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Describing the Wing Shape of three local populations of *BrontispaLongissima* Using Elliptic Fourier Analysis

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ABSTRACT

Species morphology has always been considered an important factor that affects the patterns of inter- and intra-specific competition in ecological communities. Difference in the phenotype of different populations of insects has been hypothesized to provide possible evidence of genetic differentiation or local adaptation towards their host plant thus is believed to be occurring in Brontispalongissima, a serious pest of coconut and other palms. This study was therefore conducted to determine variability in this pest by quantitatively describing the left and right wing shape variations between sexes and among geographical populations of the insect pest by applying the tools of elliptic Fourier analysis (EFA). Chain coding technique which relied on a contour representation to code shape informationwas used. The set of possible movement depends on the type of contour representation, a pixel based contour representation were used in this study. Normalized elliptic Fourier descriptors (EFD) obtained from the chain codes was calculated using elliptic Fourier transformation. Normalization of data obtained from chain codes used the first harmonic ellipse as a basis corresponding to the first Fourier approximation and utilized the 20 harmonics number to be calculated. Results showed significant variations on the shape among the three populations of B. longissima gathered from different locations. Based on the canonical variance analysis (CVA) and multivariate analysis of variance (MANOVA), the three populations were shown to differ in the costal, apex and posterior margins of the wing.While it was observed that the left and right wing shape differ the different geographical populations, variations were also detected to occur between sexes. Results of the study showed the use of Elliptic Fourier Analysis proved to be useful in quantitatively describing even subtle shape variations that may aide in understanding the nature of B. longissima. The significant variation in the shape of the inner wings between sexes within and between geographical populations in terms of the curvature in the costal, apex and posterior margin of the wings may be attributed to genetic differentiation, habitat preference, wind dispersal and host-plant relationship.

Keywords: *Brontispalongissima*, Elliptic Fourier Analysis, wing shape

INTRODUCTION

Invasive pest species are a major cause and consequence of human-mediated global change and hence one of the major challenges facing human agriculture and biodiversity conservation [1]. One of the most destructive invasive insect pest species is the coconut Hispid Beetle, *Brontispalongissima.* The beetle attacks more than 20 palm species including the coconut (*Cocosnucifera*), Royal palm (*Roystonea*sp.), Alexandra palm (*Archontophoenixalexandrae*), Sago palm (*Metroxylonsagu)*, California fan palm (*Washingtoniafilifera)*, Mexican fan palm *(W. robusta)*, Bottle palm *(Hyophorbelagenicaulis)*, Chinese fan palm (*Livistoniachinensis)*, Madagascar palm

(Chrysalidocarpuslutescens) and Areca nut palm (*Areca catechu*) [2]. This insect pest is a native of Indonesia and Papua New Guinea, including the Bismarck Archipelago but is now widespread found in Australia (Darwin, Broome, Moa Island, Cooktown, Cairns, Innisfail, Marcoola and Townsville), many Pacific Islands, Malaysia, Singapore, Cambodia, Laos, Thailand, Vietnam, the Maldives, Philippines, Myanmar and China (Hainan, Guangdong and Taiwan provinces, with Hainan Islands, the worst affected) [2].

B. longissima was introduced in the Philippines in 2005 and has then greatly affected the coconut industry [3] creating serious leaf damage retarding the growth, reduces the fruit production of coconut palms, and occasionally causes tree death [4]. Despite the serious macroeconomic and environmental impacts of this pest, the scientific information on it has had only been a few reports [5]. In the Philippines, ongoing studies on this insect are focused on survey and mapping of its occurrence, its hosts and indigenous natural enemies,mass production of promising natural enemies, crop loss assessment due to infestation, field testing and integration of control strategies against the pest, information campaign and IPM promotion in *Brontispa*-infested areas in the Philippines and effects of gamma irradiation on the sterility of the pest (*B.longissima* (Gestro) [6]. The results of these studies are yet to be reported and published. It is argued however that in order to control the attack of this beetle, basic biological information is needed such as the nature of sex ratio [7], life tables [8]-[9], and demography [10]-[11]. Since insect species morphology has always been considered an important factor that affects the patterns of inter- and intra-specific competition in ecological communities and that insect wings have large contributions to their unparalleled success and bring superiority and competence in the field of foraging, calling, finding places for spawning and avoiding predators [12] and evolve rapidly to respond to various environmental conditions[13], studying the nature of variations in this structure in *B. longissima* may provide some information on its invasiveness and widespread distribution.

