Describing Sexual Dimorphism in Inner Wings of *Brontispa longissima* Using Landmark Based Geometric Morphometric Analysis

Bryan George D. Belleza and Cesar G. Demayo

Abstract—**Sexual dimorphism is a widespread phenomenon among groups of animals that describe variation in morphology between individuals of different sexes. Differences in wing shape morphology among sexes of the same species of insects often reflects disparity in flight performance and flight range which might be of considerable significance in the monitoring and control of pest species. This study was conducted to determine the differences in wing morphology between sexes of coconut hispid beetle (*Brontispa longissima*) by looking at the variations in the shapes of the entire wing using geometric morphometrics. The results obtained showed noticeable variation in the left and right inner wings between female and male samples as shown in the relative warp analysis. Discriminant function analysis, MANOVA/CVA scores, and Kruskal-Wallis test showed statistically significant variation between sexes establishing the presence of sexual dimorphism within the species of coconut hispid beetles.**

Index Terms—***B. longissima*, geometric morphometrics, sexual dimorphism, wing morphology.**

I. Introduction

Sexual dimorphism, defined as the variation in morphology between individuals of different sexes that belongs to the same species, is a widespread phenomenon among different groups of animals [1]. It has been considered as one of the most interesting sources of phenotypic variation among organisms and has become an increasingly important area of study in evolutionary biology [2]. The direction of the differences is, whether, males or females are larger or they differ in distinctive groups [3].

Insect wings, one of the most important organs of insects, have large contribution to them to become the most prosperous biological community. Wing shape morphology using geometric morphometrics has been extensively studied in the field of entomology to clarify the relationship between closely related taxa and to help in identifying population within and between species of insects. In recent years, more attention has been paid to morphometric approaches, due to new methods of analysis such as geometric morphology [4]–[6]. These methods allow the study of form without the effects of scale, rotation and translation of objects, thus they have a better biological interpretation and are a graphical tool for visualization and quantification of morphological variation in different ecological and evolutionary contexts [7], [8].

Coconut Hispid Beetle (*Brontispa longissima*) is worthy of being investigated for wing shape variation since it is considered to be a serious pest that has greatly affected the coconut industry [9]. It has been suggested that the wing of *B. longissima* might be considered as an ontogenetically modular structure primarily divided into different compartments which is a considerable evidence indicating difference in wing morphology [10]. By quantifying morphological variation, this could contribute to easier identification in the relationship between morphology and ecology which furthermore can serve more informed deduction on species' evolution [11]. Differences in wing morphology between sexes of the same species of insects often reflect disparity in flight performance and flight range which might be of considerable significance in the monitoring and control of pest species [1]. Hence, this study was conducted to describe and determine the presence of sexual dimorphism in different populations of *B. longissima* based on the shape variation of its inner wings.

II. Methodology

A. Sample Collection and Processing

Coconut Hispid Beetle (*Brontispa longissima*) sample were collected from three different provinces in the Philippines: Aloran, Misamis Occidental, Calube, Zamboanga del Norte and San Vicente, Butuan City. Specimens were placed in properly labeled containers filled with 70% ethanol. Sex of samples was identified through visual inspection of the genitalia under a stereo microscope.

The inner wings were detached and were mounted neatly in clean and clear glass slides and were properly labeled. Digital images were captured using Olympus E-410 DSLR Camera attached on a Leica Stereomicroscope.

B. Organizing and Digitalization

Inner wings were grouped according to sex (male and female) and divided separately (left wing and right wing) in different provinces. Triplicate of an image was made to insure consistency. The images of grouped samples were digitized using tpsUtil [12] and saved as thin-plate splines (TPS) files.

C. Landmark Selection

Landmarks together with pseudolandmarks were assigned

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to the prominent features in the inner wings of *B. longissima*. A total of seventeen (17) landmarks were chosen on the left and right inner wings for their capacity to define the major elements of shape and for their reliability as homologous structure (Fig. 1). The chosen landmarks are described in Table I.

