Journal of Nature Studies 10 (2): 44-52
ISSN: 1655-3179

DESCRIBING HEAD SHAPE VARIATION BETWEEN SEXES AND AMONG TWO COLORMORPHS OF THE RICE BUG, *Leptocorisa oratorius* Fabricius, 1794 (*Hemiptera:Alydidae*)

Clifford P. Bendoy, Mark Anthony J. Torres and Cesar G. Demayo
Department of Biological Sciences
College of Sciences and Mathematics
Mindanao State University-Iligan Institute of Technology
Iligan City, Philippines
Author for Correspondence: cliffordbendoy@yahoo.com, cliffordbendoy@yahoo.com, torres_markanthony@gmail.com, c_gdemayo@yahoo.com

Abstract - Two color morphs of the rice bug, *Leptocorisa oratorius* (Fabricius), exist in local populations in Northern Mindanao, Philippines – one with distinct ventrolateral abdominal spots and the other without. A complete understanding of the biology of these color morphs is necessary in the formulation of management strategies against this rice pest. Thus, this study was conducted to determine differences in the shape of the head capsule between sexes and between the two color morphs of rice bugs collected from three sites. Images of the head capsule were digitized using the TPS series producing seventy Cartesian coordinates which were then analyzed using Relative Warp Analysis. Relative warp scores were subjected to Canonical Variate Analysis (CVA) and Discriminant Function Analysis (DFA). Results showed significant differences in the shapes of the head capsule between sexes and among color morphs, which are consistent among the three populations of rice bug from Pinuyak in Lala, Sta. Elena, Steeltown in Iligan City, and Maigo, Lanao del Norte. Observed variations might have resulted from adaptation to environment that amplify their fitness to the host plant. The results of the research warrant further studies into the species status of the two color morphs of rice bugs from Northern Mindanao. Likewise, a complete understanding of the biological basis of the observed differences is necessary for the effective management of this species.

Keywords: Relative Warp Analysis,Geometric Morphometrics, CVA, DFA

INTRODUCTION

One of the most important things that gained an attention in rice production, which is considered to be the staple food of more than 60 per cent of the world's population especially for the people in South-East Asia [1], is the loss in production because of pests [2]. The average annual loss of rice was around 1.32 million tons due to the attacks of several insects which include rice bugs accounting 8.10 % of the annual loss [2]. The most important rice bugs in the subtropical and tropical rice areas belong to the genus *Leptocorisa*. In Indonesia *Leptocorisa oratorius* (Fabricius, 1794) commonly found in the rice field in the end of rainy season [3]. In Northern Mindanao, Philippines two color morphs of *L. oratorius* exist, one with distinct ventrolateral abdominal spots and one without ventrolateral spots on the abdomen.

The major measure to control this insect pest depends upon application of chemical pesticides. However, insecticidal control has led to several problems in insect pest management such as insecticide resistance in pests [4] because insect populations have a wide range of genetic variability that maximizes their fitness in the presence of genetic diversity of host plants [5].

Consequently, the search for new environmentally safe methods is being intensified [4] and reduction of insect pest damage and increase in the yield of rice requires the need for an integration of the fundamentals of rice insect taxonomy, its biology and ecology, several various insect control strategies, and development of protocols into a successful rice insect management program [5]. Successful control of any pest is based on correct identification. Inability to recognize distinct populations can have drastic and costly consequences for pest management [6].

Thus this study is intended to explore and quantify variations in the shape of the head capsule of the rice bug, *Leptocorisa oratorius* (Fabricius) using geometric morphometrics (GM). Utilization of this GM provides statistically considerable data or facts in identifying and quantifying the nature of morphological variations within species [7-8] on the basis of homologous anatomical landmarks in the shape of the head capsule of rice bugs. A comprehensive systematic understanding of these remarkable differences or variations could lead to an efficient development of management methods for Paddy Earhead bug, *L. oratorius*.

