Article

Disparity in the pronotal shapes between sexes in the two populations of *Brontispa longissima* (Gestro, 1885) from Caraga region, Philippines using landmark-based geometric morphometrics

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Abstract
The study was conducted to describe the disparity in the pronotal shapes of *Brontispa longissima* populations in Caraga region, Philippines. Relative warp analysis was utilized for quantifying the shape while Canonical Variance Analysis (CVA) was used to compare shape variation between populations. Results from MANOVA test showed that individual symmetry has high significant values ($p<0.05$). In terms of determining the different variations between populations, pooled males have the p value of 7.62×10^{-15} and pooled females have 8.54×10^{-6}, wherein the two values display high significant scores between the two sexes. Moreover, determining the disparity of both sexes from the two geographical locations, Surigao population has the highest p value of 7.63×10^{-15} compared to Agusan population which obtained 6.072×10^{-12}, manifesting that populations from the two locations vary in the shape of the pronotum. CVA scatterplot also generates population discrimination indicating the presence of sexual dimorphism in pronotal shapes, while biogeographical factor may affect the shape structure in the pronotum of *B. longissima*.

Keywords geometric morphometrics; *B. longissima*; pronotum; shape disparity; Caraga.

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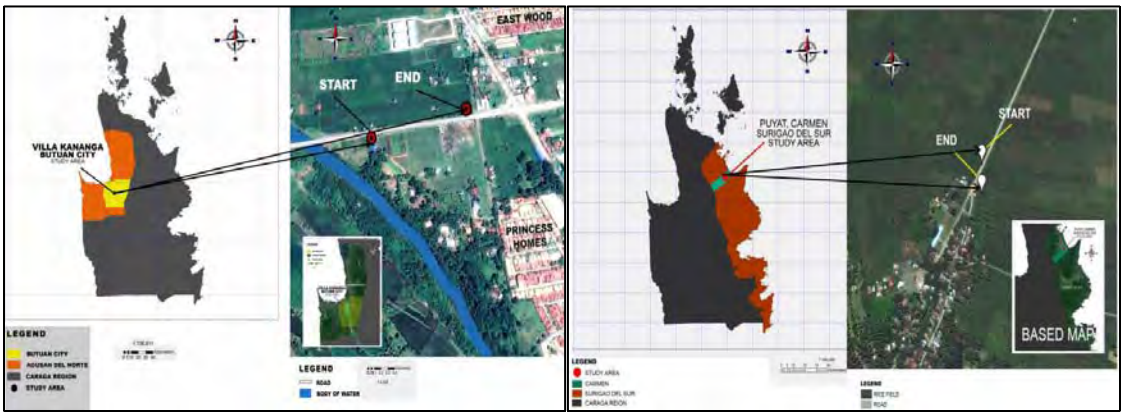
1 Introduction

The coconut plant plays an important role in the economy of many Asian countries by providing foods and becomes the main source of family income especially in the Philippines which is known in the world as the biggest exporter of coconut productions (Cuyacot et al., 2014). There are about 61.16 billion nuts of global coconut production from an area of 12.06 hectares, wherein in APCC (Asia and Pacific Coconut Community), 88% of many countries' annual production occurs. However, insect pests are the major constraints of enhancing coconut productivity and there are about 1,000 species associated to coconuts around the world. Due to a serious outbreak of the coconut hispine beetle, *Brontispa longissima* is considered a leading pest to a threatened economy of the country (Durst, 2007). *Brontispa longissima,* known as the coconut hispine beetle is one of the most damaging pests of coconut palms and commonly found on folded coconut palm leaflets (Acevedo, 2015). *B. longissima* is endemic in Papua New Guinea and Indonesia, and due to weak regulation of the palms, intensive transportation of coconuts has been disseminated in many countries especially in Southeast Asia such as the Philippines, Maldives and Cambodia (Fenner and Brown, 2014). Both adult and larvae feeds on buds of unopened leaf that causes leaves to turn brown in color and leads to death of the tree (Dujardin, 2011). They feed through chewing the youngest leaf in the throat of the palm, and removes the tissues of leaves and destroy its growing parts**.**Infestation of the palms caused by these beetles reduces photosynthesis, and worse, reduced into zero photosynthesis (Tabugo et al., 2012). By examining the sixth sternite of the beetle, sexes were differentiated from its extra tergite found at the ventral side of abdominal apices wherein visible to males but not in females (Barbon et al., 2014). On the other hand, one important structure in the understanding of the ecological distribution, behaviour and adaptation of insects is to study various insect body plans such as pronotum, which is one of the most important morphological structure that serves as an armor of segments in the pterothoracic region and initially serves as the attachment of muscles for locomotion of the front legs(Acevedo, 2015). Landmark-based analysis is used to quantify the co-variation of variables that involves biological shape and its shape variation (Webster and Sheet, 2010). The emergence on the statistical analysis for biological forms revolutionized our method and understanding in the shape disparity at the population level (Adams et al., 2004; Tabugo et al., 2017). Meanwhile, this study aims to determine the pronotal shape variations of *B. longissima* between the two populations from the provinces of Agusan del Norte and Surigao del Sur, Caraga Region. The determination in the variation of the pronotal shapes of *B. longissima* is an essential information in the understanding in the ecology and evolution of the organism as well as may help in the area of biomonitoring and pest control management.