With the advances in statistics, geometry, and biology, analysis of shapes have interestingly become more quantitatively described, leading to the development of geometric morphometrics (GM), a statistical analysis of shape that made possible for the fast and reliable way of studying biological forms [14]. It was found to be a good tool in elucidating variations in organisms especially between-populations variations [15]. We used the method of geometric morphometrics to quantitatively describe the shape of the wings of *B. longissima* since there were difficulty in detecting and describing the differences between the two sexes in field populations unless the genitalia characters are examined. To date, only a little information is known on the shape and structure of the wingof *B. longissima*. While there are arrays of GM methods,we used Elliptic Fourier Analysis (EFA) to describe the shape of outline of the insect wing[16]. We explore the nature of variations in the wings by the application of the elliptic Fourier analysis, a tool in geometric morphometric found to be very useful for structures that are generally homologous (in biological sense) or comparable in the geometric sense, but where individual of homologous points analysis of curve may be absent which could not be detected through landmark based analysis [17].

MATERIALS AND METHODS

B.longissima) samples were randomly collected from three different provinces in the Philippines: Aloran, Misamis Occidental, Calube, Sibutad, Zamboangadel Norte and Pagadian City, Zamboanga del Sur (Figure 1). 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. Thirty males and thirty females of the studied species were collected per locality, pooling a total of 180 samples (N=180). These 180 samples were then divided into left and right wings pooling a total of 360 samples overall (N=360).

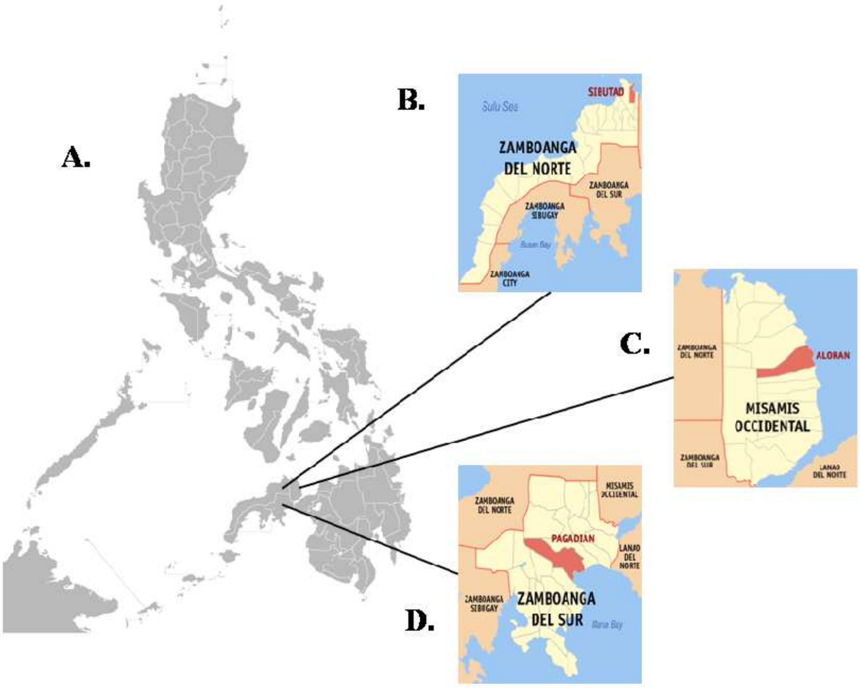

Figure 1. (A.) The Philippine Island showing the three sampling locations: (B.) Calube, Sibutad, Zamboangadel Norte, (C.) Aloran, Misamis Occidental and (D.) Pagadian City, Zamboangadel Sur.