MATERIALS AND METHODS

A. Collection and Processing of Scales

To cite this paper: Bendoy, C., Torres, M.A. and Demayo, C. 2011. Describing head shape variation between sexes and among two colormorphs of the rice bug, *Leptocorisa oratorius* Fabricius, 1794 (*Hemiptera:Alydidae*) *Journal of Nature Studies*. 10 (2): 44-52

Collection of samples was done by swaying the insect sweep net to and fro the rice paddies throughout the rice field. Three different geographical locations in Northern Mindanao, Philippines comprised the sampling sites of the rice bug namely; Pinuyak, Lala, Lanao del Norte; Maigo, Lanao del Norte, and Sta. Elena, Steeltown, Iligan City (Figure 1).

Figure 1. Map of Northern Mindanao, Philippines showing the geographical locations of the three different sampling sites (darkened bits) namely; (a) Pinuyak, Lala, Lanao del Norte, (b) Maigo, Lanao del Norte, and (c) Sta. Elena, Steeltown, Iligan City.

Figure 2. Photograph of the rice bug *Leptocorisa oratorius* Fabricius (dorsal view) showing its various body parts including the head capsule (*www.forumkhonbaakpae.com/board/image_upload/8*)

Specimens were collected in three separate days, and the specimens obtained were placed in a bottle filled with 10% ethanol. Samples were then brought to the laboratory. A total sample of 1995 rice bugs were collected from three different sampling sites, 1089 of which were classified as males while the remaining 866 were classified as females. Prior to dissection, specimens were systematically identified up to the species level thus revealing the species' scientific name as *Leptocorisa oratorius* Fabricius (Hemiptera: Alydidae), commonly known as Paddy Earhead bug (Figure 2). After which, sexing of specimens was done. Using compound microscope, males were differentiated from females based on the external structure of their genitalia or terminalia located at the last or eleventh segment of the insect's abdomen. Males have blunt end while females have nearly pointed end (Figure 3). Dissection of the specimen involved removal of the head capsule from the body through the use of forceps and dissecting needles. Two color morphs were observed from the rice bugs samples collected (Figure 3). One morph had distinct ventrolateral spots on their abdomen and the other had no distinct ventrolateral abdominal spots.

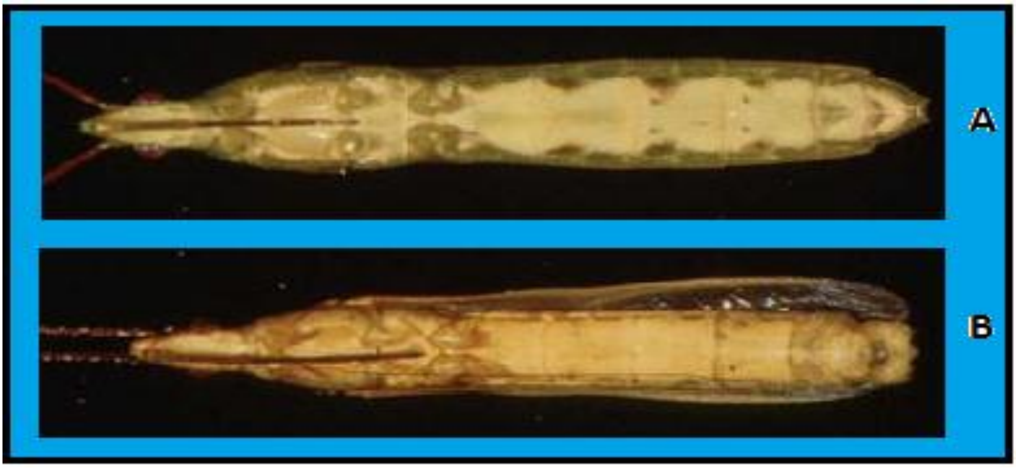

Figure 3. Photographs of a (a) male and (b) female rice bugs, *L. oratorius*. Two color morphs of rice bugs can also be summarized in this figure. *The uppermost figure represents the morph of rice bugs with no distinct ventrolateral abdominal spots while the lowermost panel represents the morph with distinct ventrolateral abdominal spots.* (*Jahn et al, 2003*)

Acquisition and Analysis of Data

Initially, images of the head capsule were taken using a Macron Cam attached to a Stereomicroscope, and digitized using TPS series. Thirty five (35) landmarks were defined in each specimen obtaining seventy (70) Cartesian (x- and y-) coordinates (Figure 4).