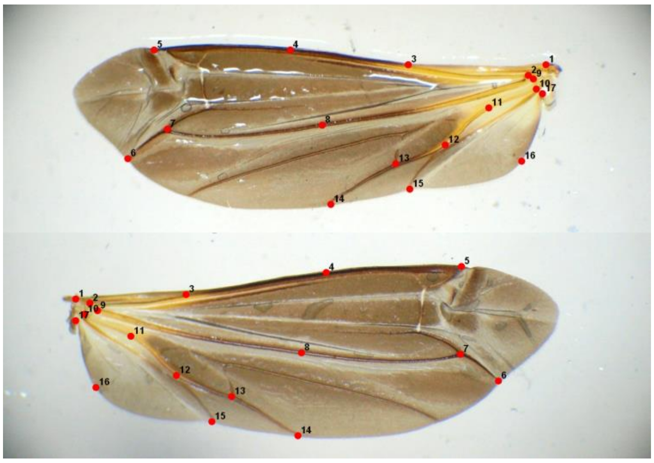

Fig. 1. Digitized image of the left and right wing showing the location of landmark points.

D. Model Testing

Digitalized images were subjected to landmark acquisition using tpsDig version 2.12 [13] to collect the x, y coordinates of each landmark within the wing structure. Thin-plate spline deformation grids were produced to visualize the wing shape differences between male and female coconut hispid beetle. The thin-plate spline technique [4] consisted in fitting an interpolating function to the landmark coordinates of each specimen against the reference configuration so that all homologous landmarks coincide. The projection of the superimposed specimens onto the principal warps produced the partial-warp scores, which described their deviations from the reference configuration and that can be used as variables in subsequent multivariate statistical analyses [14].

E. Statistical Analysis

The relative warps analysis and computation of partial-warp scores were done using tpsRelw program version 1.46 [15] in order to yield information on the variation in local shape, which involved fitting and interpolation function to homologous landmarks for each specimen sample. From the result of the relative warps of the wing shape, histogram and box-and-whiskers plots were generated using the Paleontological Statistics (PAST) software version 2.17 [16]. The most informative warp scores (first and second relative warps) were subjected to Canonical Variance Analysis (CVA) to determine whether the shape of the inner wings differ significantly between the female and male population. Hotelling's pairwise comparisons (post-hoc) test which is a cross validation test using discriminant function analysis was then used to confirm or reject the hypothesis that two populations are morphologically distinct by classifying each specimen into one of the two groups (male or female). Variations between populations were considered statistically significant if the percentage of correctly classified specimen was greater than 75%. The first and second relative warp scores of the pooled population data was subjected to Kruskal-Wallis test in order to compare independent groups of sample data and, in this case, determined the significance of difference (at 0.05 level of significance) in the shape variations of wings [17]. All statistical analyses were performed in PAST software.

TABLE I: DESCRIPTION OF ANATOMICAL LANDMARK POINTS ASSIGNED ON BOTH LEFT AND RIGHT INNER WINGS RESPECTIVELY

Landmark	Anatomical Description
1	Proximal end of the Costa + Subcosta (C+Sc)
2	Proximal end of the Radius
3	Costa-subcosta and median proximal end of Radius connection
4	Median distal end of Radius
5	Distal end of Radius (R)
6	Distal end of Media (M)
7	Median distal end of Media
8	Midpoint of Media
9	Proximal end of the Radius + Media (R+M)
10	Proximal end of Cubitus and 1^{st} Anal vein connection (Cu+A)
11	Proximal end of Cubitus
12	Midpoint of Cubitus
13	Median distal end of the Cubitus
14	Distal end of the Cubitus
15	Distal end of the 1^{st} Anal vein
16	Distal end of the 2^{nd} Anal vein
17	Proximal end of the 2^{nd} Anal vein

