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Body Shape dimorphism in the Adult coconut leaf beetle *Brontispa longissima* Gestro

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ABSTRACT

Sexual dimorphism is one of the most generalized and notable sources of phenotypic variation in Brontispa longissima and has become a subject to geometric morphometrics studies. Studies have also shown body sizes in Brontispa longissima varies but no study was done on the body shape. In this study, body shape in male and female B. longissima is quantitatively described by the application of relative warp analysis. Statistical results using relative warp scores indicate significant differences between sexes although some misclassified female individuals of B. longissima were grouped as males which can be due to several factors including differences in development and fecundity of the females. Nevertheless, the results of the study suggest that GM tools specifically relative warp analysis help make a good quantified description of the body shapes of the two sexes.

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INTRODUCTION

The coconut hispine beetle, *Brontispa longissima* (Gestro) is one of the most damaging pests of coconut and other palms. It is reported to be found almost worldwide where coconut is being cultivated (Ayri & Ramamurthy, 2012). *B. longissima* was originally described from the Aru Islands. It is native to Indonesia, possibly to Irian Java, and also to Papua New Guinea, including the Bismarck Archipelago, where it seldom causes serious problems. It was reported from the Solomon Islands in 1929 and from Vanuatu in 1937 (Risbec, 1942), New Caledonia (Tahiti) (Cohic, 1961), American Samoa (Long, 1974) and Western Samoa (Anon, 1981), northern Australia (Fenner, 1984) and Taiwan (Shiau, 1982, Anon 2004a), Hong Kong in (Lau, 1991). Though it was eradicated, some reports show its establishment in Hong Kong, Nauru, (Anon 2004b), Maldives Island (Anon 2004c) and Philippines in 2005 (Nakamura *et al*). In the Philippines, from August 2007 to March 20l1 the pest has already affected 3 million coconut trees in Pangasinan, Region 4-A In Southern Palawan, Maasin City, Southern Leyte Province, Samar Province and in the SOCCKSARGEN Region (Phil. Senate, 2014). A decline in the yield of coco production happened because the larvae and adults severely damage young coconut palms by feeding on the unopened leaflets. The adults chew narrow lines parallel to the midrib causing the leaf to look striped. It seems that the *Brontispa* was carried via imported ornamental plants brought in the country from infested sources.

Outbreaks of *B. longissima* have aroused the interest of many entomologists looking into possible cause for their dynamics, unique adaptations, density dependence, life tables, stability and diversity (Acevedo *et al*., 2014). One of these is the association of anatomical characters between individuals such as body size and shape considered as the most striking and significant traits of all organisms (Sukhodolskaya & Eremeeva, 2013) establishing physiological, ecological, evolutionary causes and consequences of development (Gaston & Blackburn, 2000). Since morphological traits such as body shapes frequently go through strong selection due to reproductive mechanism or during ontogenetic development as a consequence of environmental pressures (Zinetti *et al*., 2013; Benítez *et al*., 2013), these have become the bases of an individual’s fitness for survival. Likewise, individuals with certain characteristics are more likely than other individuals to obtain a mate or be a mate choice that results in sexual dimorphism (Campbell *et al*., 2009; Hedrick & Temeles, 1989).

While most insect species vary greatly in the expression of sexual traits (Benítez, 2013), the expression of such traits is discontinuous that results in the co-occurrence of two or more discrete phenotypes within one sex (Gross, 1996), we delimit the study of sexual dimorphism only on the beetle’s body shape. It is believed that differences in body shapes between sexes might be due to various biological processes such as disease or injury,

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ontogenetic development and fecundity which will influence population structure and tendency for an outbreak, adaptation to different selection pressures such as development of resistance to specific pesticides or chemicals. In this study, we employ a quantitative approach in describing the shape of the body of the 2 sexes of the beetle known as the landmark-based geometric morphometric (GM) method of analysis. The tools of GM was known to be efficient in detecting variations within and between morphological structures extending up to populations of the organisms being investigated (Benítez *et al*., 2013; Tabugo *et al*., 2012; Belleza & Demayo, 2014).