These anatomical landmarks that were transformed to Cartesian coordinates were then analyzed using Generalized Orthogonal-Least Square Method and Relative Warp Analysis. Consensus configuration of all the head capsules was then obtained and relative warps scores were then subjected to Thin-plate Spline Transformation Grid, Canonical Variate Analysis (CVA) and Discriminant Function Analysis in order to quantify variations in the head shape of the rice bugs between sexes and among two color morphs.

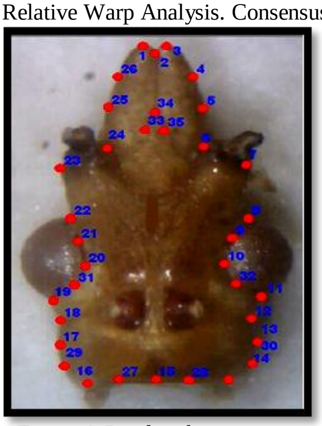

Figure 4. Landmark assignments and the locations of the thirty five (35) biological landmarks (red dots) on the head capsule of the rice bug, *L. oratorius* (F.).

Figure 5 shows the summary of procedures done to determine sexual dimorphism and color polymorphism in rice bugs, *L. oratorius* (Fabricius).

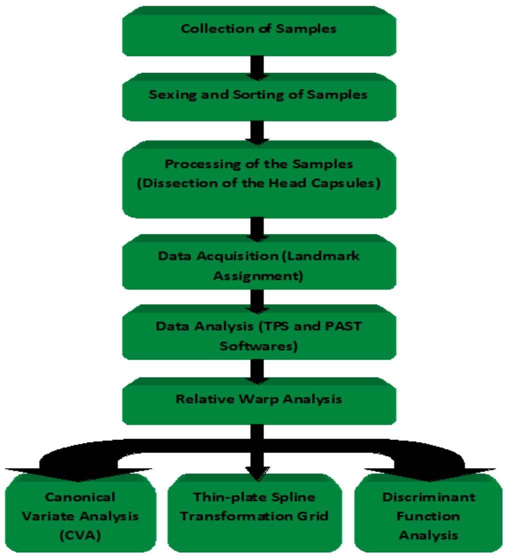

Figure 5. Summary of the procedures done to determine sexual dimorphism and color polymorphism in the rice bug, *L. oratorius* (Fabricius).

B. Thin-plate Spline Analysis

Patterns of head shape changes were analyzed using the thin-plate spline technique. Shape changes were shown as deformations and differences in shape among the specimens are expressed as a bending energy matrix. The eigenvectors of the bending energy matrix are known as principal warps. The eigenvalues associated to each principal warp are inversely related to the spatial scale of shape change, so that large eigenvalues describe small-scale deformations, whereas small eigenvalues indicate large-scale deformations.

C. Canonical Variate Analysis (CVA)

CVA was done using the Paleontological Statistics (PAST) software in order to determine variations among groups relative to the pooled within-groups variation. The canonical variates were displayed as an ordination and were scattered within groups.

D. Discriminant Function Analysis

Discriminant Function Analysis also known as Hotelling's Test was used not only to determine equality of the means of the two groups but also to reclassify specimens to previously defined groups. This type of statistical tool was performed using PAST in order to verify whether the observed variations between two color morphs as a result of CVA are statistically significant. This analysis is a standard method for visually confirming or rejecting the hypothesis that two groups are morphologically distinct [9,5].Two groups of multivariate, marked with different colors were used and analyzed using the paired Hotelling's test.

RESULTS AND DISCUSSION

Results of the Thin-plate Spline Analysis

Shown in Figure 6 were the non-affine or non-uniform shape changes expressed as deformations of the pooled female and pooled male rice bugs with ventrolateral spots on the abdomen. The two plates at the center are used as the reference configuration where all shape configurations of the head capsule are compared relative to it. Looking at the male and female rice bug plates from Maigo, it can be observed that females have stronger deformations, coupled with a bending energy of 0.17152, compared with those of the males which have lesser deformations with a bending energy of only 0.09229. There was a strong compression on the rightmost portion of the head in females while males have their leftmost portion of the head strongly compressed.