2 Materials and Method

2.1 Sample collection

The study was conducted in two coconut plantation areas in Caraga Region. These plantation areas are from Villa Kananga, Butuan City, Agusan del Norte which lies between 8˚ 55' 19.91" and 8˚ 55' 20.27" North latitude and 125˚ 32' 12.81" and 125˚ 32' 21.26" East longitude, and Barangay Puyat, Carmen, Surigao del Sur that lies between 9˚ 13' 44.94" and 9˚ 13' 42.39" North latitude and 125˚ 59' 48.09" and 125˚ 59' 47.23" East longitude.



(A) **(B)**

Fig. 1 Map showing the collection of *B. longissima* from two different locations in Caraga Region, Philippines. (A) Villa Kananga, Butuan City, Agusan del Norte, and (B) Puyat, Carmen, Surigao del Sur.

Adult samples with 120 individuals of *B. longissima* were randomly collected (males and females) from each population between the months of December 2016 and January 2017.The collected samples were placed in vials and were preserved in 70% ethanol with 30% glacial acetic acid. Sexes were determined using dissecting microscope (Censico 27211). The differentiation between males and females were based on the extra tergite found in the ventral side of the abdominal apices of the male.

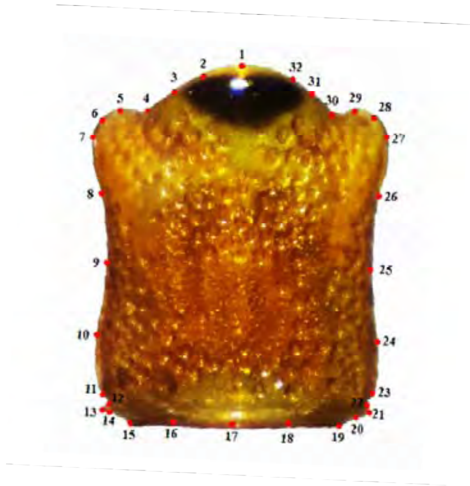


Fig. 2 Landmark points in the pronotal shape of *B. longissima* adult individual.

2.2 Processing of samples

The beetle samples were dissected to detach the pronotum from its body. Each pronotum was placed on a glass slide under the microscope for visualization. Each sample was taken for image using a digital camera (Nikon D3100). The images were then processed for orientation and digitisation errors. The captured images were converted to TPS format using tpsUtil program and subjected to tpsDig2 for the assignment of corresponding landmarks.

Table 1 Description of landmark points in pronotum of *B. longissima* adapted from (Polly, 2012).

Coordinates	Location
1	First LM in the tip of the anteriorpart of the pronotum
2	LM in the anterior margin of the pronotum between LM 1 and LM3
3	LM in the left juncture of the head andthe pronotum
4	LM in the diverging point betweenthe mid-anteriorand the left upper curve of the pronotum
5	LM in the left anterolateral marginof the pronotumbetween LM 4 and LM 6
6	LM in the tip of the left upper curve of the pronotum
7	LM in the left anterolateral margin of the pronotum between LM 6 and LM 8
8	LM in the curving point between theleft anterolateral margin and the left lateral margin
9	LM in the left lateral margin between LM 8 and LM 10
10	LM in the curving point between the left lateralmargin and the leftposterolateral side
11	LM in the curving point between LM 10 and LM 12
12	LM in the anterior base of tip of theleft posterolateralside of the pronotum
13	LM in the tip of the left posterolateral side of the pronotum
14	LM in the posterior base of tip of theleftposterolateral side of the pronotum
15	LM in the diverging point betweenthe leftposterolateralmargin and themid-posterior margin

