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Elliptic Fourier analysis in describing Shape of the Mandible of the Larvae of the Coconut Leaf beetle *Brontispa longissima* Gestro, 1885 (Chrysomelidae: Hispinae) collected from Plants with varying Degrees of Damage

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Abstract

This study was conducted to describe the mandibles of the larvae of coconut leaf beetle (Brontispa longissima) (CLB) populations collected from plants with various degrees of damage (heavy, moderate and low) using elliptic Fourier analysis. It was hypothesized that differences in phenotypic form of populations may provide evidence of possible genetic differentiation or mere adaptations of the pest to available food types with varying degree of resistance. Results show considerable differences in mandible shapes of the three populations of the beetle collected from coconuts with different degrees of damage. Based from the canonical variate analysis (CVA), the three populations were found to be distinct from each other indicating that variations in mandible shapes can be a result of the different level of resistance of the coconut plants. The results of the study show the utility of the method of Geometric Morphometrics specifically elliptic Fourier analysis in describing shapes of mandibles of B. longgisima attacking coconut trees with different level of damage. Future studies should be directed towards unraveling the observed disparity in the shapes of the mandibles between sexes and those attacking other plant species.

Keywords: Mandible, coconut leaf beetle larvae, geometric morphometrics, brontispa longissima.

Introduction

The coconut leaf beetle (*Brontispa longissima*) (CLB) (fig ure 1), is one of the most damaging pests of coconut and other palms. The beetles attacked all ages of coconut, although more damage is found in coconut plantation between four to five years old, especially in drying areas[1]. Damages caused by *Brontispa* were first recorded in Narathiwas province, the border area near Malaysia, in 2000. Damages range accordingly, from severe heavy, moderate to light moderate. Severe damage by the beetle can cause significant production losses, and high infestation levels may result in plant death[2,3]. It has been reported that both larvae and adults of CLB, impair young coconut leaves, by feeding on the soft tissues of plants such as the epidermis and parenchyma of young unfolded coconut leaflets which later become brown, dry and die[4,5,6]. Beetle larvae chew large areas of the leaflets killing underlying tissues and reducing leaf photosynthesis; photosynthesis may be reduced to zero in the case of multiple beetle attacks[7]. Severe infestations may result in the complete defoliation of the palm[8]. In the worse cases palms, especially young ones die from severe infestation[6,9,10]. Frequent and sporadic infestation were also recorded[11,12]. However, the larvae eat stronger than adults. The younger coconut trees were more heavily attacked.

It is hypothesized that the observed variability in damage could be brought about by variability of *Brontispa* populations. Since mandibles are central to the understanding of adaptive modifications in insects as they are used as major feeding apparatus, fundamental understanding of mouthpart structure and function is important in understanding host-insect relationship[13].

Understanding variability in responses of insects to plant genotypes/cultivars can lead to possible emergence of populations that can overcome resistance in plants. This association is widely assumed to be characterized by gene-for-gene relationship expressed through a polygenic system resulting to the visible phenotypic effect[14-18]. Studies on mandibular morphology in grasshoppers[19] and cockroaches[20] showed a diet-related influence on shape variability. This may also hold true for CLB thus this study was conducted.

With advances in statistics, geometry and biology, analysis of shapes have become more quantitatively described. The development of geometric morphometrics (GM), a statistical analysis of shape has made possible for a fast and reliable way of studying biological forms[21-23]. It was also found to be a good tool in elucidating variations in organisms especially between-population variations[24]. Elliptic Fourier descriptors for example (EFDs), originally proposed by Kuhl and Giardina[25], can delineate any type of shape with a closed two dimensional contour. EFDs have been effectively applied to the analysis of various biological shapes in animals[26-31] and plants[32-43]. The principal component scores obtained can be used as observed values of morphological features in subsequent analysis, such as analysis of the shapes of biological organs. Thus, this can be used in the analysis of mandibular shapes in *Brontispa*.