Image Acquisition

Wings of *B. longissima* were viewed under a Leica stereomicroscope and digitized images were taken using Olympus E-410 DSLR Camera (Figure 2). Images were later transferred into a laptop and were then edited and cropped using Picasa 3.0 software for better outlining. The full colored images of the inner wings produced were then converted to 24-bitmap type, binary (black and white color) images. The outlines of each individual wing image were digitized using the software SHAPE v1.3 [18] for examination of shape variation and were recorded as chain codes [19].

Figure 2. Digitized image of left and right wing of*B. longissima*.

SHAPE Analysis

The objects of interests were distinguished via segmentation techniques through a "threshold procedure" where a parameter called the brightness threshold is manually chosen from brightness histogram and applied. Undesirable marks also termed as "noise" were consequently eliminated by erosion-dilation filter process. After noise reduction, the closed contour shape of the wings was extracted via edge detection and the contours were stored in the form of chain codes [20]. Chain coding technique was used which relied on a contour representation to code shape information. This method tracks the shape of the wings and represents each movement by a chain code symbol ranging from 0-7. The set of possible movement depends on the type of contour representation, a pixel based contour representation were used in this study. Normalized Elliptic Fourier Descriptors (EFD) obtained from the chaincodes was calculated using Elliptic Fourier transformation [21]. Normalization of data obtained from chain codes used the first harmonic ellipse as a basis which corresponds to the first Fourier approximation and utilized the 20 harmonics number to be calculated as suggested by [16]. Principal component analysis (PCA) was used to summarize independent shape characteristics [15].

Statistical Analysis

Shape variabilities among *B. longissima*wings were determined and subjected to Multiple Analysis of Variance and Canonical Variate Analysis (MANOVA/CVA). Wilks' lambdia and Pillai trace values were obtained. Box–and–whiskers and scatters plots showing variations were generated so as to visualize the shape variation distribution via the principal component scores. Since many species of insects vary greatly in the expression of sexual traitsshowing co-occurrence of two or more discreet phenotypes within one sex [22], analysis for both male and female inner wings was done separately. Likewise, since symmmetric morphology and function are commonly observed in bilateral organisms [23]-[24], separate analysis was done for both left and right inner wings.