16	LM in the posterolateral margin of the pronotum between LM 15 and LM 17
17	LM in the middle of the posterior part of the pronotum
18	LM in the posterolateral margin of the pronotum betweenLM 17 and LM 19
19	LM in the diverging point between the right lateral marginand the mid-posteriormargin
20	LM in the posterior base of tip of the rightposterolateral side of the pronotumparallel to LM 14
21	LM in the tip of the right posterolateral side of the pronotum
22	LM in the anterior base of tip of the rightposterolateral side of the pronotum parallel to LM 12
23	LM in the curving point between LM 22 and LM 24
24	LM in the curving pointbetween the right lateral margin andthe rightposterolateral side of the pronotum parallel to LM 10
25	LM in the right anterolateral margin ofthe pronotum between LM 24 and LM 26
26	LM in the curving point between the rightanterolateral margin and the right lateralmargin parallel to LM8
27	LM in the right anterolateralmargin of the pronotum parallel to LM 7
28	LM in the tip of the right upper curve of the pronotum parallel to LM6
29	LM in the right anterolateral margin parallel to LM 5
30	LM in the diverging point between right upper curve and the mid-anterior of the pronotum parallel to LM4
31	LM in the right juncture of the head andthe pronotum parallel to LM 3
32	LM in the anterior margin of the pronotum between LM 31 and LM 1

2.3 Data analysis

In landmark-based analysis, pronotal shapes of *B. longissima* were subjected to relative warp analysis to determine the disparity of the samples based from the digitized landmarks which includes consensus shape, centroid size and relative warp scores. It also shows the positive and negative deformation of the pronotal shape described in the grid square. The relative warp scores were recorded in WPS spreadsheets and were utilized as a source of analysing the variation in the pronotum of *B. longissima*. By using Paleontological/ Statistical Statistics Analysis (PAST), the results from relative warp analysis were generated in histograms and box-plots. The relative warp scores were then subjected to Canonical Variance Analysis (CVA) and Principal Component Analysis (PCA) (Polly, 2012).

3 Results and Discussion

The results from MANOVA test determines the significance of the pronotal shapes between the different sexes of *B. longissima.* Table 2 shows the significant values in the pronotal shape from the pooled females and pooled males. From the p values ($p < 0.05$), both sexes shows significant values wherein male population ($7.6x10^{-15}$) is relatively higher than females ($8.54x10^{-06}$), manifesting shape disparity among individuals were also observed between sexes in *B. longissima*.

Table 2 Results for MANOVA test for the pronotal shape of male and female populations of *B. longissima*.

Population	Wilk's Lambda	F	p-value
All Pooled Females	0.7474	6.364	$8.54x10^{-06}$
All Pooled Males	0.5203	21.02	$7.62x10^{-15}$

$p<0.05$: highly significant

Table 3 Results for MANOVA test for the pronotal shape of *B. longissima* from two different locations in Caraga region.

Population	Wilk's Lambda	F	p-value
Agusan del Norte	0.5872	16.03	6.07×10^{-12}
Surigao del Sur	0.6679	11.34	7.63×10^{-15}

$p<0.05$: highly significant

Also, Table 3 determines the significant values of *B. longissima* populations using pronotal shape variation from two different provinces in Caraga region. Both populations are significantly different wherein the Surigao del Sur population has p value of 7.63×10^{-15}, while Agusan del Norte has 6.07×10^{-12}.

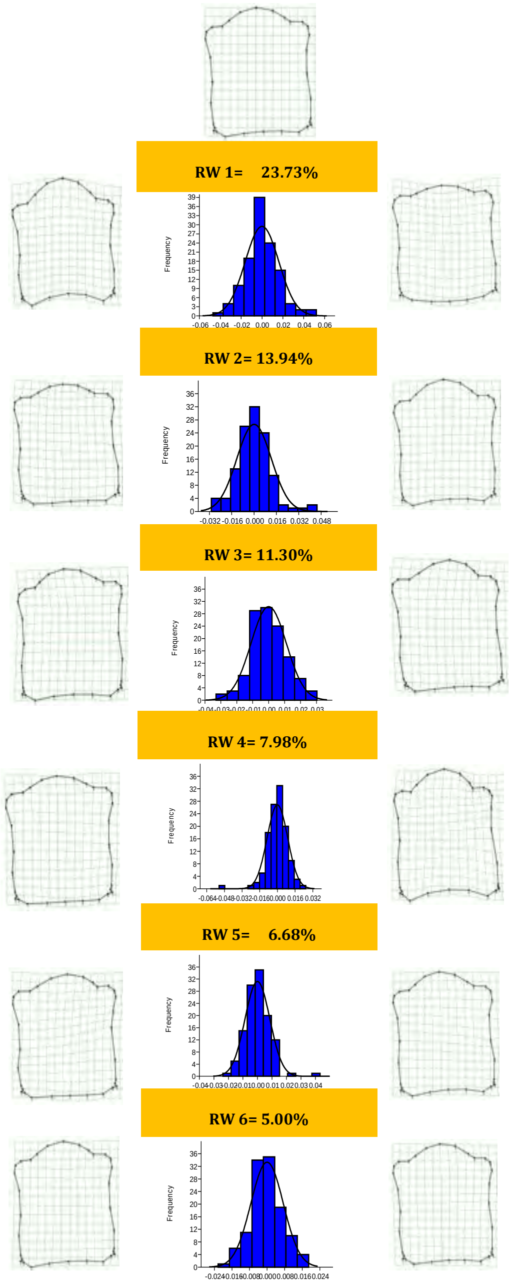


Fig. 3 Box plot showing the consensus pronotal shape of *B. longissima* of all-pooled females, left side (-) and right side (+).