Figure-1
The coconut leaf beetle *Brontispa longissima* larvae

Material and Methods

Coconut plants were quantitatively and qualitatively assessed to describe the extent of damage on the young leaves of the sampled coconut trees (4-6 yrs. old) infested by *B. longissima* larvae. Numerical values were assigned from 1 to 3 with descriptions: 1- light moderate damage (1-2 young leaves colored brown); 2- moderately damage (3-4 young leaves colored brown); 3-heavy/severely damage (the infestation resulted in the complete defoliation of the leaves or dying of leaves) (figure 2).

The coconut leaf beetle larvae (*Brontispa longissima,* Gestro) (CLB), were randomly collected from different sites in Lanuza and Tandag City, Surigao del Sur from coconut host plants. The collected individuals (N= 180) were placed in plastic containers containing prepared fixative (70% ethyl alcohol + 30% glacial acetic acid) for preservation. The larvae were processed by boiling it in 5% NaOH solution until it will become transparent and the mandibles were removed (figure 3). Image acquisition was done using a Digital Microscope (20-400x) connected to a laptop. The full colored images of mandibles produced were converted to 24-bitmap type, binary (black and white color) images. The outlines of each mandible were digitized using the software package SHAPE version 1.3[44] for examination of shape variation and were recorded as chain codes [45]. Herewith, 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 each mandible was extracted via edge detection and the contour will be stored as chain codes[46]. Chain coding technique was used which relied on a contour representation to code shape information. This method tracks the shape of the mandible 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 were calculated using Elliptic Fourier transformation as suggested by Kuhl and Giardina[25]. 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 Iwata and Ukai[44]. Principal component analysis was used to summarize independent shape characteristics. The differences in shape among mandibles were determined and subjected to Multivariate Analysis of Variance and Canonical Variate Analysis (MANOVA/CVA). Wilks’ lambda and Pillai trace values and p values were obtained. Box- and whiskers and scatter plots showing the variations were generated, this is to visualize the distribution of shape variation using the principal component scores. The procedures are summarized in figure 4.

Figure-2
Varied extent of damage of *Brontispa longissima,* Gestro larvae on its host *Cocos nucifera*: a.) light moderate -slightly damage, (1-2 young leaves colored brown); b.) moderate damage, (3-4 young leaves colored brown) ; c.) severe to heavy damage, (the infestation resulted in the complete defoliation or dying of the leaves) (see arrow)

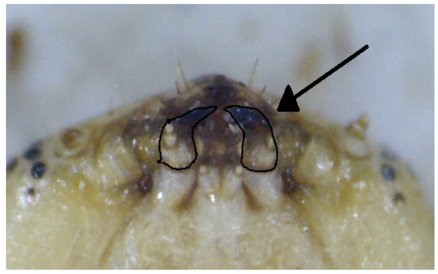

Figure-3
Typical mandible of *Brontispa longissima,* Gestro (Coconut leaf beetle larvae), appearing as large pincers; pair of hard, often tooth-like structures that move horizontally to grasp, crush, or cut food

