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Describing head shapes of white stem borers (*Schirpophaga innotata* Walker) that are able to survive on different rice types using Landmark based geometric morphometrics

Queenilyn B. Albutra, Mark Anthony J. Torres, and Cesar G. Demayo

Department of Biological Sciences, College of Science and Mathematics, MSU-Iligan Institute of Technology, Iligan City, Phillippines. Corresponding author: Queenilyn B. Albutra queenilyn_albutra@yahoo.com

Abstract. Rice stem borers are considered as the most serious insect pest of rice in Asia. It infects its plant host by burrowing into the stem using its mandible. However, apart from the mandible, the head of rice stem borers is also associated in the incursion process since it facilitates the entry of larvae to the rice plant. Differences in the head capsules have a direct effect on the ability of the insects to ingest hard foods rapidly. Different rice varieties in the Philippines serve as plant host for this pest and infestation occurred in different geographical location. Variations in habitat and plant host were thought to generate environmental variation in morphometric traits and host adapted herbivore phenotype respectively. Landmark based geometric morphometric analysis was used to assess the hypothesis that the head shape of white stem borer differ between populations with respect to different rice varieties and geographical location where it was obtained. Relative warp analysis showed variation in the head shape between different white stem borer (*Schirpophaga innotata* Walker) populations infesting different varieties of rice. Non-significant head shape variations were obtained between geographically separated populations. These results indicate that the rice host varieties play an important role in the selection of individuals that are able to counteract the resistance factors in plants.
Key words: *Schirpophaga innotata,* Landmark based geometric morphometric analysis, Relative warp analysis, MANOVA.

Introduction. One of the major crop production constraints in the Philippines is the damage caused by rice stem borers. The damage can decrease yield by as much 50%. Host plant resistance was argued to be one of the major deterrence to infestation but still many of the varieties produced only showed moderate resistance. Many deployed rice varieties suffered from stemborer outbreaks and in the Philippines especially in Mindanao, the white stem borer *Scirpophaga innotata* (Walker), is considered one of the major pests. This insect feeds exclusively on rice attacking the plant at all stages of growth (PhilRice 2007). Attacks during the vegetative stage will result to the death of the youngest shoot known as “deadhearts”. When the plants are infested during the reproductive stage, affected plants produce panicles with empty grains known as “whiteheads” (Rapusas et al 1999). The mandibles of rice stem borers allow them to bore and feed regularly in the rice plants which results in dying of the affected plants. However, apart from the mandible, the head of rice stem borers is also associated in the incursion process since it facilitates the entry of larvae to the rice plant. According to Bernays (1986), differences in the head capsules, with the correlated differences in mandibular power, have a direct effect on the ability of the insects to ingest hard foods rapidly. It is argued that when an insect population has different host plant, the possibility arises that gene flow is restricted between groups on different hosts that are subjected to divergent natural selection for host adaptations (Berlocher & Feder 2002). Although no rice variety that is completely resistant to rice stem borer’s attack, there are sufficient differences among varieties to make varietal resistance an important factor in insect control (Chandler 1967). According to Fordyce et al (2006), plant traits that confer resistance against herbivores can serve as strong selective agents on herbivore physiology, life history, behaviour, and morphology. Variation in plant resistance traits can lead to locally adapted herbivore phenotypes.

It was also argued that populations of the pests differ between geographical locations thus may explain variations in the response of the rice hosts to the insect pests. Since stem borers are highly mobile pests, variation in the quality of habitats may also be able to generate environmental variation in morphometric traits of the pests (Smith & Patton 1988). It is for this reason that geographic variation could have locally adapted populations that follow unique and divergent evolutionary path (Thompson 1988). Analyses of geographic variation in body size and the size of other functional parts are important for understanding locally variable adaptations, which may produce morphological diversity within insect species and potentially may lead to speciation. It is on this context that we investigated the shape of the head of rice stem borer's larvae that not only associated with the rice variety it infests but the geographical location where they were collected. We applied the Landmark-based method in geometric morphometric (GM) analysis to determine head shape variation between populations of white stem borer. The method of GM is considered one of the best approaches to have a better comparison of shapes and would no longer rely on qualitative descriptions that usually are being interpreted differently by individual scientists (Adams et al 2004). By the use of quantitative approach like GM in describing morphological variations, it will be easier to determine relationships between morphology and other variables thus one could make more informed deduction on organism's evolution (Adams 1999).