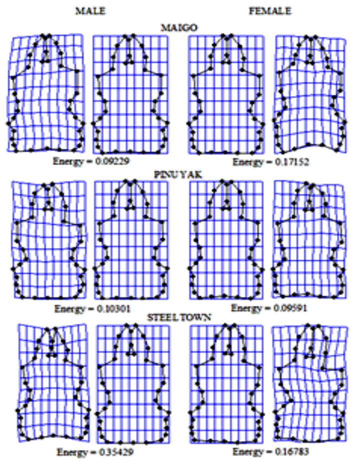

Figure 6. Results of the Thin-plate Spline Transformation grid of the three populations of rice bug without distinct ventrolateral abdominal spots showing patterns of head shape variation between sexes – male (leftmost panels) and female (rightmost panels).

Also, clear variations on the shape of the vertex and the labrums can be seen between sexes. Labrum of the females was slender and pointed while that of the males, was broad. The vertex of the female samples was greatly curved outward or rounded as compared to slightly curved vertex of the male samples.

Assessing the deformed male and female plates of the rice bugs from Pinuyak, a strong compression on the anterior midpoint of the head of the female rice bugs can be detected. In male samples, compression can be seen on the anterior leftmost portion of the head. Variations on the labrum and vertex can be observed also in these plates wherein females have shorter labrums and rounded vertex while males have larger labrums and plain vertex. Furthermore, the given bending energies indicate that males (with bending energy of 0.10301) have stronger deformations than females (bending energy of 0.09591).

Finally, comparing the plates of male and female rice bugs from Steeltown, it can be seen that males coupled with bending energy of 0.35429 have stronger deformation than that of the females which have bending energy of only 0.16783. Clear variations in the labrums and vertex can be seen. Labrums of the male individuals were smaller and pointed while those of the females were larger and slender. Also, the vertex of the male rice bugs was curved while that of the females was slightly bulged.

Presented in Figure 7 below is the transformation grid of the male and female rice bugs without distinct ventrolateral abdominal spots from three different sites. The first figure shows that female rice bugs from Maigo have stronger deformation as seen in the plate with bending energy of 0.11228 compared to male rice bugs with only 0.05815 bending energy. Detected in the male deformation plate was a slight inflation at the central portion of the head while a strong inflation at the anterior zone of the head was detected in the females. Slightly curved vertex can be seen in the females while on male individuals, they were more or less straight vertex.

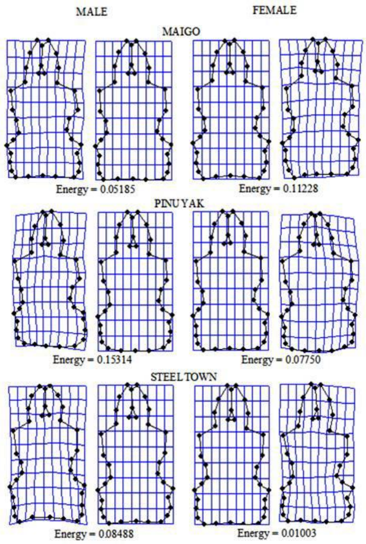

Figure 7. Results of the Thin-plate Spline Transformation grid of the three populations of rice bug without distinct ventrolateral abdominal spots showing patterns of head shape variation between sexes – male (leftmost panels) and female (rightmost panels).

Meanwhile, looking at the male and female plates of the Pinuyak rice bugs, the effect of deformation was not so strong in female samples with a bending energy of only 0.07750, in contrast with the strong deformations on the male samples with bending energy of 0.15314. A lateral variation was detected in the deformed shape wherein the left lateral portion was strongly compressed in males while females have their right lateral portion of the head strongly compressed. Also, variations on the labrums can be observed. Labrums of the males are larger with the right division bigger than the left one, whereas labrums of the females were smaller with the left division bigger than the right division.

