

**Molecular and Quantitative genetic Variation of Shape and Size of Wings for Populations of *Ch. Megacephala* by Using Outline-Based Geometric Morphometric Technique**

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**Abstract:**

There are several different methods of classifying Insect. One of these methods that were used Geometric Morphometric of wings technique to study the impact of Imipramine tab. on variation the shape and size of the wings of populations of *Ch. Megacephala* ,The results showed that the average Centroid size of the left wing of populations that treated were 1077.3 and 1055.8 uM for the population were un treated and did not show any significant differences in the average of the centriod size for the left front wing for two colonies of populations of *Ch. Megacephalan* . When reclassification percent using discriminate analysis, the results showed that all specimens of populations are completely identical that mean the populations represent the same species. Furthermore, results of the statistical analysis by using ANOVA test showed that there were no significant differences in the average of the centriod size for the left wing for two colonies of population of *Ch. Megacephala*

**Keywords:** Diptera, Calliphoridae, *Ch. Megacephala* and Imipramine tab.

**Introduction:**

The larvae of the majority of metal fly species feed on carrion or on the decaying tissue of the host , these species are attracted to carrion and can be reared on such substances in different regions of the world , and the success of their breeding on such carrion is influenced by several factors and the most important are temperature , relative humidity and rainfall( 1 ) .

Both researchers ( 2 ;3 ) pointed out that there is agreat similarity between the two species *Ch. megacephala* (Fab.) ; *Ch.bezziana* (Vill.) and the ease of overlap in diagnosis between them , the larg –headed mayasis fly *Ch. megacephala* (Fab.) is spreading in many countries of the world as well as spreading locally, is one of the Facultative parasite it is possible to rearing their larvae on carrion and the



tissues of the host in very large quantities, as they are widespread in the environment in which humans live, especially in poor regions as a result of poor health conditions and the abundance of decaying substances, the mayasis fly was widespread in Latin America, and this type arrived in the United States of America (4;5).

This type of fly has recently entered the new world after the increase in livestock trade between Brazil and Europe. These cases and animal bites are not transported to veterinary clinics where sheep account for the largest proportion in terms of the number of injuries recorded in veterinary clinics compared to other animals ((6)

Geometric measures have been used by diagnosing the shape and composition of the wing Geometric Morphometric, known for the science that studies the metric measurement of the composition or shape, the quantitative analysis of the form and the implementation of this technique of analysis in general on all living organisms and benefits in the analysis of the fossil record of it and the knowledge of the differences between environmental factors and form and that the main benefit of this technique is that it uses all the information available about the events of the features and thus determine the changes of shape (7). The aim of the study is to study the effect of the pills Imipramine affecting nervous in differences in the shape and size of the wing of insects *Ch. megacephala* using the geometric scale outline –Based Geometric Morphometric, as well as to compare the individuals of the same population group.

**Material and methods :**

**Sample collection :**

A calf lung was used for the purpose of attached Blue fly after being placed in a black bag inside a plastic container placed on a wooden table inside an iron cage, after laying eggs by adults that were lifted using a flat metal playground and sterilized on a piece of calf liver inside a plastic container in the laboratory the resulting larvae were taken and followed up and raised and divided into two groups:

one group consisting of twenty gm of good carp and blended calf liver meat .2ug emprammeen tab .

two group : The larvae were placed on 20 gm of non- drug- treated veal liver after the larvae were raised and the adults of *Ch megacephala* were completed growth

The method was followed in the preparation of glass slides of the wings (8), 32 insects 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 :**

After photographing the wings of samples , the data of each sampels were collected separately by using CLIC program (collecting landmark for and characterization) specialized for morphometric analysis that was described by ( 9 ) . The program is available and feely downloaded from the website [http://www.mpl.ird.fr / Morphometrics](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**

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

The distance between each pair of landmarks were 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 comparison 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 competition :

The shape ( 1-2 ) shows the left wing of a fly *Ch. megacephala* , explaining the coordinates of the Landmark and by using the geometric scale system of the wing classification insects and finding variations between the treated and non-treated communities depending on the size and the shape of the wings compared to insects *Ch. Megacephala* by principle component analysis as well as comparing the sample pf the population by Disseminate analysis based on the central size of each wing . the shape ( 3 ) showing the rate of Landmark of 32 point for 16wngs , the green color represents the rate of comparative samples and yellow color represents the rate of parameter coordinates for 16 wings and when conducting the process of matching the wings to use the geometric scale system of the shape or structure of the wing by unit ( MOG ) and as shown in the figure that the wings of insect ( 4 ) of the two shapes are almost identical in most landmark.