Methodology:

Insects were collected from infected coconuts from Aloran, Misamis Occidental, a 4th class municipality in the province of Misamis Occidental, Philippines (nautical grid coordinate of 8°25'6" north latitude and 123°49'13" east longitude). Coconut plantations have the largest area of 101,784 hectares or 96.87% of commercial crops. It is still the main cash crop of the province, even if some considered it a "sunset industry" because of the apparent decline of fruit yields per year due to *B. longissima* infestation.

Fig. 1: The sampling is located at Aloran, Misamis Occidental, Philippines with a nautical grid coordinate of 8°25'6" north latitude and 123°49'13" east longitude.

Opportunistic samplings of adult insects were done on randomly selected infected coconuts. A total of 60 adult specimens (30 males, 30 females) of *B. longissima* were used for the comparative analysis of the body shape. Male or female individual adults were classified by examining the sixth abdominal sternite where it is visible in males but invisible in females (Ayri & Ramamurthy, 2012). The appendages which were not needed were carefully removed with the aid of forceps and a clear digital image of the ventral body was taken using an HP2410 flatbed scanner at 2400 resolution (dpi).

Landmark which refers to a two or three-dimensional point described by a tightly defined set of rules were being established in the body of the insect. The first order landmarks (type I) are shown in figure 2, these being located at fixed points while the other landmarks (type II) were located at points of inflexion (Tabugo *et al*., 2014). Landmarks were captured using the computer program tpsDig, version 2.10 (Rohlf, 2006). The 21 landmarks identified were: 21 Landmark 1 is in the tipmost portion of the anus, Landmark 2 to 13 are in the 6th to 1st sternites, landmarks 14 and 15 are the lining in procoxal cavity in the 1st sternum, landmark 16 and 17 is lining in the procoxal cavity in the metasternum, landmark 18 and 19 is the joint between prosternal epimeron and mesoepisternum, and landmark 20 and 21 is located on the joint between gena and prosternum.

Thin-plate spline deformation grids were produced to visualize the ventral body shape differences between male and female *B. longissima*. After landmarking, Tps file of the images of male and female *B. longissima* was subjected to Relative warp analysis using TpsRelww32 ver. 1.46 software (Rohlf, 2008) with a link file for the variation in shape. This extracts x and y coordinates of landmarks on the images. The relative warps analysis and computation of partial-warp scores were obtained. Since the x and y coordinates processed would vary, these were standardized in order to remove biases in size, shape and rotation using Procrustes superimposition method (Rohlf, 1999). The differences in spline shape were then used to describe the scale shape differences between sexes of each species. The relative warp scores recorded were converted in MS Excel worksheet and were then used in analyzing intraspecific variation in body shape using the paleontological statistics (PAST) software (Hammer *et al*., 2001). The relative warp scores were used to generate values that constitute the multivariate shape data sets.

Discriminant Function Analysis (DFA) was used to best discriminate (separate) scale shape difference between sexes and equality of the means of the compared groups was tested using Hotelling's t-squarred showing a p value ($p < 0.05$) . Multivariate Analysis/Canonical Variate Analysis (MANOVA/CVA) was used for the analysis of these relative warp scores for the ventral body shape of the different sexes (Hammer *et al*., 2001).

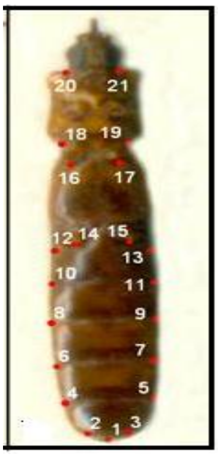

Fig. 2: Landmarks in the ventral body of *B. longissima*.