Relative warp analysis were used to show the summary of the patterns in the disparity of pronotal shapes. The negative and positive deformation of the pronotum shape defined in the grid square was also shown through frequency histograms of the relative warp scores (Fig. 3). A total of six relative warps for the pooled female from the two study areas which explains the variance of five percent and more. RW 1 has 23.73% variation. Most of the disparities are found on the anterior tip of the structure which is slightly convex while disparity on the posterior is also observed to be more concave. On the other hand, differences are observed on the tip of the anterior described as very blunt with a slight curvature on the posterior region. RW 2 obtained 13.94% variation in the point of curvature between the right lateral margin. Variability in the shape is noticed as wider versus slightly concaved. RW 3 obtained a variance of 11.30% in the left and right junctures of the pronotal head indicating as more pronounced. Also, RW 4 is 7.98% which includes the differences in the right anterolateral margin, middle of the posterior portion of the pronotum and variation in the diverging point between the mid-anterior and the left-upper and right-upper curvatures that are slightly compressed. RW 5 has 6.68% variation affecting the right anterolateral margin, right juncture of the pronotal head and the mid-posterior portion. While RW 6 which has only 5.00%, also affects the mid-posterior region with a more pronounced structure and a wider right-upper curvature of the pronotum. The figure located at the topmost is the mean shape of the population.

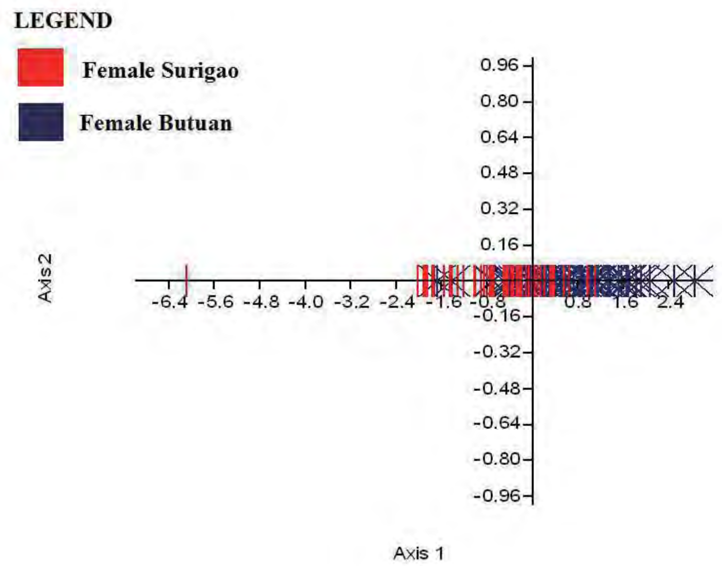


Fig. 4 CVA scatter plot of the landmark coordinates of all-pooled females in two different locations (Surigao and Butuan) showing pronotal shape variation of individuals.

Fig. 4 illustrates the comparison of the pronotal shape disparity of *B. longissima* between the females from different location in Caraga region. The female individuals represented by blue coordinates are from Butuan, Agusan del Norte, while red coordinates are the females from Carmen, Surigao del Sur. The plot displays population discrimination of females between the two locations, while few individuals from the two populationsmay observed overlapping with one another.