In the last plates, still in figure 7, head shape variations within sexes of the rice bugs collected in Steeltown can be observed wherein female rice bugs have stronger deformations than the males, 0.1003 and 0.08488 correspondingly. Strong compression on the anterior zone of the head can be seen in the female deformation plate while slight inflation on the posterior most portion of the head was detected in the male samples. Also, variations on the labrum and vertex can be clearly seen wherein males have small labrum and curved vertex, compared to the large labrums and rounded vertex of the female samples. Shown in Figure 8 is the deformed plates of the pooled male and female rice bugs showing morphological variation on the shape of the head capsule between the two observed color morphs of rice bugs, with and without distinct ventrolateral abdominal spots. It can be observed in the deformed plates of the pooled male rice bugs that the two color morphs have only slight deformations with bending energies of 0.05919 and 0.04115, for with and without distinct ventrolateral abdominal spots respectively. Though the bending energies of the two color morphs were close to each other, this does not imply that there were no variations on the shape of the head capsule. By looking at the plates, it can be distinguished that rice bugs with distinct ventrolateral abdominal spots have larger and more slender labrum compared to the other morph.

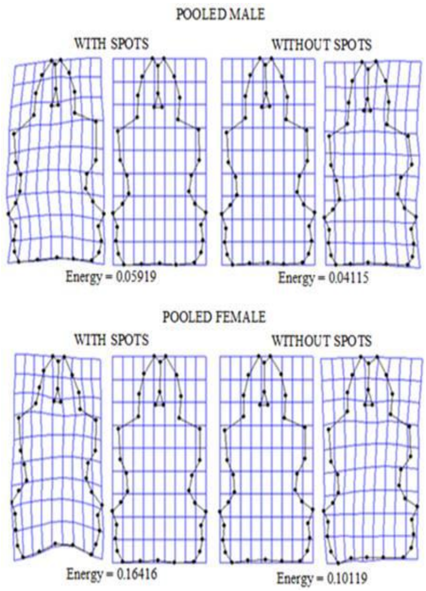

Figure 8. Results of the Thin-plate Spline (TPS) Transformationof the pooled male and female rice bugs showing patterns of head shape variations between the two color morphs as confirmed by the different deformations of the homogenous deformable metallic plates.

Also, a strong anterior compression of the head can be spotted in the deformation plate of the rice bugs without distinct ventrolateral abdominal while those of the rice bugs with distinct ventrolateral abdominal have their anterior zone of the head slightly extended/expanded.

On the other hand, a strong deformation of both color morphs can be observed in the deformed plates of the pooled female rice bugs. Rice bugs with distinct ventrolateral abdominal spots have stronger deformations with bending energy of 0.16416 compared to those without distinct ventrolateral abdominal spots with bending energy of 0.10119. Variations or deformations occurred mainly in the labrums and the vertex. Labrum of the rice bugs with distinct ventrolateral abdominal spots was smaller and slender while that of the rice bugs without distinct ventrolateral abdominal spots was bigger and more likely rounded. A deeply curved vertex was detected in rice bugs with distinct ventrolateral abdominal spots, compared to a slightly curved vertex of those without distinct ventrolateral abdominal spots. Also, a weak compression on the anterior part of the head can be found on the plate of the rice bugs with distinct ventrolateral abdominal spots while a strong dilation or expansion on the same area can be observed on the morph without distinct ventrolateral abdominal spots.

Results of the Cannonical Variate Analysis

Results of the CVA are scatter plot (Figure 9) with convex hulls and analyzed using covariance structure approach. This type of analysis is a method of multivariate analysis and is closely related to multivariate analysis of variance (MANOVA), hence, the values of the Wilk's lambda and p(same) or Hotelling's T2 are very essential in determining the relationship between variables and testing the significance of the observed variations respectively. As the value of Wilk's lambda approaches to zero, the relationship between variables gets stronger. On the other hand, observed variation was considered to be significant if the p(same) value was less than the threshold value 0.05.