Results:

Figure 3 graphically show the results of the relative warp analysis of the shape in the 2 sexes of *B. longissima*. A total of 63.24 % variation was obtained from the first five relative warps in females. In males, four significant relative warps accounted for 67.35% of the total variation. The descriptions of the shapes are presented in Table 1. Variations in shapes within sexes were observed although majorities show the consensus shape. To compare the shapes between sexes, CVA and MANOVA (Hammer *et al*., 2001) of the relative warp scores were done and results indicate significant differences in body shapes of the 2 sexes of the insect pest (Tables 2 and 3, Fig. 4). These results indicate that sex differences are apparent in *B. longissima*. What is notable however is the generated results from the discriminant analysis. While males were significantly classified correctly, the females have higher frequency of being misclassified as males (Table 3, Fig. 5).

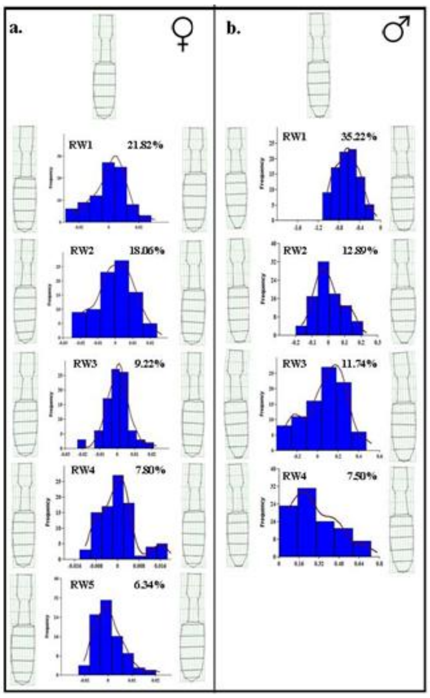

Fig. 3: Summary of the geometric morphometric analysis on the ventral body shape of (a) female and (b) male *B. longissima* from Aloran, Misamis Occidental. It shows the consensus morphology (uppermost figure) and the variation produced. The female results show 5 relative warps explaining more than 5% of the variance (63.24 % total variation). For the male, four relative warps with 67.35% of the total variation were determined.

Table 1: Descriptions of body shapes within sexes of *B. longissima* based on the significant result of the relative warp analysis.

	Female	Male
RW1	Observed variation from mean show bodies with a shorter distance from the joint between prosternal epimeron and mesoepisternum, and gena and prosternum. It has much elongated and larger abdomen.	Individuals have wide proternum and abdomen. The 6th sternum is much wider.
RW2	Observed variations show individuals with observed last sternum that is much wider and not pointed as well as more of an oval abdomen and there also a broad prosternum.	Variation show body shapes with a wider joint between prosternal epimeron and mesoepisternum. The prosternum is near to be perpendicular. Shape of the abdomen is slender.
RW3	Variant forms have the slender prosternum and slim abdomen.	More individuals has wider proternum, joint between prosternal epimeron and mesoepisternum and 6th sternum.
RW4	More variant forms have the shape of joint between prosternal epimeron and mesoepisternum that is not well pronounced. It has much elongated and larger abdomen.	Individuals' have much wide proternum and narrow metasternum. The abdomen is shown to be slender as well as the 6th sternum.
RW5	Variant forms have a well-defined joint between prosternal epimeron and mesoepisternum. The prosternum is wider and the abdomen elongated and larger.	

Table 2: Results of MANOVA test for significant variation in the shape of the ventral body in a population of female and male of *B. longissima*.

	value	df1	df2	F	p(same)
Wilk's Lambda	0.758	4	175	13.97	6.57 x 10 -10
Pillai trace	0.242	4	175	13.97	6.57 x 10 -10