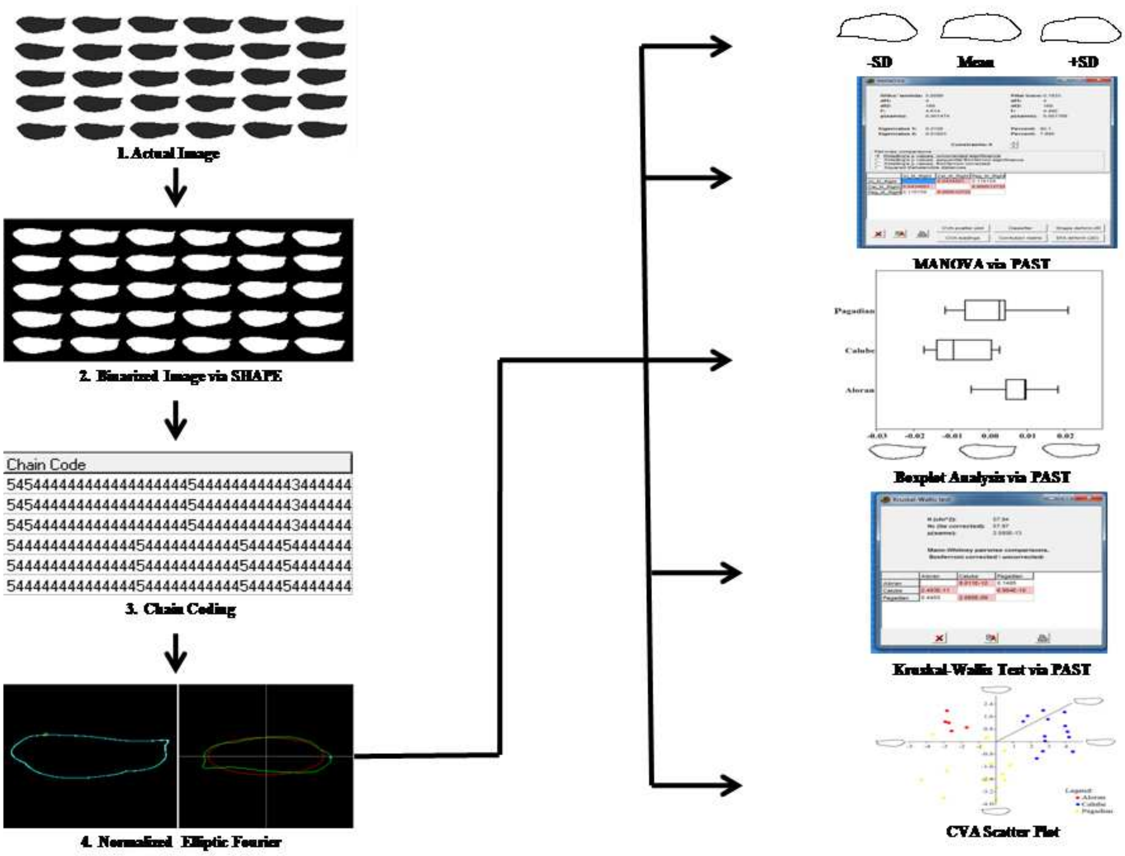

Figure 3. Outline of the Elliptic Fourier analysis of the wing shape of B. longissima.

RESULTS AND DISCUSSION

Significant differences in wing shapes were observed in individuals in three populations of *B. longissima* based on the distribution of the individuals along the first two canonical variant axes(Figure 4).Individuals belonging to the CV1 axis described the variation on the anterior and posterior portion of the wing that ranged from straight costal margin to curve, blunt to more pointed apex and more pronounced curve posterior margin to less curve while those individuals in the CV2 axis vary in the length-width aspect ratio.