Head shape variation between two color morphs via the CVA was illustrated in a CVA scatter plot in Figure 9. It shows an overlapping among morphs which was consistent throughout the three locations indicating variation between groups was quite small. Three group variations showed uniqueness since minor overlapping of variables was observed. Despite this, they still tend to have a group structure which could be inferred to be similar since overlapping occurred. These include the overlapping of Steeltown with spots and Steeltown without spots, between Steeltown with spots and Maigo without spots, and that of the Steeltown without spots and Maigo without spots. However, these observed variations were said to be significant since the p(same) value is lesser than the threshold value ($1.21e^{-29}<0.05$).

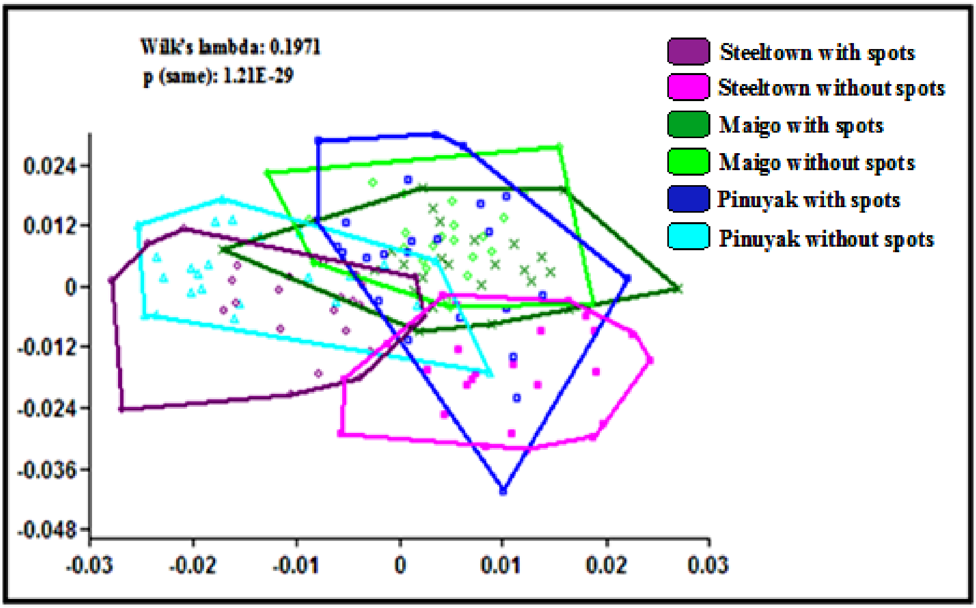

Figure 9. CVA scatter plot of the pooled male populations collected from 3 sites showing relationship between two color morphs - with and without distinct ventrolateral abdominal.

From Figure 10, it is very evident that there was a clear separation between Steeltown with spots and Pinuyak with spots, as well as between the Steeltown with spots and Maigo without spots correlations. Results indicate that there were highly significant variations between these groups. Also, Hotelling's Pairwise comparison value which was lesser than the threshold value. This result is consistent among the pooled populations of male and female rice bugs of the said sampling area.