III. RESULTS AND DISCUSSION

Following a series of analysis made in the left and right inner wings of coconut hispid beetle (*Brontispa longissima*), it was found out that there was a shape variation between the female and male inner wings, consequently suggesting the presence of sexual dimorphism. The results of relative warp analysis on the left and right inner wings of the pooled population located at three different sampling locations showed at most 5 significant relative warps which defined the wing shape variation in both male and female population (Fig. 2). It can be observed clearly that the left and right wings for male and female population differed distinctively from each other as they yielded different shapes as presented by the negative and positive warps. The result of histogram and box-and-whiskers plot displayed a multimodal variation in the mode of distributions of wing shape and the mean values of left wing were close to the mean value of the right wing of one population described indicating a high degree of intrapopulation variation. In addition, the mean values of one population varied significantly to the mean values of the other population indicating a high degree of interpopulation variation in the beetle. As described in Table II-Table IV, majority of the variations in *B. longissima* wing shape morphology were found in the apical and basal region of the wing. Observable variations were based on the location of landmark points within the wing's structure.

Another tool used in this study is discriminant analysis that supplements the results obtained in the relative warp analysis for the presence of sexual dimorphism in coconut hispid beetle based on its wing shape. The visual result of the analysis is shown in a form of histogram (Fig. 3).

The histogram shows the difference between the shape of female and male's left and right inner wings in each of the population. Based on the graphs, they positively show that sexual dimorphism is an evident in all population of *B. longissima* from three different locations though there having been slight overlaps. The presence of the gap between the two components in right wing samples from San Vicente, Butuan City, exhibits the greatest sexual dimorphism among them all. The farther the components are from each other, the greater the difference is in the shape of the wings.