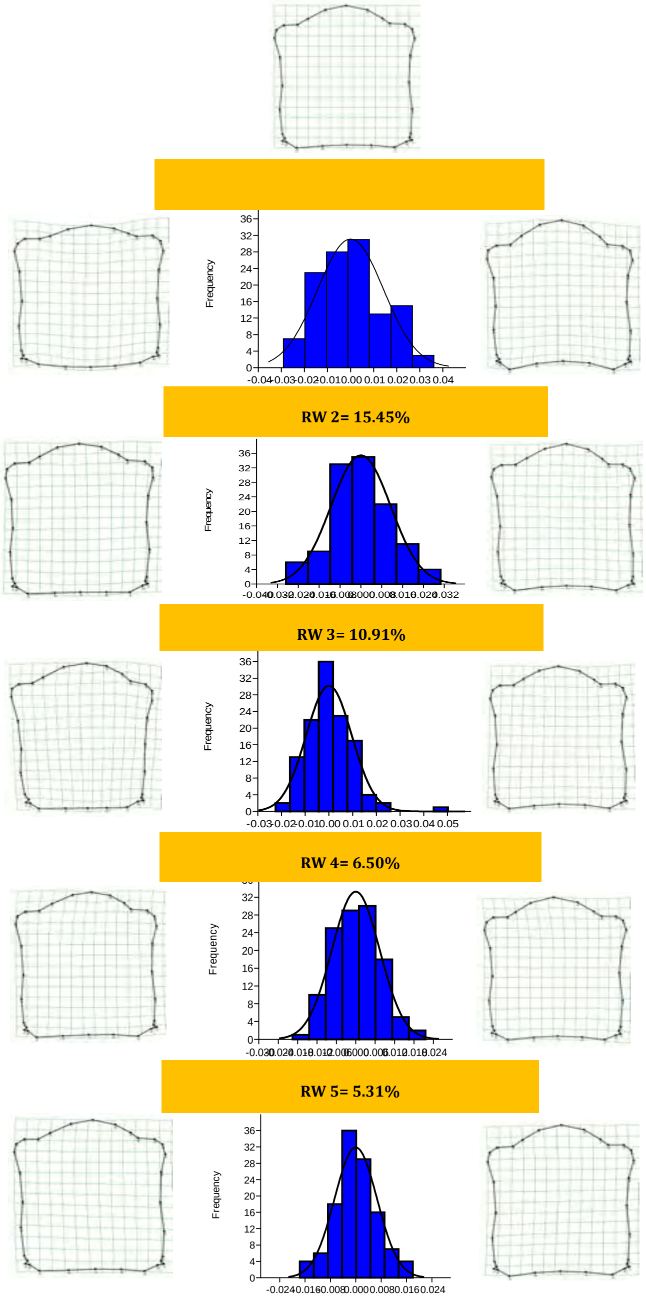


Fig. 5 Box plot showing the consensus pronotal shape of *B. longissima* of all-pooled males, left side (-) and right side (+).

The summary of disparity in the pronotal shape of pooled males of *B. longissima* pooled male was shown in Figure 5. Five relative warp variationswere noticed in *B. longissima* males from the two different locations. RW 1 varies in the posterolateral region of the structure described with blunt to concave margin with 24.26% score. Variation is also observed in the curvature point between the lateral margin which is described as pronounced, while the mid-posterior of the pronotum is slightly concaved. RW 2 has 15.45% score and observed to have pronounced posterolateral side. Variation is also determined in the curving point between the left lateral margin and the left anterolateral portion wherein observed to be more slender towards the base. The tip of the anterior region is also pronounced while the middle posterior part of the structure is slightly concaved. For the RW 3, disparity is on the curvature of the right lateral margin which is noticed to be more slender towards the base with 10.91%. Also, in RW 4 with a score of 6.50% varies on the anterior tip of the pronotum with an appeared to be wider against the more pronounced tip. Variation in the middle posterior part of the pronotum is also slightly concaved. In RW 5, a wider tip on the anterior tip and a wider pronotum on the posterior region is noticeable with a 5.31% score.

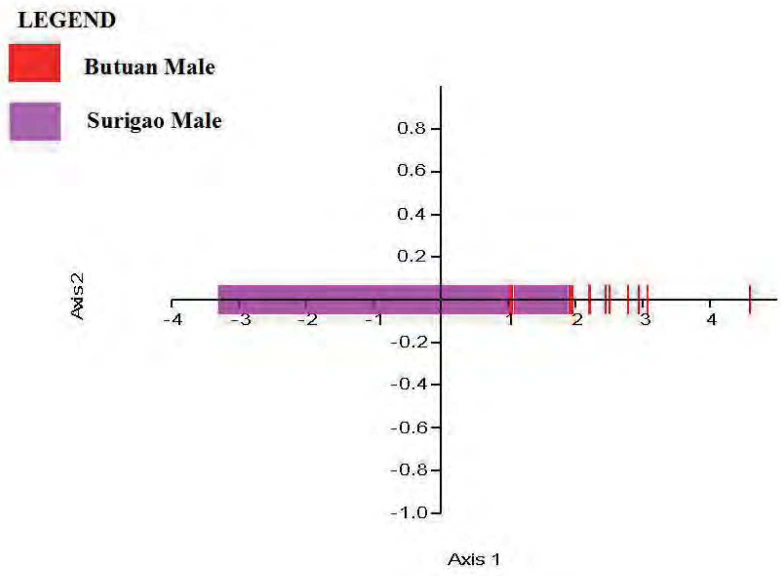


Fig. 6 CVA scatter plot of the landmark coordinates of all-pooled males in two different locations (Surigao and Butuan) showing pronotal shape variation of individuals.

Fig. 6 shows the pronotal shape variation comparing the populations of *B. longissima* males in two different areas of Caraga region. The male population from Carmen, Surigao del Sur is shown in purple coordinates situated on the left side of the plot, while male population from Butuan City, Agusan del Norte is located on the right side of the plot represented by red coordinates. The result of the plot appears to discriminate the two male populations from different locations indicating that most of the individuals in Butuan, Agusan del Norte is discriminated from Carmen, Surigao del Sur individuals, while a few individuals from Butuan population has overlapped with the Surigao population.