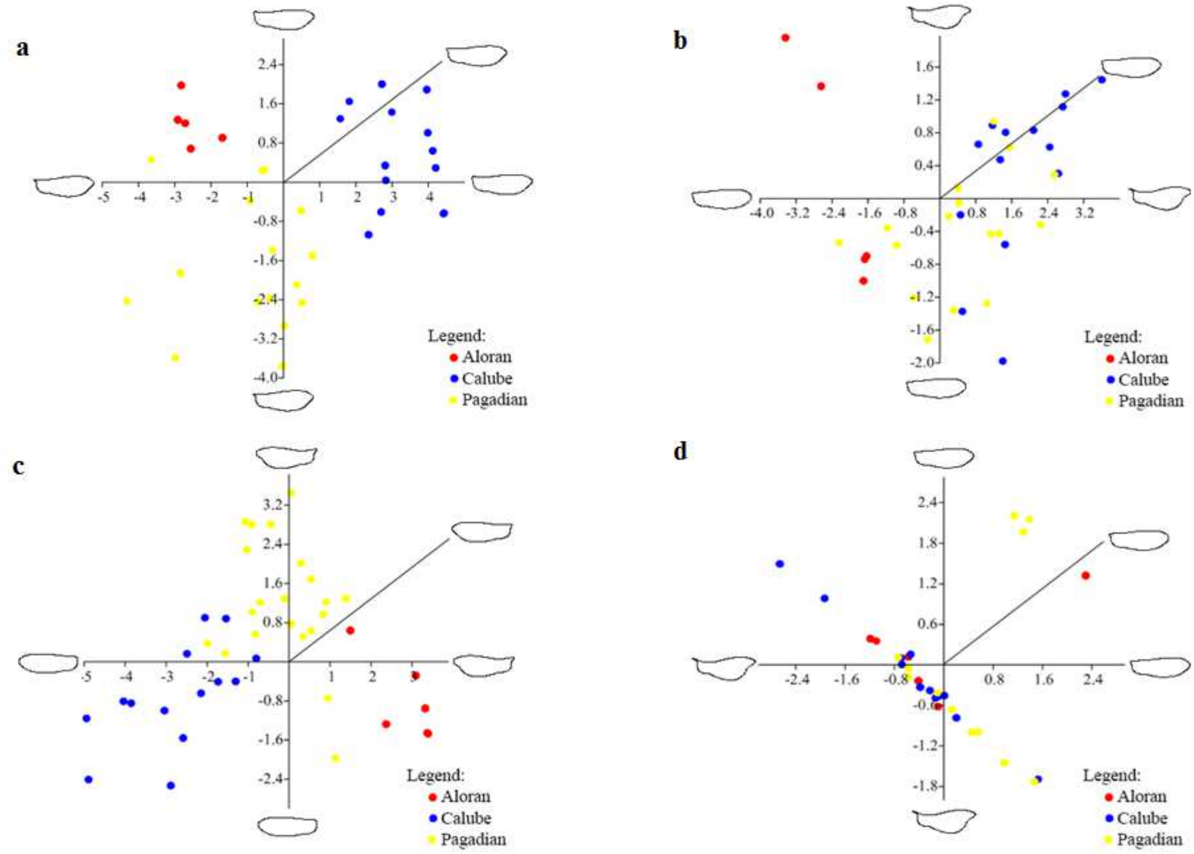

Figure 4. Plots of the first two canonical axes showing significant difference in the shape of the left and right inner wings among the three male and female populations of B. longissima:

(A.) All-female left wing - Wilks' lambda: 0.0544; p-value: 1.324-37; Pillai trace: 1.4730; p-value: 2.5290-34; (B.) All-female right wing - Wilks' lambda: 0.2398; p-value: 3.4690-23; Pillai trace: 0.8206; p-value: 5.7130-17; (C.) All-male left wing - Wilks' lambda: 0.0856; p-value: 3.4140-33; Pillai trace: 1.3140; p-value: 1.5950-28; (D.) All-male right wing - Wilks' lambda: 0.8099; p-value: 0.0014; Pillai trace: 0.1933; p-value: 0.0017.

To describe the shape variation between three populations of *B. longissima*, the principal component scores and calculated standardized elliptic Fourier coefficients were used to restructure the wing consensus morphology with the positive (+) and negative (-) deviations derived along with the mean wing shape. The procedure's purpose is for comparison of the mean shapes to the deviations in order to explain the possible underlying relationship. Significant differences in the wing shape of the three populations of *B. longissima* were observed (Figures 5, 6, 7 and 8), with the shape descriptions summarized in Table 1.

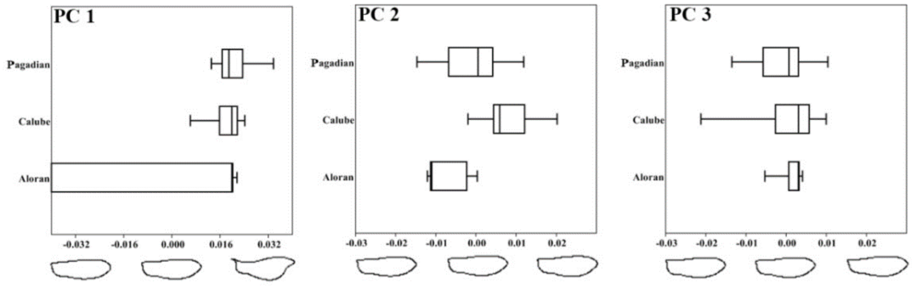

Figure 5. Box-and-Whiskers plots showing the significant differences in the right wing shape among the three populations of female B. longissima.