Results of the Discriminant Function Analysis

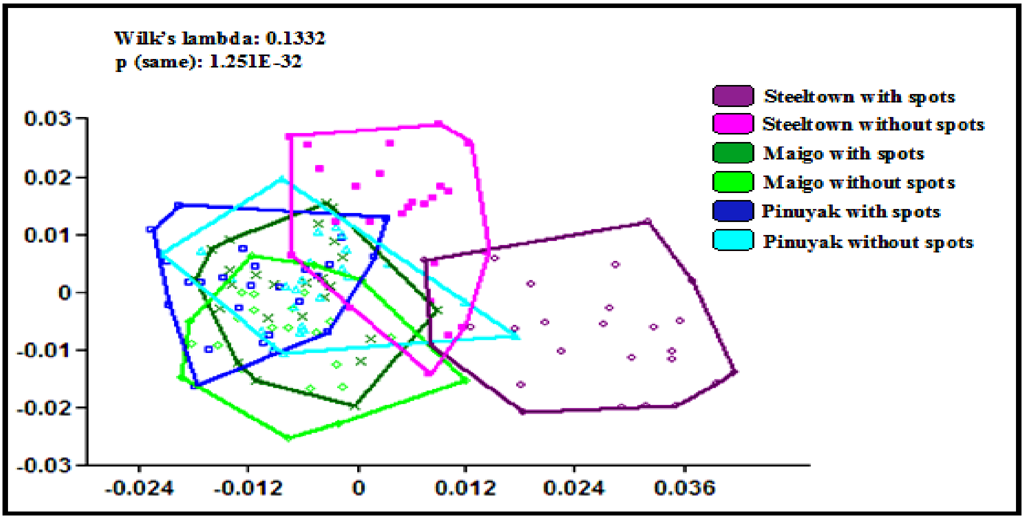

Figure 10. CVA scatter plot of the pooled female populations collected from 3 sites showing relationship between two color morphs - with and without distinct ventrolateral abdominal

minor overlapping of variables can be observed between Steeltown with spots and Maigo with spots. Other than these, majority of the Hotelling's Pairwise comparisons show overlapping of convex hulls. This means that only small amount of variation was observed. Otherwise, these observed variations were said to be significant since p(same) which is $1.25e^{-32}$ value is lesser than the threshold value.

It can be seen in the two CVA scatter plots presented that correlations between two color morphs of rice bugs from Steeltown have the highest level of significance, as confirmed by the

Table 1 shows the reclassification of the pooled rice bugs with ventrolateral spot of the three locations using Discriminant function analysis, explaining 74 % of the original group cases were correctly classified. It can be seen that the highest percentage of group reclassified were from Steeltown, wherein 22 % of the group was classified to Maigo and 26 % to Pinuyak. Also, the group that has the lowest percentage of reclassification was from Maigo which reclassifies only 6 % of its original population to Steeltown.

Shown in Table 2 is the reclassification of the pooled rice bugs without distinct ventrolateral abdominal spots among the three populations

Table 1. Reclassification of The Pooled Rice Bugs with Distinct Ventrolateral Abdominal Spots among The Three Populations

	Predicted Group Membership				
	Sampling Sites	***Maigo***	***Pinuyak***	***Steeltown***	***Total***
Original Count	***Maigo***	35	3	12	50
	Pinuyak	4	36	10	50
	Steeltown	11	13	26	50
%	***Maigo***	70	24	6	100
	Pinuyak	8	72	20	100
	Steeltown	22	26	52	100

74 % of original group cases correctly classified.

Table 2. Reclassification of The Pooled Rice Bugs Without Distinct Ventrolateral Abdominal Spots among The Three Populations

	Predicted Group Membership				
	Sampling Sites	**Maigo**	**Pinuyak**	**Steeltown**	**Total**
Original Count	**Maigo**	40	3	7	50
	Pinuyak	2	41	7	50
	Steeltown	4	8	38	50
%	**Maigo**	80	6	14	100
	Pinuyak	4	82	14	100
	Steeltown	8	16	76	100

82.67 % of original group cases correctly classified

explaining 82.67% of the original group cases correctly classified. It is evident that the Steeltown group had the highest percentage of rice bugs reclassified to another group, which includes the reclassification of 16% of the total population from Pinuyak, wherein only 4 % were reclassified to Maigo and 14% to Steeltown.

SUMMARY AND CONCLUSION

Sexual dimorphism and color polymorphism in the rice bug, *L. oratorius* were the primary concerns of this study. These were done by quantifying variations in the shape of the head capsule of the rice bug using series of geometric morphometric tests. A total of 1995 rice bugs was collected from three different localities in Northern Mindanao, Philippines in which, 1089 were classified as male, and only 866 individuals were classified as female. Two major morphs or populations of rice bug were evaluated in this study namely; those with distinct ventrolateral abdominal spots and the other without. Images of the head capsule were taken using a Macron Cam coupled with Leica ES2 microscope, and digitized using the TPS series (Rohlf, 2004). Thirty-five (35) landmarks forming 70 Cartesian coordinates were analyzed using Relative Warp Analysis, and shape variability was summarized using Thin-plate spline transformation grid. These relative warp scores were also subjected to CVA and Discriminant Function Analysis using PAST software.