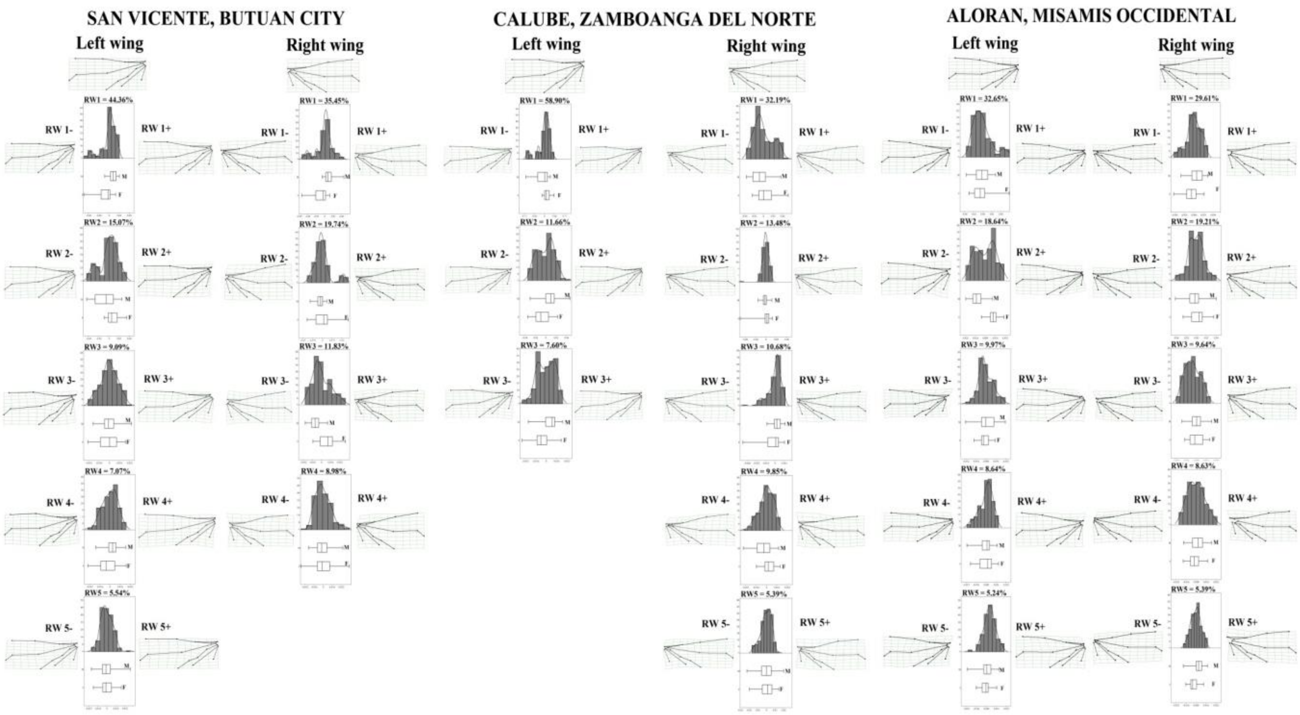

Fig. 2. Summary of the landmark based geometric morphometric analysis showing the consensus morphology (uppermost panels) and the variation in the shape of left and right inner wings between female and male populations of coconut hispid beetle (*B. longissima*) collected from different locations (Aloran, Misamis Occidental, Calube, Zamboanga del Norte and San Vicente, Butuan City) explained by each of the significant relative warps. Legend: F = female; M = male.

TABLE II: VARIATION IN THE LANDMARK'S POSITION WITHIN THE LEFT AND RIGHT INNER WINGS BETWEEN MALE AND FEMALE POPULATION SAMPLED AT ALORAN, MISAMIS OCCIDENTAL AS DEFINED BY EACH OF THE SIGNIFICANT RELATIVE WARPS

LEFT WING		RIGHT WING	
RW1	Variation found in the apical and basal region of the wing. The distance of the landmark at the C+Sc and the median proximal end of the radius connection is farther in females than in males.	RW1	Variation in the apical and basal region of the wing. The distance of the landmark at the beginning of media and the junction between Cu and anal vein is farther in females compared to males.
RW2	Variation in the apical and basal region of the wing. The landmark at the distal end of the radius is farther in females than in males. In males, the landmark at the junction of Cu and 1st anal vein is farther compared to females.	RW2	The distance of the landmark at the distal end of the media is farther in females than in males. In males, the position of the landmark at the beginning of the costa-subcosta is located superior compared to females.
RW3	In males, the distance of the landmark at the median and distal of the radius is farther compared to females.	RW3	Variation in the apical and basal region of the wing. The landmark at the distal end of the anal veins, cubitus and media is farther in males than in females.
RW4	Variation in the apical and basal region of the wing. The distance of landmark at the distal end of radius is farther in females than in males. The position of landmarks in cubitus and anal veins is located farther in females compared to males.	RW4	The landmark at the proximal end of the 2nd anal vein and the juncture of Cu+M is located farther in males than in females.
RW5	The distance of the landmark at the midpoint of media is farther in females than in males. In males, the distance of the proximal and distal end of the 2nd anal vein is farther compared to females.	RW5	The distance of the landmark at the C+Sc and the proximal end of the radius is shorter in females than in males. Landmark located between the proximal and distal end of the cubitus is farther in females compared to males.

In contrast, there were instances wherein sexual dimorphism was probable even if overlapping occurred in the histogram. This can be explained by the values given in the discrimination score table where the percentage of correctly classified items was given (Table V). This percentage shows how correctly female wings were classified as female and how correctly male wings were classified as males. If the discriminant analysis was efficient for a set of data, the classification table of correct and incorrect estimates would yield a high percentage. In the given set of data, the result showed that in left wing for the different populations, 95%, 87.78% and 91.67% were classified correctly in Aloran, Misamis Occidental, Calube, Zamboanga del Norte and San Vicente, Butuan City respectively. On the other hand, the results for the right wing show that 88.89%, 77.78% and 100% were classified correctly for the three locations respectively. It is observable that there is a number of mistakenly classified male and females for Calube, Zamboanga del Norte right wing. But, despite this discrepancy, it is still safe to say that the degree of shape variation in the right primary wing was relatively high since the minimum percentage of correctly classified items should be 75% for it to be considered significant.