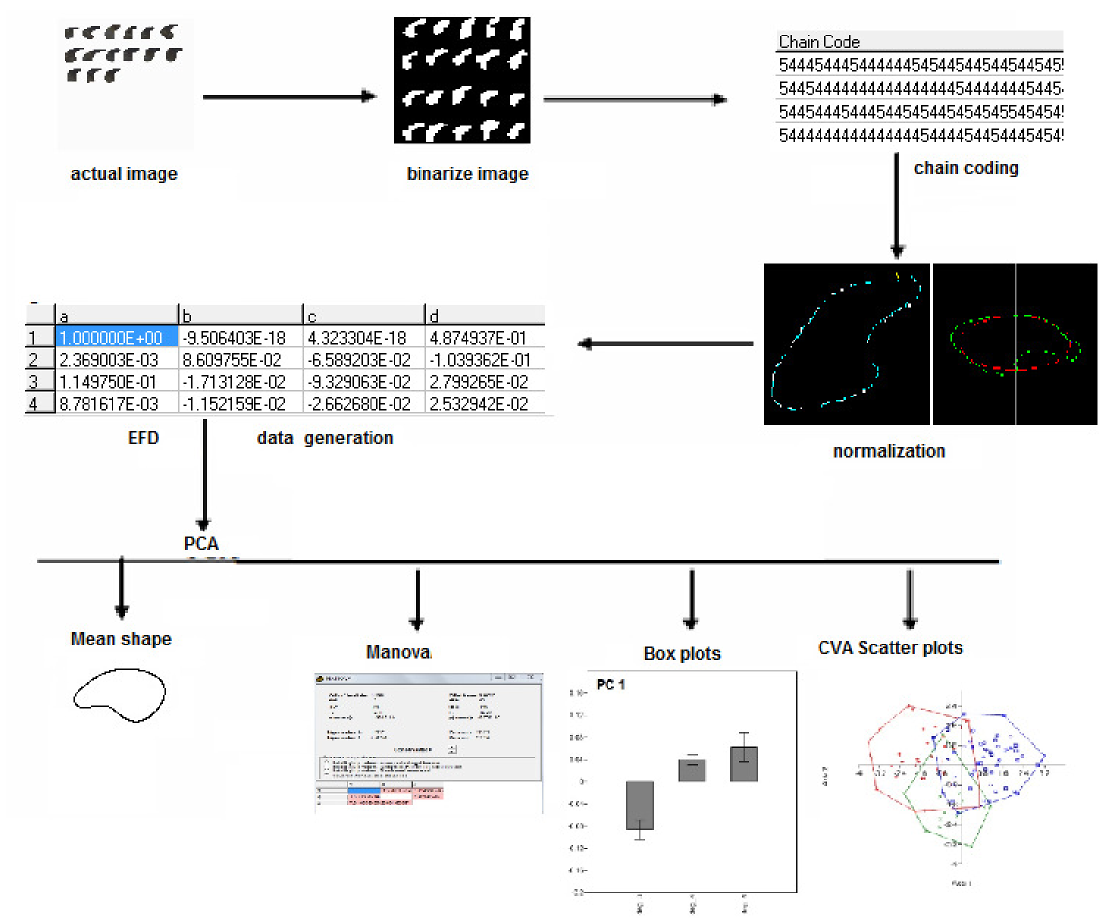

Figure-4
Outline of the Elliptic Fourier analysis of mandible shape of *B. Longissima* larvae

Results and Discussion

Mouthpart morphology is argued to be pronouncedly adapting and evolving to specific food types[47,48]. The mandibles in particular, are central to the understanding of adaptive modifications as they are used as major feeding apparatus. The typical mandible of *B. longissima* larvae, appears as a pair of hard large pincers; often tooth-like structures that move horizontally to grasp, crush, or cut food.

Significant differences in mandible shapes were observed in individuals in three populations of *B. longgisima* based on the distribution of the individuals along the first two canonical variate axes (Right mandible- Wilks' lambda: 0.2329; p-value : 6.625×10^{-25} ; Pillai trace: 1.016; p-value: 1.943×10^{-24}; left mandible - (Wilks' lambda:0.305; p-value:1.554×10^{-19}; Pillai trace: 0.8707; p-value: 6.92×10^{-19}) (figure 5 A,B). Since asymmetric morphology and function are commonly observed in bilateral animals[49,50], separate analysis was done for both right and left mandibles. For the right mandible, CV1 accounts for 72.66% and CV 2 explains 27.34% of the overall variation respectively. The left mandible CV1 accounts for 68.25% and CV2 explains 31.75% of the overall variation. Individuals in the CV1 axis described the variation on the anterior portion of both mandibles that ranged from a more pointed to a blunt end while those individuals in the CV2 axis vary in the length-width aspect ratio.