The results were consistent in showing significant variations in the shape of the head capsules between sexes and among two color morphs of rice bugs across three sites. The largest variations were associated with differences in the shape of the labrums and vertexes, and slight variations were also observed at the curvature or outline of the compound eye. For the color polymorphism test, the results of the CVA showed that the observed two color morphs differed from each other based on their head morphology as seen on the convex hulls in the CVA scatter plot and confirmed by their Hotelling's Pairwise correlation value. Also, the results of the thin-plate spline analysis showed that the two color morphs have variations in the shape of their head capsule and these variations occured mainly on the labrums. Rice bugs with distinct ventrolateral abdominal spots have generally strong bending energies, associated with strong compression on the anterior zone of the head, than with the rice bugs without distinct ventrolateral abdominal spots.

Rice bug feeding patterns and habitat preference might have influenced these observed variations in the labrums. Genetic factor can also be attributed to the variation in the labrums of the head capsule of this pest. Also, ecological stresses such as habitat destruction or eradication might have caused the variations in the vertex since these pests are the ones frequently being eliminated in rice fields. Likewise, the variations observed among the populations might also indicate a wide range of genetic variability in rice bugs that maximizes their fitness in the presence of genetic diversity of host plants [5].

Many other factors, such as evolutionary, genetic or ecological might have also caused these observed differences in the shape of the rice bugs. To ascertain these factors, however, more specific and thorough studies are needed. Although the obtained results were only preliminary and exploratory, they have demonstrated that even slight variations in the shape of the head capsule of a rice bug could be detected by geometric morphometric analysis. Nevertheless, the results of the study warrant further studies into the species status of the two color morphs of rice bugs from Northern Mindanao. Also, a complete understanding and proper identification of the biological basis of the observed differences is elemental for the effective management of this species. Furthermore, the consistency of geometric morphometric in providing significant results indicates that it can become an important tool in taxonomic studies of the genus *Leptocorisa* and in studying morphological variations of other biological forms.

Further studies toward this goal should be carried out trying to correlate the observed differences or variations to environmental and/or genetic factors such as habitat selection and food ecology. To obtain a more credible and conclusive results, several types of statistical analysis should be performed.

ACKNOWLEDGMENT

The authors would like to acknowledge the Department of science and Technology (DOST) of the Philippines for the scholarship grant.

REFERENCES

Shanmugam, T.R., Sendhil, R. and Thirumvalvalavan, V. 2006. Quantification and prioritization of constraints causing yield loss in rice (*Oryza sativa*) in India, *Agricultura Tropica et Subtropica*, 39(3).

Supriatna, A. 2003. Integrated pest management and its implementation by rice farmer in Java. *Jurnal Litbang Pertanian*, 22:109-115.

Pathak, M.D. and Khan, Z.R. 1994. *Insect Pests of Rice*. IRRI, Manila.

Loc, N.T. and Chi, V.T.B. 2005. Efficacy of some new isolates of *Metarhizium anisopliae* and *Beauveria bassiana* against rice earhead bug, *Leptocorisa acuta, Omonrice* 13: 69-75.

M.A.J. Torres, J.K. Lomansoc and C.G. Demayo. 2010. Variability in head shapes in three populations of the Rice Bug *Leptocorisa oratorius* (Fabricius) (Hemiptera: Alydidae), Egypt. Acad. J. biolog. Sci., 3 (1): 173 – 184.

S.B.J. Menken and S.A. Ulenberg. 1987. Biochemical characters in agricultural entomology. *Agricultural Zoology Review* 2: 305-360.

K. Caley, J. Grahame and P.J. Mill. 1995. A geographically based study of shell shape in small rough periwinkles. Hydrobiologia, 309: 181-193.

P. Conde-Padin, J.W. Grahame,and E. Rolan-Alvarez. 2007. Detecting shape differences in species of the *Littorina saxatilis* complex by morphometric analysis. Journal of Molluscan Studies, 73: 147-154.

Ø Hammer, D.A.T. Harper and P.D. Ryan. 2001. PAST: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontologia Electronica, 4(1):9. http://palaeo electronica.org/2001_1/past/issue1_01.htm.

JOURNAL OF NATURE STUDIES
(formerly Nature's Bulletin)
ISSN: 1655-3179