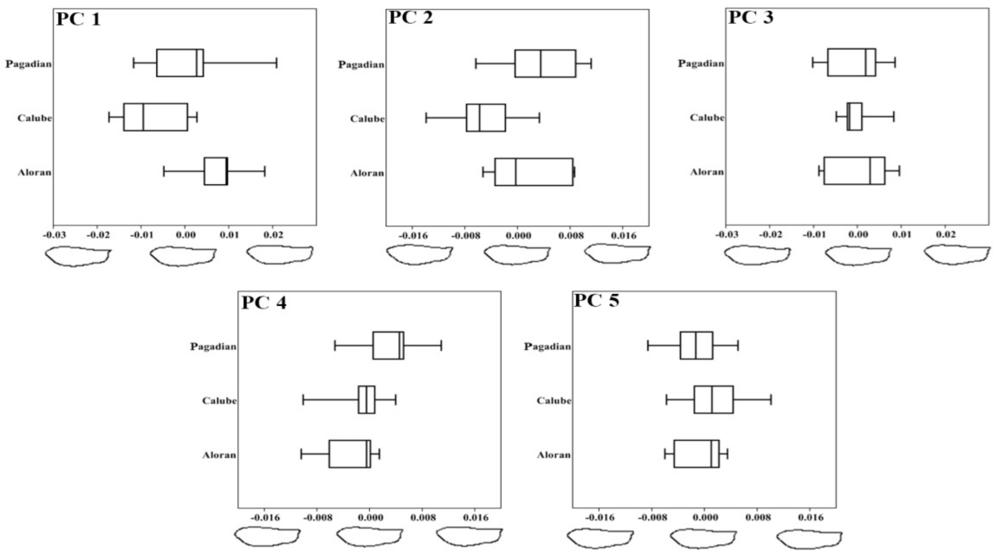

Figure 6. Box-and-Whiskers plots showing the significant differences in the left wing shape among the three populations of female *B. longissima*.

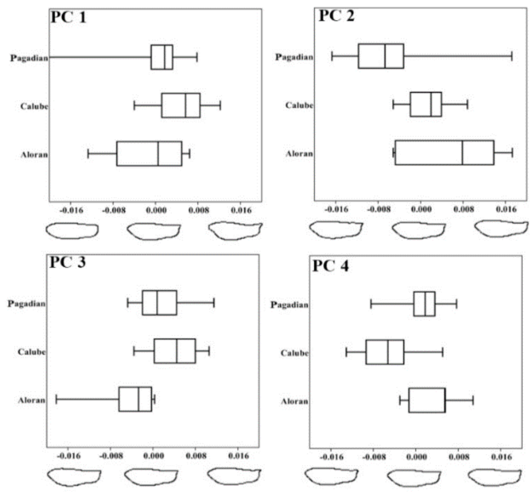

Figure 7. Box-and-Whiskers plots showing the significant differences in the left wing shape among the three populations of male *B. longissima*.

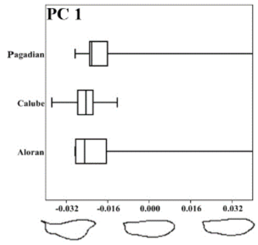

Figure 8. Box-and-Whiskers plots showing the significant differences in the right wing shape among the three populations of male *B. longissima*.

Table 1. Wing shape descriptions on the left wing among the all-female *B. longissima* populations.