To determine subtle shape variations between the three populations of *B. longgisima* larvae, the principal component (PC) scores that defined shape differences, the standardized elliptic fourier coefficients calculated were used to reconstruct the consensus morphology and the positive (+) and negative (-) deviations from the mean shape of the mandibles. This is an exploratory procedure to compare the mean shapes and elucidate the underlying relationships. Considerable differences in mandible shape on the three populations were observed (figure 6). Descriptions of the shapes are summarized in table 1.

Results of the study show differences in mandible shapes in *B. longgisima*. There are many hypotheses as to the existence of variations in shapes. One possible reason for the differences obtained could be the emergence of or over specialization in the shape of the mandible catering the most abundant food source and the evolution or possible emergence of a common phenotypic mandible shape that can cater various conditions and food types thereby, inflicting more damage on plant tissues. Plants actually present a series of potential problems that could influence the evolution of insect form and this includes the hardness and toughness of food substrates[51]. To date, there are morphological adaptations for insect feeding on plants and much emphasis is considered to two factors: the nature of plant surface and the difficulty of dealing with hard or tough food. The case on the difficulty of feeding upon certain plant tissues can be overcome in variety of ways such that mandible adaptations for rough food appeared to be minimizing handling time with a concomitant risk of predation hence, convergent evolution is present on mouthpart morphology in response to differences in host characteristics[51,52].

Variations in mandible shape may be expected also to allow optimal exploitation to certain types of food plant as individual plants also vary. Induced plant responses often yield variation in terms of nutritional status, secondary substances and physical characteristics of host plants[53,54]. The observed range of variation is presumably an effect to produce an efficient masticatory mechanism forming a continuous cutting edge producing a scissor-like cutting mechanism and as major tool feeding apparatus thus mandibles for example are subjected to continuous wear and tear in effect of the biochemical properties of the plant.

It can be seen from the results also that within-populations have some degree of continuous variation ranging from big to small. This is expected to be under genetic control but environmentally induced selection pressures such as the variations in age and phenotype of the plants contributed to the variation. It can be argued also that equally susceptible to selection while genetically determined, discontinuous variation in which distinct classes of variation are found, few intermediates can be observed. Such discontinuous variation is termed polymorphism and is also common referred to as morphs[55]. It can be argued also that host-plants are known to promote high taxonomic diversity and ecological disparity among many insect-herbivores[56] thus explains the variations observed in this study.