( 10 )The findings are consistent with the findings of those who studied the changes in the shape of the wing in an insect , noting the mismatch of some of the landmark placed on insect wing in Thailand due to different environmental conditions. The shape of the number ( 4 ) shows the method of distribution of insects *Ch.megacephala* and it is clear that the individualityis close to each other for all transactions and that the reason for matching the shapeor size of the wing between individuals is due to the similarity of the environmental conditions from which these individuals came from , but the two forms ( 5-6 ) represent the change of the central size . For the left wing of insect samples *Ch.megacephala* and according to the treatment , each box represent the middle group distributed between spring( 25 and 75 )and spring ( 10 and 90 ) and as it isclear that there is as light change in the central of the left wing between insect samples *Ch.megacephala*. as is clear , individuals are close to each other for all treatments and the reason for the matching shape or size of the wing between individualsis due to the young environmental conditionsfrom which these individualsis came from , but the two forms ( 5-6 ) represent the change of the central size of the left wing of insect samples and according to the treatment , each box represents the intermediate group distributed between spring ( 25-75 ) and spring ( 10-90 ) and is clearly the presence of asimplified central wing of the central wing of the insect samples *Ch.megacephala* . According to their nutrition , the central size of the left wing 1077.3 and 1055.8 micon for comparison and treatments respectively , and the comparison of the communities using using test ( T,F ) were their value( 2.14;1.74) and the value P 0.15, and the value absolute difference were equal to 21.51where there is no moral difference between the treatments

In comparing the central size of the left wing table ( 1 ) this indicates that it belongs to one type of geographical boundary and this study agrees with ( 11 ) where they used the Geometric morphometric of the shape and size of the wing to study the variations between the communities of honey bee which collected from different regions of Italy,carinolan and Africa there was difference in the shape and size of the wing and the rate of size of the wing between the population. the separation between the population and the reason for this difference to the environmental conditions in th difference, which is the temperature , relative humidity and the amount of rain . The Figure ( 8 ) showed the distinction that the central size of left wing of the comparative samples

1077.3. and the central size of the left wing of the treatment 1055.8 . the variations in the shape and size of the wing in the treatment are lower than the variations in the control samples . and that they don't go back to one type with a simple change for some of the population. this is clear from the matching of the landmarks of the treatment and also from the rate of central size of wing where there is no moral difference among the members of these sample when using the unit ASI .where two table 2;3 showed the results of the analysis of the contrast to the symmetry of the shape and size of the left wing and the and the absence of amoral difference between the population of the insect sample *Ch. megacephala* and the two colonies , and there are several reasons , one of these reasons physiological and is considered the main method which led to differences during the different stages of the growth, mutations and toxins also have an effect on the external form of the insect and found there did not individually , they appear in shape , but were found during the stages of growth generated to occurrence of abnormalities in small- age, and also the effect of Genes in the metabolism of the insect in the early stage, and the occurrence of random mutations with in species. From this study , we conclude that there are small , non-moral statistical differences between the two colonies ( treatment and control ) , which is belong to one species .