	All-female *B. longissima* populations (left wing)
PC1 (36.84%)	Observed variations due to the anterior portion of the wing, ranging from a straight costal margin to less curved, and on blunt to a more pointed apex. Individuals from Aloran have straighter costal margin and pointed apex compared to those from Calube with less curved costal margin and a blunt apex. Individuals from Pagadian tend to approach the mean shape.
PC2 (15.87%)	Variations observed are based on the posterior portion of the wing, ranging from a curved posterior margin to a straighter portion. Individuals from Calube have pointed apex with curved posterior margin while those from Pagadian have blunt apex with a less curved posterior margin. Individuals from Aloran tend to approach the mean shape.
PC3 (12.83%)	Individuals from Pagadian and Aloran vary by having pointed apex and straighter costal margin. Individuals from Calube have blunt apex with curved costal margin.
PC4 (9.53%)	Observed variations due to anterior portion of the wing ranging from a pointed costal tip to a blunter end and a more pronounced pointed apex. Individuals from Pagadian have more pointed costal tip and pointed apex while individuals from Calube and Aloran tend to approach the mean shape.
PC5 (7.63%)	Individuals from Aloran and Calube vary by having a less curved posterior margin and a blunt apex and costal tip. Individuals from Pagadian have more pointed apex, straighter costal margin and more pronounced curved posterior margin.
	All-Female Populations (Right Wing)
PC1 (93.06%)	Observed variations due to the anterior and posterior portion of the wing, ranging from a straight costal margin to more pronounced curve, blunt to a more pointed apex and on a straighter posterior margin to a much curved margin. Individuals from Aloran, Calube and Pagadian have pronounced curved costal margin with pointed costal tip and apex. Posterior margin vary greatly through its much curved orientation with a narrow area of location of anal veins.
PC2 (2.71%)	Variations observed are based on the posterior portion of the wing, ranging from a curved posterior margin to a straighter portion. Individuals from Calube have blunt apex with curved posterior margin while those from Aloran have pointed apex with a straighter posterior margin. Individuals from Pagadian tend to approach the mean shape.
PC3 (1.51%)	Individuals from Aloran and Calube vary by having pointed apex and straighter costal margin. Individuals from Pagadian fall under the mean shape.
	Male *B. longissima* populations (left wing)
PC1 (56.62%)	Observed variations due to the anterior and posterior portion of the wing, ranging from a straight costal margin to more pronounced curve, blunt to a more pointed costal tip and on a straighter posterior margin to a much curved margin. Individuals from Calube and Pagadian have pronounced curved costal margin with pointed costal tip. Posterior margin vary greatly through its much curved orientation with a narrow area of location of anal veins. Individuals from Aloran fall under the mean shape.
PC2 (15.11%)	Variations observed are based on the anterior portion of the wing, ranging from a straight costal margin to a less curved side. Individuals from Aloran and Calube have curved anterior margin with a narrow width of the location of anal veins while those from Pagadian have straight costal margin and a less pointed costal tip.
PC3 (8.33%)	Individuals from Aloran vary by having pointed apex and straighter costal margin. Individuals from Calube have straight apex and costal margin with a pointed costal tip. Individuals from Pagadian tend to approach the mean shape.
PC4 (6.57%)	Variations observed due to anterior portion of the wing ranging from a pointed costal tip to a blunter end and a more pronounced pointed apex. Individuals from Pagadian and Aloran have more pointed costal tip and pointed apex while individuals from Calube have blunt apex costal tip.
	All-Male Populations (Right Wing)
PC1 (94.40%)	Observed variations due to the anterior and posterior portion of the wing, ranging from a straight costal margin to more pronounced curve, blunt to a more pointed apex and on a straighter posterior margin to a much curved margin. Individuals from Aloran, Calube and Pagadian have pronounced curved costal margin with pointed costal tip and apex. Posterior margin vary greatly through its much curved orientation with a narrow area of location of anal veins.

The non-parametric Kruskal-Wallis test (all-female left wing populations, p=2.585E-13; all-female right wing populations, p=3.18E-06; all-male left wing populations, p=4.284E-05 and all-male right wing populations, p=0.0161) showed that there were significant differences between the medians of at least two populations. Tables 5, 6, 7 and 8 show the result of the Bonferroni corrected Mann-Whitney pairwise comparison of the first and second PC scores of the shape of wingsIn table 5, pairwise comparison showed that the Aloran, Calube and Pagadian female left wing populations were significantly different to the other populations. In table 6, the Aloran female right wing population showed significant difference compared to the other populations. In Table 7, Pagadian left wing male populations were significantly different to the other populations. In table 8, Calube and Pagadian right wing showed significant difference to other populations.