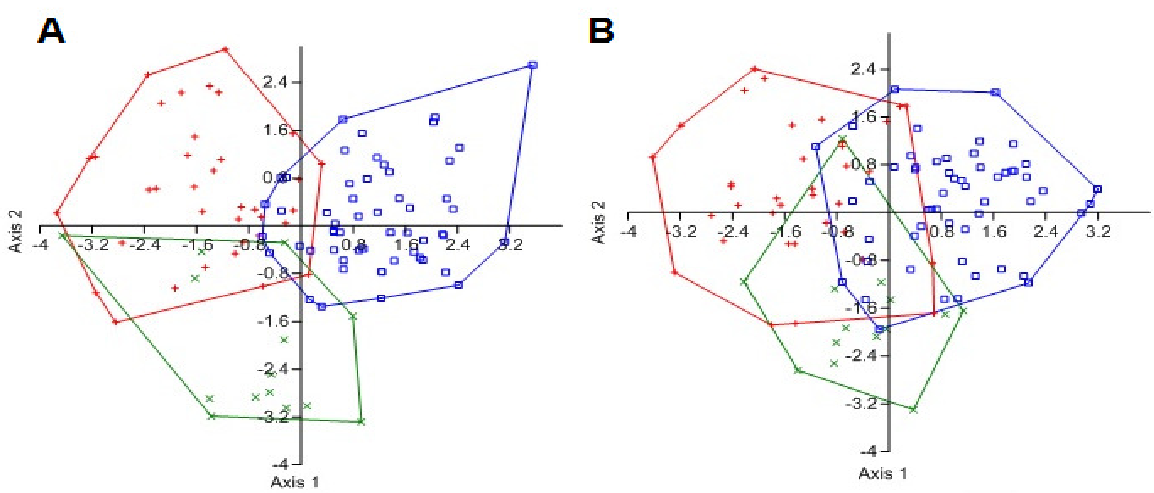

Figure-5

Plots of the first two canonical axes showing significant difference in the shape of mandibles among the three populations of the coconut leaf beetle (*Brontispa longissima,* Gestro), larvae: A.) right mandible, (Wilks' lambda: 0.2329; p-value : $6.625x10^{-25}$; Pillai trace: 1.016; p-value: $1.943x10^{-24}$); B.) left mandible, (Wilks' lambda:0.305; p-value:$1.554x10^{-19}$; Pillai trace: 0.8707; p-value: $6.92x10^{19}$)

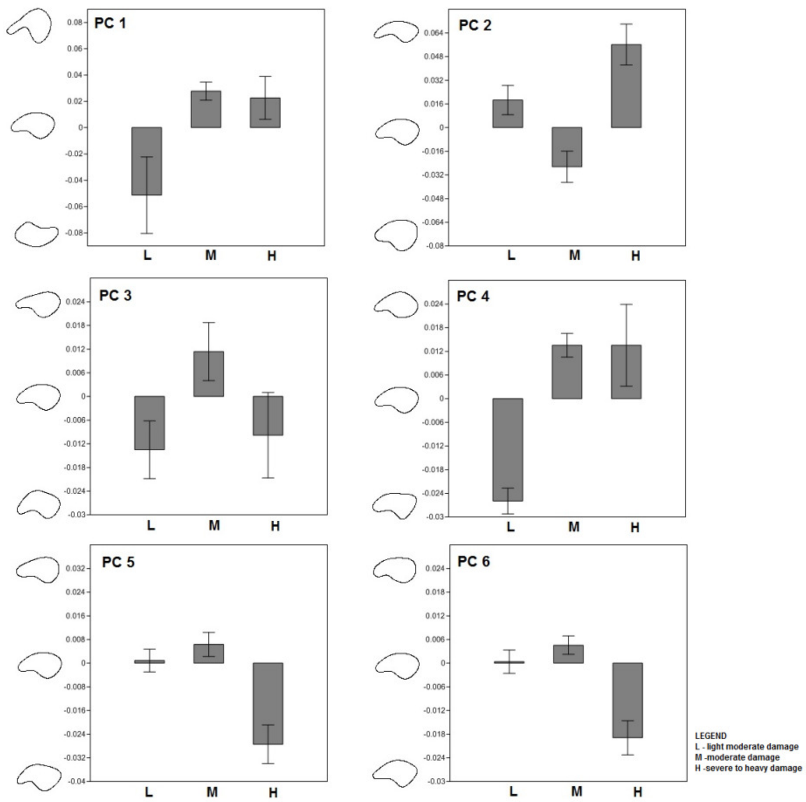

Figure-6

Box Plots showing significant difference in the shape of the right mandible among he three populations of *B. longissima*