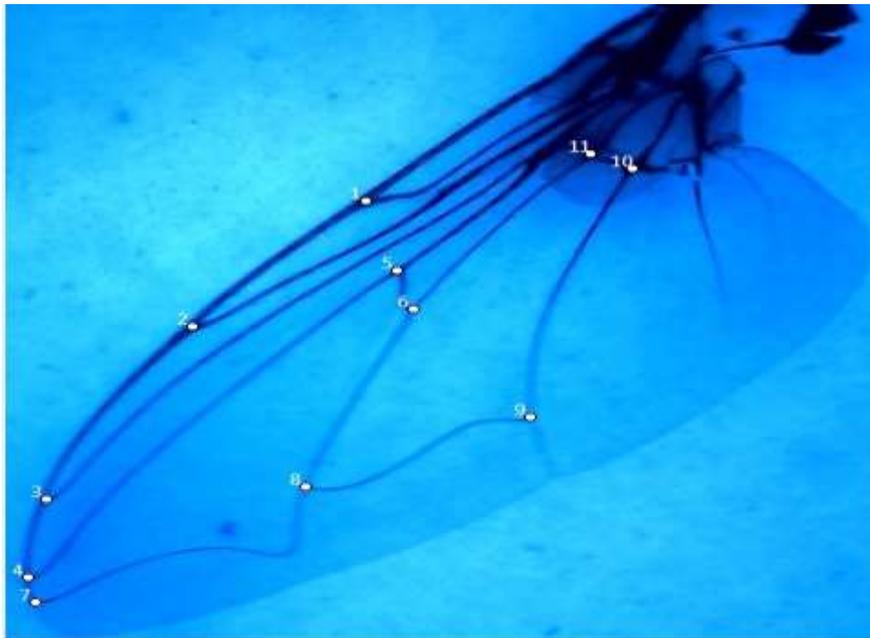


Fig.1. Eleven landmarks on the *C. megacephala* left wing (Control) no treatment used in geometric analysis

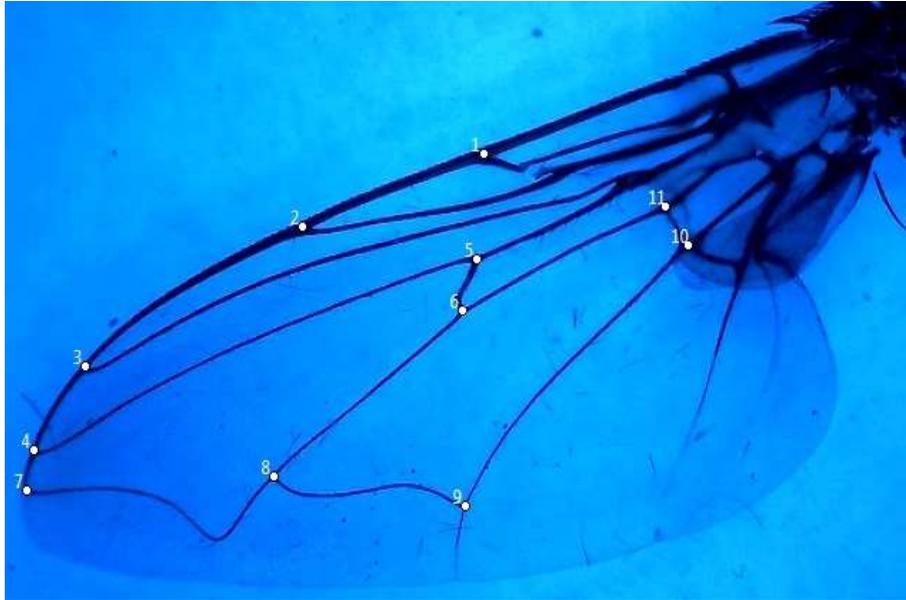
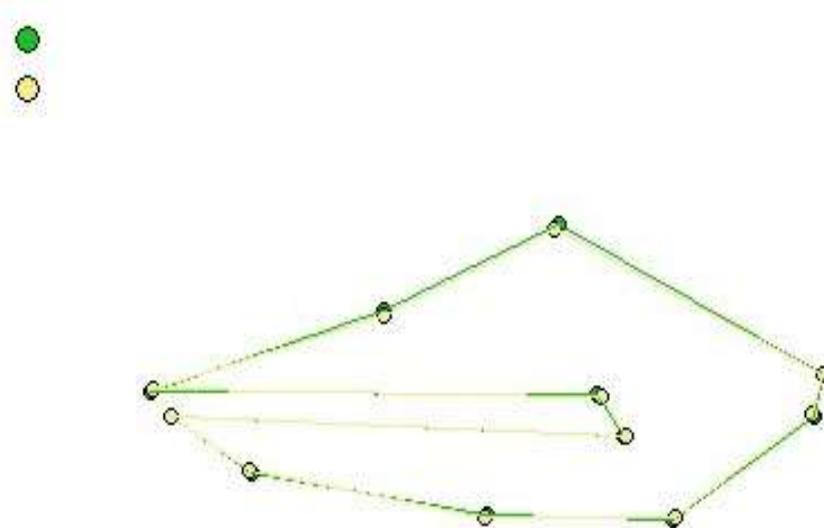
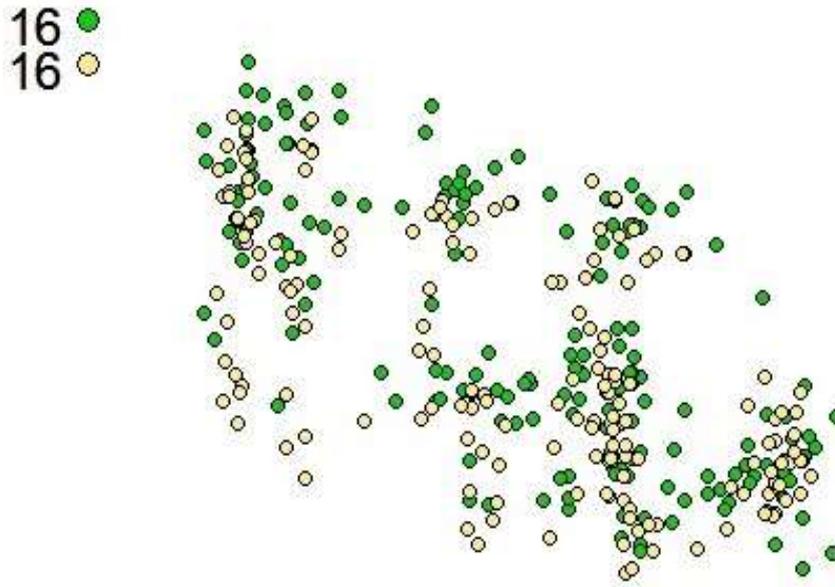