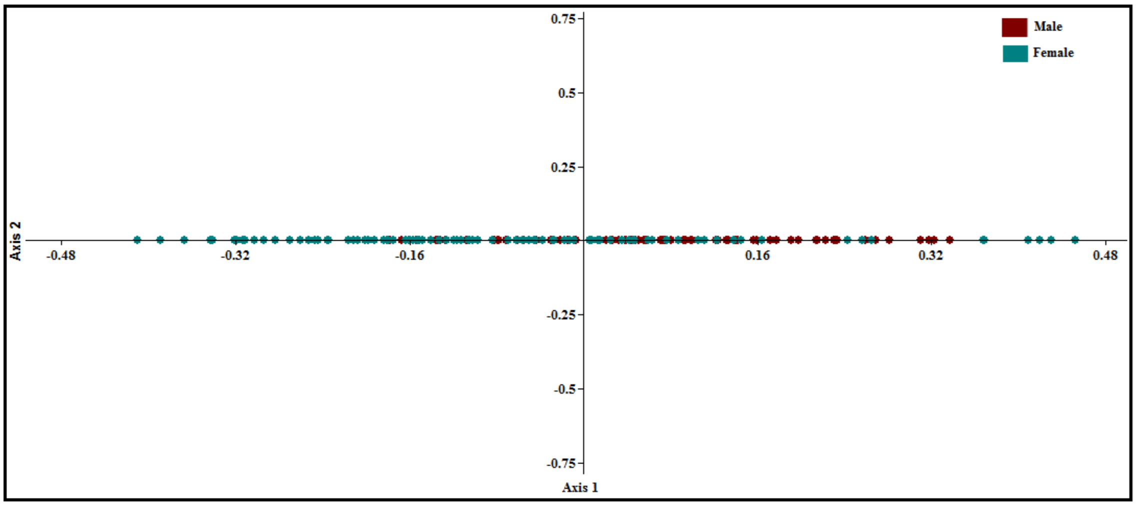

Fig. 4: CVA Scatterplot of the landmark coordinate data of male and female body shape variation for the first four relative warp scores.

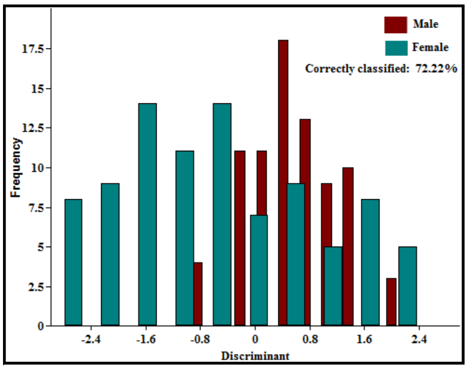

Fig. 5: Graph of Frequency Distribution of the Discriminant Scores (DFA) of the relative warp scores of highly significant scale variation between male and female *B. longissima* ($p = 6.571x10^{-10}$).

Table 3: Summary of the DFA results for *B. longissima* of female and male.

	N	Male	Female	Correctly classified (%)
Male	90	72	18	80
Female	90	58	32	64

Discussion:

Disparity between sexes in morphological characters are a widespread phenomenon in many animal taxa; the most conspicuous one is body size and the direction of the difference (whether males or females are larger) is different among species (Benitez *et al*., 2010). The results in this study show differences in the body shapes of the local populations of the male and female *B. longissima* and this can be attributed to the differences in variant forms within sexes. It is suggested that differences in development time between the sexes might be one of the major proximate mechanisms to produce sexual dimorphisms (Jarošík & Honek, 2007). The results of the DA which show differences between sexes however, also revealed a higher percentage of misclassified female individuals indicating there were individual shapes in females which are similar to that of the males. Esperk *et al.* (2007) argue that three basic mechanisms may lead to size differences between the sexes where the individuals of the ultimately larger sex could either be larger already at hatching/birth, grow faster (having higher instantaneous growth rates), or grow for a longer time (sexual maturation). These will b adding benefit for the species since females that are generally larger than males gives them adaptive advantages such as greater fecundity and better parental care (Andersson, 1994) thus may explain the results observed.

Conclusion:

Geometric morphometric (GM) analysis of body shapes of a local population of *B. longissima* indicate sexual dimorphism in the species. It was shown that variations within sexes may explain differences between sexes. The misclassification of some females as males can be attributed to several factors including differences in development and fecundity of the females. Nevertheless, the results of the study suggest that GM tools specifically relative warp analysis help make a good quantified description of the body shapes of the two sexes of the insect. Comparing variations observed between sexes using different statistical tools have helped explain the extent of the observed differences.

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