TABLE III: Variation in the Landmark's Position within the Left and Right Inner Wings between Male and Female Population Sampled at Calube, Zamboanga Del Norte as Defined by Each of the Significant Relative Warps

LEFT WING		RIGHT WING	
RW1	The distance of the landmark point between the distal end and beginning of media is farther in females than in males.	RW1	The distance of the landmark points located at the cubitus vein is farther in females than in males.
RW2	Variation at the apical and basal region of the wing. The distance of the landmark point at the junction of costa-subcosta and radius is farther in females than in males.	RW2	The distance of the landmark point between the midpoint and proximal end of the cubitus vein is farther in females than in males.
RW3	The distance of the landmark points located at the radius, media, cubitus and anal veins are farther in females compared to males.	RW3	The distance of the landmark point at the proximal end of radius and distal end of the 2nd anal vein is farther in males than in females.
		RW4	Variation at the apical and basal region of the wing. The distance of the landmark point at the median distal end of the radius and distal end of the cubitus is farther in females than in males.
		RW5	The distance of the landmark point located on the junction of C+Sc and radius is farther in females than in males. In males, the distal end of the radius is farther compared to females.

TABLE IV: Variation in the Landmark's Position within the Left and Right Inner Wings between Male and Female Population Sampled at San Vicente, Butuan City as Defined by Each of the Significant Relative Warps

LEFT WING		RIGHT WING	
RW1	Variation at the apical and basal region of the wing. The distance of the landmark point at the median distal end of the radius and distal end of cubitus and anal vein is farther in females than in males.	RW1	The distance of the landmark point at the beginning of 2nd anal vein and distal end of cubitus is farther in female than in males.
RW2	In males, the distance of the landmark point between the midpoint of radius and junction of C+Sc and R is farther in females than in males.	RW2	The landmark point at the midpoint of media is farther in males than in females. Males have slightly wider distance of juncture between radius and cubitus.
RW3	In females, the landmark point at the beginning of media, cubitus and anal veins is located slightly higher (shifted towards the apex of the wing).	RW3	Variation at the apical and basal region of the wing. The distance of the landmark point from the junction of C+Sc and radius to the distal end is farther in females than in males. In males, the juncture of Cu+A is narrower compared to females.
RW4	The distance of the landmark point at the median distal end of the media and cubitus is farther in females than in males.	RW4	The distance of the landmark point at the proximal and distal end of the second anal vein is farther in females than in males. Juncture of C+Sc and beginning of radius is narrower in males compared to females.
RW5	The distance of the landmark point at the juncture of costa-subcosta and radius is farther in males than in females.		

The CVA scatter plot (Fig. 4) shows the distribution of female and male coconut hispid beetle population based on the landmark analysis on its left and right inner wings. Comparing the shapes of left and right wings based from pooled and the most important scores, the generated CVA scatter plot and scores revealed a randomly distributed wing shape distribution suggesting high intrapopulational variation. The scatter plot also presents distinct difference between male and female wing shape among different locations which then display a high possibility of interpopulational variation. It is important to note however, that the result of the non-parametric Kruskal-Wallis test ($p = 1.694^{-46} < 0.05$ and $p = 7.811^{-17} < 0.05$) for pooled left wing and right wing respectively, showed that there were significant differences between the medians of at least two populations. Results of the Bonferroni corrected Mann-Whitney pairwise comparison of the most significant warp scores (first and second) of the wing shape for all populations in different locations are shown in Table VI. Pairwise comparisons showed that for the left wing, the male and female Aloran population ware significantly different from the other population while for the right wing the female San Vicente population was significantly different from all other populations.

It can be seen from the results obtained from landmark based analysis of the inner wings that a variation existed in the shape of left and right wings between female and male sexes. The overall landmarks located within the structure of the wings can both serve as criteria for determining wing sexual dimorphism. A significant wing shape variation has been observed between female and male *Drosophila melanogaster* [18], in *Chilo suppressalis* [19], in *Synneuria* sp. [2] and in *Schirpophaga innotata* [1]. In the last few decades, a number of hypotheses have been proposed to explain sexual dimorphism in insects [20], [21]. One of the most commonly used is the hypothesis indicating the connection between sexual selection versus natural selection and environmental variation [22] although male-male competition and the segregation of sexes due to limited resources [23] have produced notable selective differentiation.