Fig.2. Eleven landmarks on the *C. megacephala* left wing treatment used in geometric analysis



X = of -0.445 to 0.404 , Y = of -0.129 to 0.209

Fig. 3. Mean coordinates of landmark in the left wing of *C. megacephala*, Green color represents Control specimens and Yellow color represents Treatment specimens



X = of -476.761 to 480.239 , Y = of -231.761 to 506.239

Fig. 4. Output of consensus configuration by the GPA Procrustes superimposition method locating the 11 landmarks for each individual left wing of the two colonies. Green color represents Control specimens and Yellow color represents Treatment specimens.

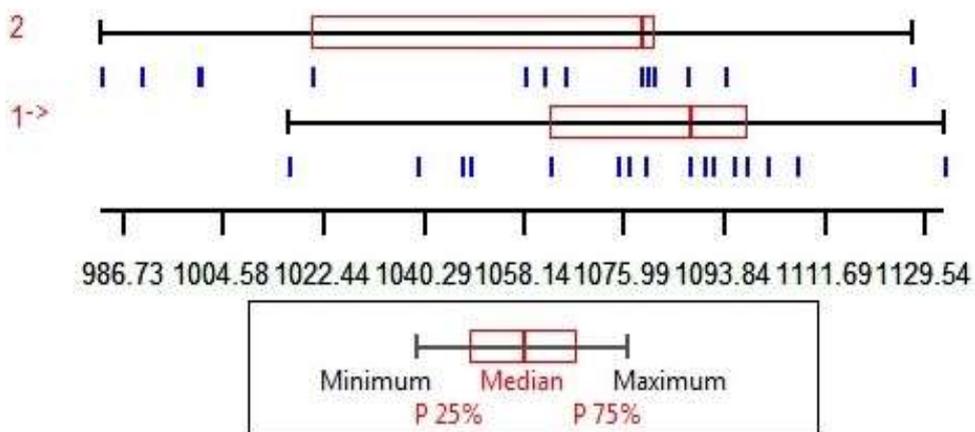


Fig. 5. Variation of the centroid size of left wings for *C. megacephala* according to feeding, Each box shows the group median separating the 25 th and 75 th the quartiles. Vertical bars under the boxes represent the wings numbers 1 and 2 in the Fig. represent Control and Treatment specimens respectively. Units are pixels. P, percentile.