Fig. 7 demonstrates the summary of variations in the pronotal shape of all males and females of *B. longissima*in Butuan City, Agusan del Norte, wherein five relative warp variationswere distinguished in both males and females of*B. longissima*. RW1 has a score of 25.07% with a disparity on the tip of the anterior portion of the pronotum. Variation in the mid-posterior is also observed as concaved and slightly curved, while the curvature point between the right lateral margin which is slender, and the posterolateral margin of the structure which is slightly curved is also demonstrated. Differences are observed in RW 2 in the posterolateral sides and the tip of the right curvature of the pronotum with a score of 14.56%. For RW 3 with 11.49%, a slender curvature between the right lateral margin and right posterolateral side of the structure is observed. More pronounced tip on the anterior and right upper curvature is observed in RW4 with a score of 6.87%, while in RW 5 with a score of 6.46%, differences in the right junction of the head of the pronotum is reduced and the anterior tip left junction of the pronotal head are pronounced.

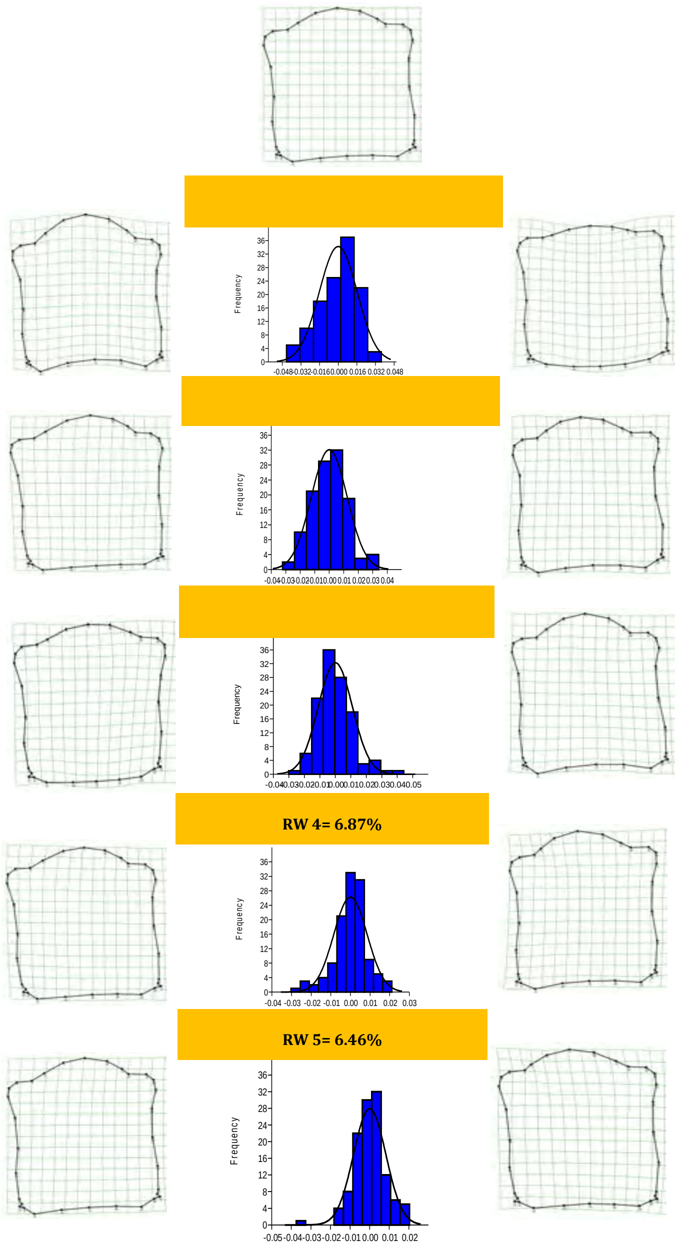


Fig. 7 Box plot showing the consensus pronotal shape of *B. longissima* of all males and females in Butuan City, Agusan del Norte, left side (-) and right side (+).

Fig. 8 displays the shape variation in the pronotum of *B. longissima* of Butuan City, Agusan del Norte comparing the populations of males and females using Canonical Variance Analysis (CVA). The coordinates in blue are the female individuals situating most of the samples on the left side of the box plot, however, male population represented by green coordinates are located on the right side of the plot, showing that there is a disparity in sexes using pronotal shapes.