TABLE V: RECLASSIFICATION OF THE COCONUT HISPID BEETLE (*BRONTISPA LONGISSIMA*) INTO FEMALE OR MALE BASED ON THE LANDMARK ANALYSIS OF THE WINGS

LEFT WING		Female	Male	Total
Aloran, Misamis Occidental	Female	88	2	90
	Male	6	84	90
	% Correctly Classified			**95.00%**
Calube, Zamboanga del Norte	Female	79	11	90
	Male	10	80	90
	% Correctly Classified			**87.78%**
SanVicente, Butuan City	Female	75	15	90
	Male	1	89	90
	% Correctly Classified			**91.67%**
RIGHT WING				
Aloran, Misamis Occidental	Female	83	7	90
	Male	13	77	90
	% Correctly Classified			**88.89%**
Calube, Zamboanga del Norte	Female	73	17	90
	Male	23	67	90
	% Correctly Classified			**77.78%**
SanVicente, Butuan City	Female	90	0	90
	Male	0	90	90
	% Correctly Classified			**100%**

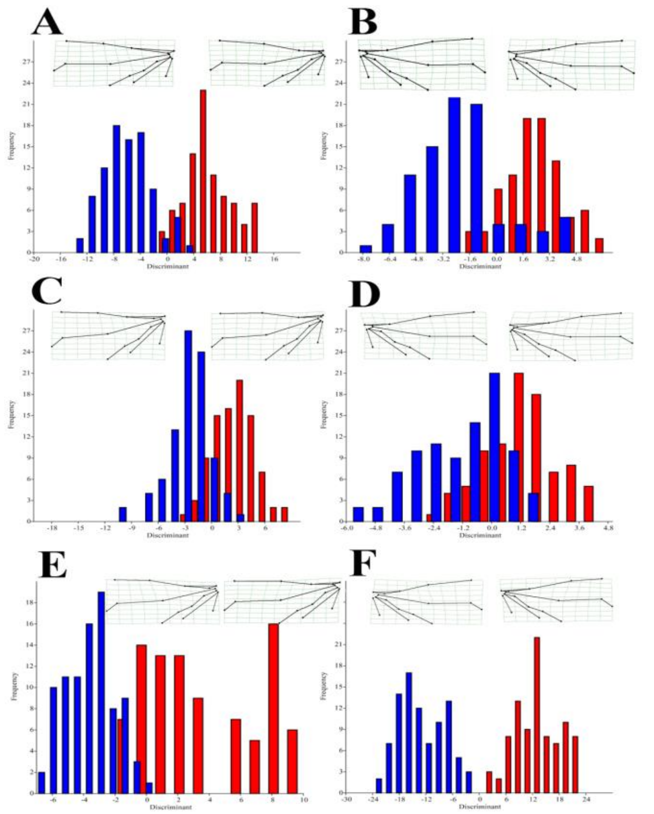

Fig. 3. Histogram showing the variation in the left and right inner wing shape of the male (blue) and female (red) species of *B. longissima* between (A) the left wing (B) the right wing from Aloran, Misamis, (C) the left wing (D) the right wing from Calube, Zamboanga del Norte and (E) the left wing (F) the right wing from San Vicente, Butuan City.