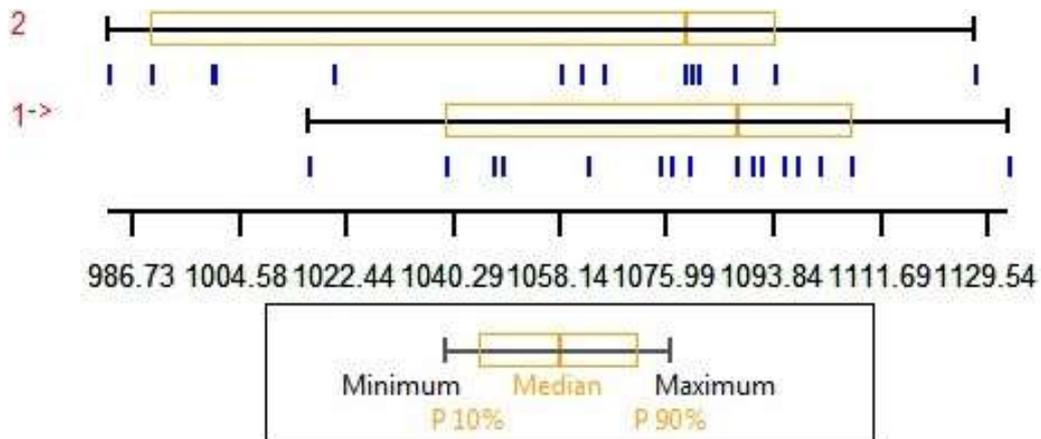
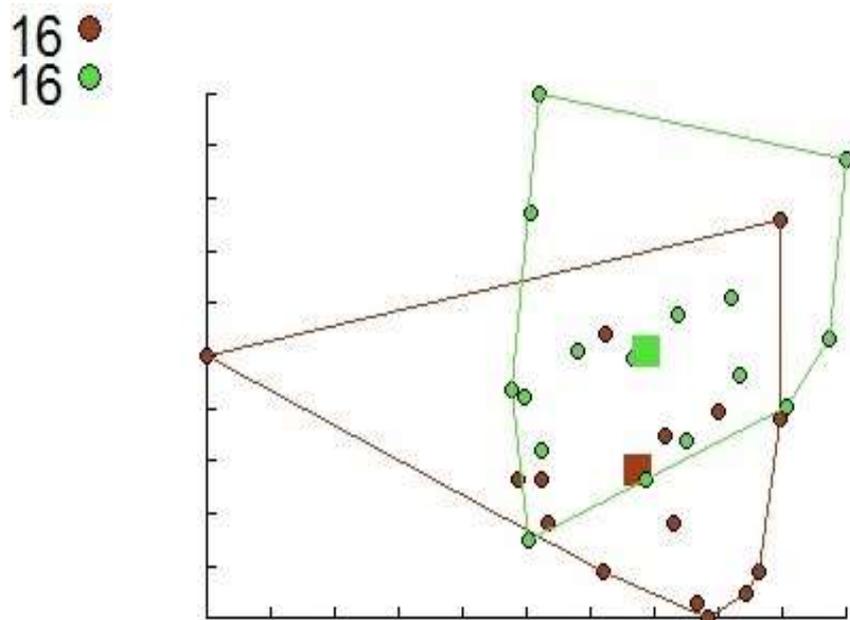


Fig. 6. Variation of the centroid size of left wings for *C. megacephala* according to feeding, Each box shows the group median separating the 10 th and 90 th the quartiles. Vertical bars under the boxes represent the wings numbers 1 and 2 in the Fig. represent Control and Treatment specimens respectively. Units are pixels. P, percentile.