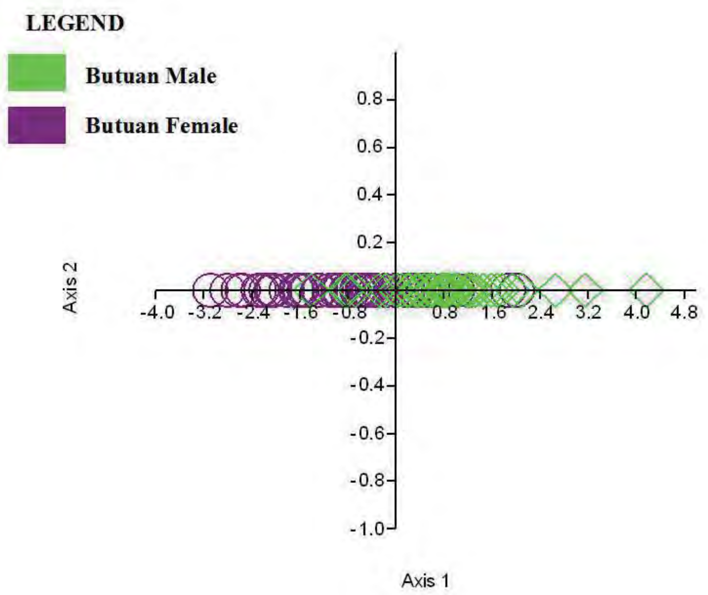


Fig. 8 CVA scatter plot distribution of *B. longissima*males and females from Butuan City, Agusan del Norte.

Fig. 9 is an illustration showing the summary of patterns in the pronotal shapes in both males and females of *B. longissima* from Surigao del Sur anda total of 5 relative warps were generated. RW 1 has 25.64% score including variations in the tip of the anterior and mid-posterior portion which is observed to be more blunt and wider as well as convex to concave-shaped. RW 2 varies on the anterior margin and the tip of the left curvature and the mid-posterior portion. Variation in the right junction of the pronotal head is also observed with a score of 14.99%. For RW 3, a total score of 9.33% is generated varying a wider pronotum on the mid-posterior portion, while convex on the anterior tip, pronounced on the left curvature and concave on the mid-posterior region. Distinction is also observed in the curvature between the right lateral and right posterolateral margin which is slender in RW4 revealing a score of 8.22%. Also, a more pronounced and concaved posterolateral margin in the tip of the upper right curvature is noticed. In RW 5, a score of 6.22% is observed wherein disparity is shown in the mid-posterior part of the pronotum as lightly concaved. Variation is also observed in the anterolateral margin of the pronotum which appeared to be more pronounced.