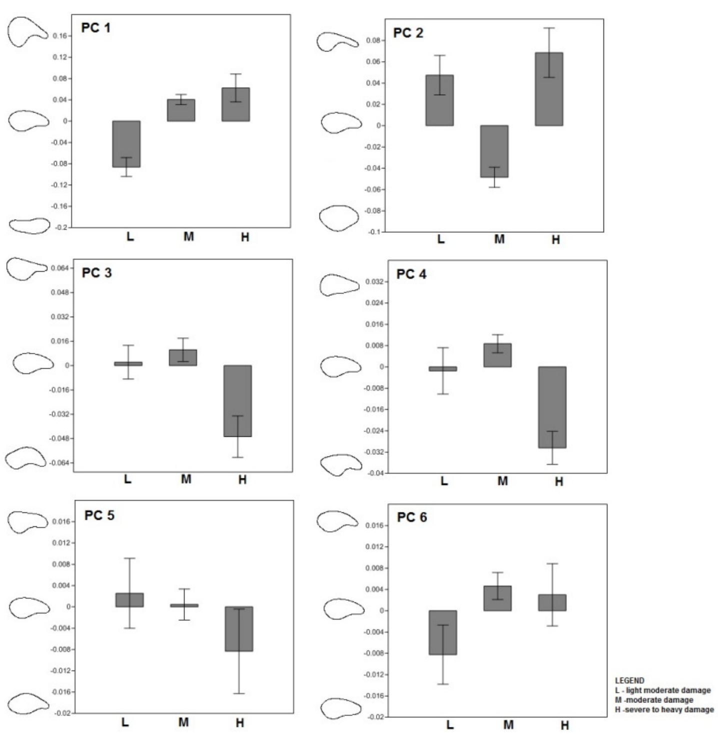

Figure-7

Box Plots showing significant difference in the shape of the left mandible among the three populations of *B. longissima*

Table-1
Descriptions of the shapes of the right and left mandibles

	Right Mandible		Left Mandible
PC 1 (53.88%)	Observed variations due to the anterior portion of the mandible ranging from a more pointed to a blunt end and the extent of the basal margin; individuals causing light damage have blunted interior and external margins towards the basal end and blunter anterior end; those individuals causing moderate and severe damage tend to approach the mean shape.	PC 1 (37.72%)	Variation is based on the anterior portion of the mandible ranging from a more pointed to a blunt end and the extent of the basal margin; individuals causing light damage vary by having a very narrow blunted interior and external margins towards the basal end and a more blunt anterior end; individuals causing moderate and severe damage tend to approach the mean shape.
PC 2 (22.47%)	Variations observed are based on the extent of the basal margin and the shape of the anterior end; a more pointed to a blunt end; individuals causing heavy damage vary by having a narrow basal margin towards a pointed anterior end; interior and external margins are relatively narrow; individuals with light and moderate damage tend to approach the mean shape.	PC 2 (33.77%)	The individuals causing light moderate and heavy damage vary by having an extremely narrow pointed anterior end but quite pronounced basal margin towards the internal margin; individuals for the moderate damage may exhibit a blunt and round shape mandible however, individuals obtained tend to approach the mean shape.
PC 3 (10.21%)	The individuals causing light damage vary by having a blunted anterior end but more pronounced wider basal margin towards the external margin; however, individuals causing heavy damage obtained tend to approach the mean shape.	PC 3 (12.49%)	The individuals causing light to moderate damage all fall under the mean shape; but for the individuals causing heavy damage exhibited a pointed anterior end with a quite broad basal margin towards the base
PC 4 (3.72%)	The individuals causing light damage vary by having a blunted anterior end towards a broader basal end as pronounced by wider basal margin towards external margin; however, all individuals still tend to approach the mean shape.	PC 4 (4.85%)	The individuals causing light to moderate damage all fall under the mean shape; but for individuals causing heavy damage the individuals exhibited a pointed anterior end with a broad external and internal margin towards the base
PC 5 (3.40%)	The individuals causing light to moderate damage have mandibles with the mean shape; but for those causing heavy damage exhibited a pointed but quite blunt anterior end with a broader basal margin towards the external margin.	PC 5 (2.88%)	The individuals causing light to moderate damage have mandibles fall on the mean shape; but for individuals from the heavy damaged plants exhibited a minimally pointed anterior end with a broad external and internal margin towards a rounded base
PC 6 (1.41%)	The individuals causing light moderate damage all fall under the mean shape; but for individuals causing heavy damage exhibited a more pointed anterior end with a broader basal margin towards the base	PC 6 (2.14%)	The individuals causing moderate to heavy damage have mandibles with the mean shape; but for individuals causing light damage have the mandibles of the mean shape with a blunt rounded anterior end with a quite broad external and internal margin towards a rounded base

Conclusion

The results of the study show the utility of the method of Geometric Morphometrics specifically elliptic Fourier analysis in describing shapes of mandibles of *B. longgisima* attacking coconut trees with different level of damage. Future studies should be directed towards unraveling the observed disparity in the shapes of the mandibles between sexes and those attacking other plant species.

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