X = of 0.048 to 0.023 , Y = of -0.020 to 0.031

Fig. 7. Scatter plot of the principle component analysis of *C. megacephala* specimens according to feeding based on Geometric Morphometric, Brown spots represent Control specimens and Green spots represents Treatment specimens, Brown square represent mean centroid size of the left wing for Control specimens = 1077.3 and Green square represent mean centroid size of the left wing Treatment specimens = 1055.8

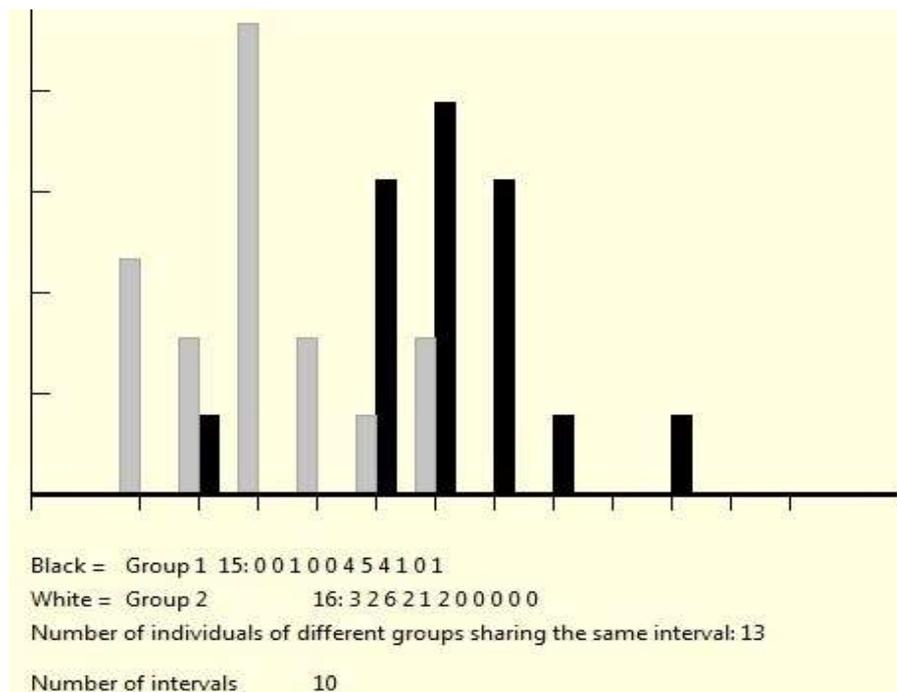


Fig. 8. Discriminate analysis of two groups for *C. megacephala*, Mahalanobis distances between centroids size were as follows: Control specimens to Treatment specimens = 2.61, Black color in this figure represented to Control specimens and White color represented to Treatment specimens. Simple re-classification The classified individuals were part of the discriminant analysis First Correct assignments : Black group 15/16 = 93% , White group 13/16 = 81% \*

Table 1. Compared the centroid size of the left wing for *C. megacephala* between Control and Treatment specimens .

Group	M.CS.	St.D.	Va.	F	P	T	P	A.D.
Control	1077.3	27.92	779.41	2.14	0.15	1.74	0.09	21.51
Treatment	1055.8	40.84	1667.72					

M.CS: Mean centroid size, St.D: Standard Deviation, Va.: Variance, P :Probability, A.D : Absolute differences .

Table 2. Analysis of variance for asymmetry left wing size of *C. megacephala* Control and Treatment specimens.

Source	SS	df	MS	F	Signification
Model	0.0002	3	0.00007	0.61	0.61
Individual	0.0002	1	0.0002	1.63	0.21
Side	0.0000	1	0.0002	0.16	0.69
Side*I	0.0000	1	0.000005	0.05	0.83
Residue	0.0026	24	0.000109		

Table 3. Analysis of variance for asymmetry left wing shape of *C. megacephala* Control and Treatment specimens

Source	SS	df	MS	F	Signification
Model	0.0017	54	0.00003	0.9	0.78
Individual	0.0008	18	0.00004	1.2	0.27
Side	0.0006	18	0.00003	0.9	0.61
Side*I	0.0003	18	0.00002	0.5	0.79
Residue	0.0160	432	0.00004		

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