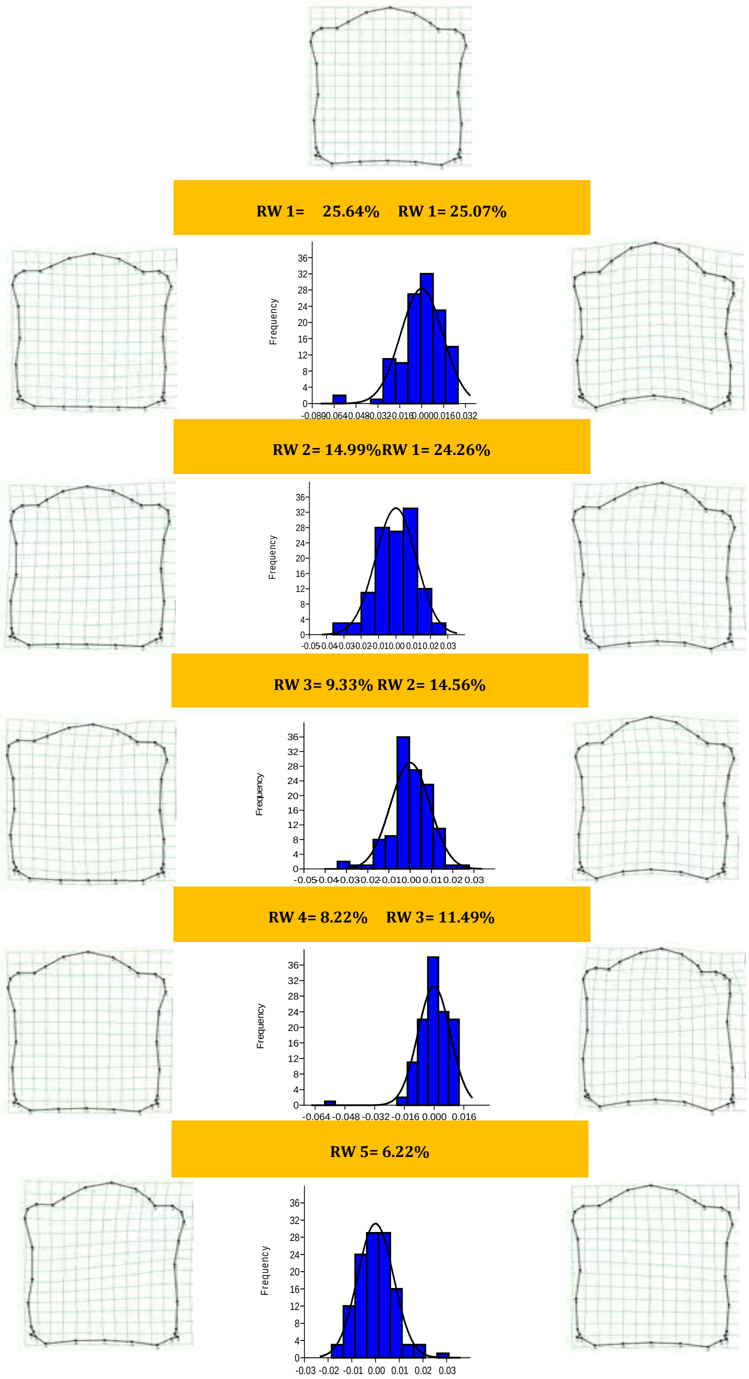


Fig. 9 Box plot showing the consensus pronotal shape of *B. longissima* of all males and females in Carmen, Surigao del Sur, left side (-) and right side (+).

Fig. 10 displays the pronotal shape disparity of both males and females of *B. longissima* from Carmen, Surigao del Sur comparing the two sexes using Canonical Variance Analysis (CVA). The green coordinates are represented by male individuals while females are denoted in blue coordinates, showing population discrimination of sexes using pronotal shapes.

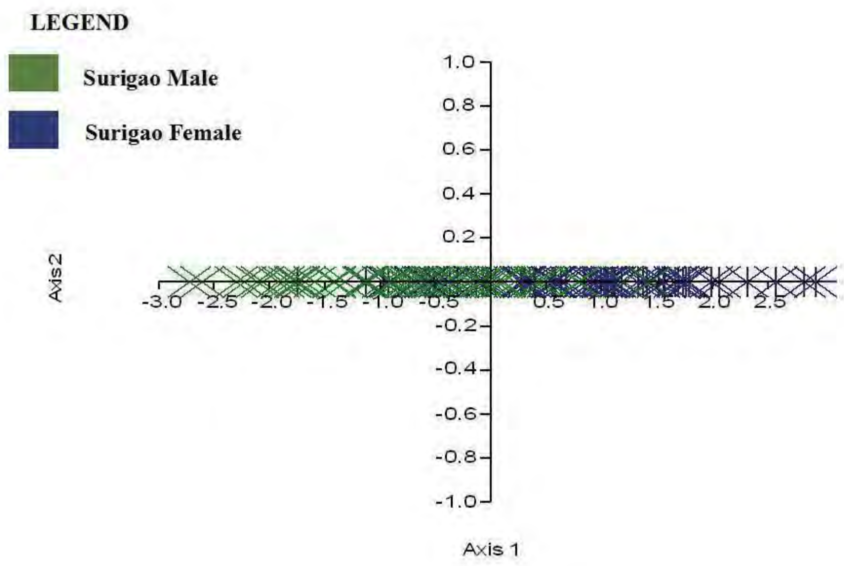


Fig. 10 CVA scatter plot distribution of *B. longissima*males and females from Carmen, Surigao del Sur.

The results of therelative warp analysis show disparities in the pronotal shapes between the two sexes of *B. longissima* from two different locations. The study revealsthe differences in the pronotal shapes of both male and female *B. longissima* from local populations showing that pronotum may play a vital role in the phenotypic variation within populations from different geographical locations. It has also been suggested that dissimilarities in the development between the sexes can lead to an ultimate mechanism that can create sexual dimorphism (Liebergts et al., 2006). There are several factors that attribute the variability of the shape such as environmental, genetic or behavioural features. According to (Lumnetut, 2013), variation in the structure may be obtained from intraspecific genetic differences, physiological status or adaptive changes. Also, biogeographical barrier and variety host plant may also play a vital part in the disparity of the structure in an organism (Berns, 2013), while dispersal and mean distance between connected individuals is also a premier importance to insect’s capability to succeed (Polly, 2012).

4 Conclusion

The pooled males has the highest p value ($7.62x10^{-15}$) than pooled females ($8.54x10^{-06}$), however both populations are significant. While discriminating the two populations from location, Carmen, Surigao del Sur has the highest p value ($7.63x10^{-15}$) than in Butuan City, Agusan del Norte ($6.07x10^{-12}$). The result from the relative warp analysis shows that there is sexual dimorphism among the individuals using pronotal shapes, as well as population discrimination between populations from different locations. While the study demonstrated the utilization of RW on determining the morphological disparity in the pronotal shapes of *B. longissima*, the result of the study may be helpful tool in understanding the role of geographical location towards phenotypic trait variation.

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