analysis of right hind leg size among species A. Florea, A. Mellifera and L. Aegyptiellum using Model II oneway ANOVA (Arnqvist and Märtensson).

Source	Ss.	df	Ms.	f.	P-value
Model	0.0061	5	0.001221	0. 39	0. 8508
Individual	0.0041	2	0.002051	0. 66	0. 5235
Side	0.0020	1	0.001999	0.640	0.4281
Side*i	0.0000	2	0.000002	0.00	0.9992
Residue	0.1120	36	0.003112		

Morphometric analysis of honeybees considered the gold standard for this purpose [23]. Geometric morphometric shifted the way morphological studies are performed, using analyses based on landmarks and outlines [25]. It can be useful as a tool for addressing biological questions [26]. According to the results of this study Geometric Morphometric proved that can be used as a taxonomical tool to identify and distinguish the species of social bees A.florea, A.mellifera and solitary bee L.aegyptiellum and its an inexpensive, rapid and Time-efficient tool.

4 CONCLUSIONS

This study highlights the efficiency of legs geometric morphometric it's not only an experience and rapid alternative DNA analysis it’s a high accuracy discriminate taxonomical tool within bee species in diyala Governorate the results approved significant statistical difference in shape and size hind right legs of workers bees, A.florea was dominates in centroid size on both A.mellifera and L.aegyptiellum ,the study reveal the morphological characteristics for legs are strong key genetic ,that make it a reliable source in Biodiversity doesn’t affect by temporary environment factors Geometric Morphometric analysis has fundamental role to distinguish between species and genus, this approach can be used in wide field studies to classify bee species to support ecosystems and food security stability due to its accuracy to separation the convergent genetically groups.

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