Table 5.Kruskal-Wallis test result on the wing shape among the three all-female left wing populations based on the first and second PC scores. Bold values indicate significant difference (level of significance, p=0.05).

Location	Location		
	Aloran	Calube	Pagadian
Aloran		**8.01E-12**	0.1485
Calube	**2.40E-11**		**6.98E-10**
Pagadian	0.4455	**2.10E-09**	

Table 6.Kruskal-Wallis test result on the wing shape among the three all-female right wing populations based on the first and second PC scores. Bold values indicate significant difference (level of significance, p=0.05)

Location	Location		
	Aloran	Calube	Pagadian
Aloran		**2.504E-06**	**0.0001691**
Calube	**7.513E-06**		0.207
Pagadian	0.0005072	0.6209	

Table 7.Kruskal-Wallis test result on the wing shape among the three all-male left wing populations based on the first and second PC scores. Bold values indicate significant difference (level of significance, p=0.05)

Location	Location		
	Aloran	Calube	Pagadian
Aloran		0.2703	**0.00367**
Calube	0.8109		**6.027E-06**
Pagadian	**0.01088**	**1.808E-05**	

Table 8.Kruskal-Wallis test result on the wing shape among the three all-male right wing populations based on the first and second PC scores. Bold values indicate significant difference (level of significance, p=0.05)

Location	Location		
	Aloran	Calube	Pagadian
Aloran		0.1469	0.195
Calube	0.8109		**0.003448**
Pagadian	0.585	**0.01035**	

It can be seen from the results of the study that significant variations in the wing shape among the three populations of *B. longissima* were quantitatively described. Male and female individuals also showed significant variations. Differences between populatiosn were observed specially on the right wing shape where a pronounced curved costal margin with pointed costal tip and apex was greatly exhibited. CVA scatter plot also showed that individuals from different geographical locations have distinct morphology since they were most likely distributed separately and did not overlap. Male and female individuals within a population also possess significant variation as female individuals from Calube have less curved costal margin and blunt apex while males of the same population exhibited more pronounced curved costal margin with pointed and costal tip and apex. Also, females from Aloran have straighter costal margin and pointed apex while males of the same population fall under the mean shapethus, there is an observed possibility for the presence of sexual dimorphism.On a similar study [25]-[26], multivariate analysis of the female and male wings of *C. gelidus* showed a significant differentiation. Similar study on eight populations *Apiscerana*worker's wings using multivariate morphometrics showed morphological differentiation. Principal component and factor analysis showed that eight populations differed significantly from one another.

Variations in morphological shape is frequently argued to be dependent on environmental condition where the organism is found [27]. 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. It was reported in a study that environmental factors such as 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 [28]. There are reports however that geographically close populations are more varied than those geographically distant. Morphological variation couldalso be under the influence of physiological (or pathological) status, adaptive changes or genetic differences [29]and habitat preference. Animals actively select habitats to which they are best adapted morphologically and physiologically[30]. Another possible reason could be insect wind dispersal. Dispersal, a vital aspect of zoogeography, is of prime importance if the insect is to succeed [31]. Wind dispersal map possibly play an important role in the transport of the coconut leaf beetle individuals into their different geographical locations. Localization of differences may have taken place in which metric differences found in organs responsible for mechanical reproductive barriers may be given more evolutionary significance than to differences located elsewhere [29]. Environmentally induced selection pressures such as the variations in age and phenotype of the plants may have contributed also to the observed variation [32]. Interactions between insect pheromones and host-plant semichemicals have manifested effects on insect physiology and behavior [33]. Host-switching has been studied to cause non-random patterns of phylogenetic congruence [34].

CONCLUSION

Results of the study showed the use of Elliptic Fourier Analysis proved to be useful in quantitatively describing even subtle shape variations that may aide in understanding the nature of *B. longissima*. The significant variation in the shape of the inner wings between sexes within and between geographical populations in terms of the curvature in the costal, apex and posterior margin of the wings may be attributed to genetic differentiation, habitat preference, wind dispersal and host-plant relationship.

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