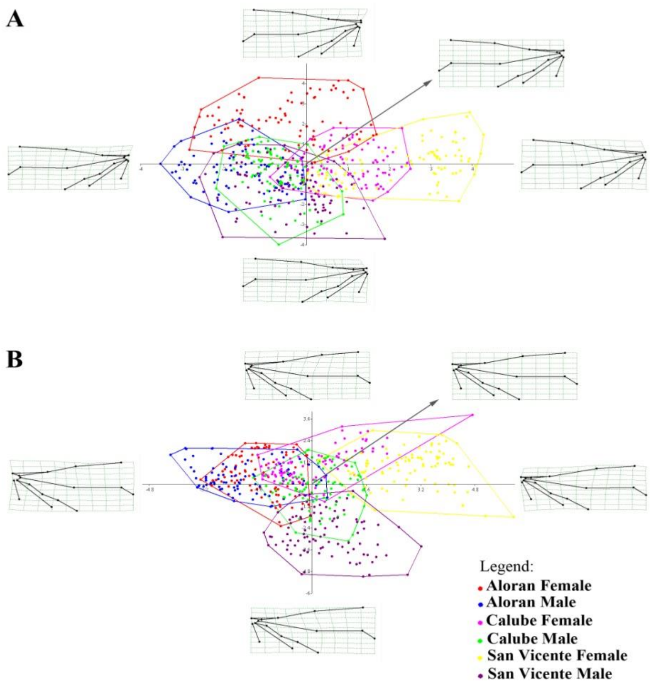

Fig. 4. CVA scatter plot showing the distribution of wing shapes (A) left wing (B) right wing of samples from different provinces of the Philippines based on landmark based geometric morphometric analysis with corresponding shape of each axis and the mean shape indicated by the arrow. Results of MANOVA test in wing shape: Wilk's Lambda= 0.0961, df1= 25, df2= 1970, F= 43.51, and p(same)= 6.553^{-248}.

TABLE VI: RESULT OF KRUSKAL-WALLIS TEST ON WING SHAPE IN DIFFERENT LOCATIONS BASED ON THE FIRST AND SECOND RELATIVE WARP SCORE. MANN-WHITNEY PAIRWISE COMPARISON (BONFERRONI CORRECTED) OF THE WING SHAPE (0.05 LEVEL OF SIGNIFICANCE)

Left Wing						
	A (F)	A (M)	C (F)	C (M)	SV (F)	SV (M)
A (F)	-					
A (M)	**2.57^{-04}**	-				
C (F)	**2.38^{-15}**	**2.09^{-05}**	-			
C (M)	**1.56^{-07}**	**8.63^{-04}**	1	-		
SV (F)	**2.47^{-34}**	**6.88^{-34}**	**1.07^{-11}**	**1.81^{-08}**	-	
SV (M)	**1.40^{-10}**	**1.95^{-05}**	1	1	**3.86^{-13}**	-
Right Wing						
A (F)	-					
A (M)	**7.684^{-03}**	-				
C (F)	1	**2.11^{-05}**	-			
C (M)	1	**1.245^{-05}**	1	-		
SV (F)	**3.21^{-07}**	**3.66^{-14}**	**1.49^{-05}**	**7.65^{-07}**	-	
SV (M)	1	0.09134	0.3147	1	**5.28^{-09}**	-

Individual variation in shape which is strongly dependent on environmental conditions is frequently argued [24]. The environment of the living organisms, with rather few exceptions, is spatially and temporarily diverse resulting to a continuous movement of organisms to colonize empty habitat and to offset the inevitable local extinctions. [19] reported in their study that environmental factors (geographic condition and host type) were considered in asserting that the phenotypes of an individual is the result of the interaction between genotype and environment showing that the most geographically distant population are also the most morphologically varied.

Another suggested factor that possibly explains sexual dimorphism in insects is the variation in the functions performed between sexes [25]. In insects, flight is the most likely considered selective pressure influencing the evolution of sexual shape dimorphism in the wing. Since characteristic flight behaviour in females is to search for host plants for oviposition sites and in males for the nuptial flight, territoriality and search mating opportunities, flight requirements and optimal wing shapes may differ for sexes. Therefore, the selection would act on wing shape to optimize flight characteristics [26].

IV. Conclusion

Analysis of the wing shape morphology of coconut hispid beetle (*Brontispa longissima*) found variation between the female and male inner wings, consequently suggesting the presence of sexual dimorphism. This study has demonstrated the effectiveness of geometric morphometric in describing morphological variation within and between populations of organisms.

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