Materials and Methods. The white stem borer larvae were collected from different sampling sites in three provinces of Mindanao. They were Lanao del Norte, Misamis Occidental and Zamboanga del Sur respectively (Fig. 1). Aside from different sampling areas, samples were also obtained from different varieties of rice plant as shown in Table 1. A mature stage of rice plant was chosen in each of the sampling site. Rice stalks which appeared white in color and bearing no rice grains compared to the others were uprooted. With the aid of a knife, each stalk was longitudinally divided. Thus, revealing a colony of stem borer's larvae inside. The samples were then preserved in 95% ethyl alcohol.

Figure 1. Topographic view showing the sampling site in Lanao del Norte (Kapatagan), Misamis Occidental (Plaridel) and Zamboanga del Sur (Kumalarang).

Table 1

List of PSB/BPI/NSIC/IR rice variety and their corresponding characteristics

Rice Variety	Ave. Yield (t/ha)	Growth Duration	Height (cm)	Susceptibility
IR 72	5	112	88	MS
PSB Rc 18	5.1	123	102	I
PSB Rc 26H	5.6	110	88	I
BPI Ri 10	4.7	108	84	MR
NSIC Rc 158	7	113	NA	MR

Legend: MR- Moderately Resistant; I – Intermediate; MS – Moderately Susceptible.

For simplicity, different populations were labeled as Kap158, Kap18, Kap10, Kum26, Pla158, Pla26 and Pla72. Kap, Kum and Pla stands for Kapatagan, Kumalarang and Plaridel respectively where the samples were taken. 158, 18, 10, 26 and 72 stands for the NSIC Rc 158, PSB Rc 18, BPI Ri 10, PSB Rc 26H and IR 72 rice varieties respectively which serve as plant host of the white stem borer larvae.

Each larva was place in the test tube which contains 5% sodium hydroxide. A stereomicroscope was used to magnify the anterior portion of the larva. The image of the larvae's head capsule was captured using micronCAM attached to the stereomicroscope.

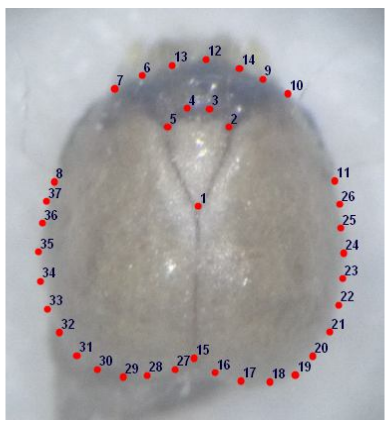

Figure 2. Digitized image of white stem borer's head showing the location of 37 anatomical landmarks.

Landmark based geometric morphometric analysis was employed in this study to determine and compare the shape of the head between populations of *S. innotata* taken from different localities and infest different varieties of rice. Two-dimensional Cartesian coordinates of the selected landmarks in the head capsule of the white stem borer's larvae were digitized using the program tpsDIG version 2.12 (Rohlf 2008a). The raw coordinate data were then subjected to generalized Procrustes analysis (GPA) to superimpose the landmark configurations using least-squares estimates for translation and rotation parameters (Adams et al 2004). GPA is an important procedure because it eliminates any variation due to differences in translation, orientation, and size, and would superimpose the objects in a common coordinate system.

The coordinates of superimposed configurations in all aligned specimens were used for the thin-plate splines relative warp analysis (Bookstein 1991) in order to analyze and display the direction of shape differences among species. The thin-plate splines technique (Bookstein 1991) consists of fitting an interpolating function to the landmark coordinates of each specimen against the reference configuration so that all homologous landmarks coincide. Thin-plate spline deformation grids were produced to visualize the wing shape differences between male and female white stem borers. The relative warps analysis and computation of partial-warp scores were done using tpsRelw program, version 1.46 (Rohlf 2008b). The projection of the superimposed specimens onto the principal warps produces the partial-warp scores, which describe their deviations from the reference configuration and that can be used as variables in subsequent multivariate statistical analyses (Rohlf 1999, 2004).

The relative warp scores were subjected to MANOVA and Kruskal-Wallis test using PAST software version 1.91 (Hammer et al 2001) to determine whether the head shape of white stem borer differ significantly between different populations. When MANOVA showed significant results, the analysis would proceed to Hotelling's pairwise comparisons (post-hoc) test. Kruskal-Wallis test was also done to determine whether the shape variations observed in each of the significant relative warps are also considered statistically significant.

Results and Discussion. Using the thirty-seven landmarks, the CVA scatterplots revealed no significant variations between geographically different populations of the stem borer attacking varieties of the same rice variety (Fig. 3A,B, Table 2). However, when populations of the stem borer attacking different varieties were compared, variations in the head shapes observed (Fig. 3C, D, Table 2). Comparison of head shapes are described based from the relative warp analysis (Figs 4 and 5, Table 3). Kruskal-Wallis test was employed to verify whether the shape variations observed in each of the significant relative warps can be considered statistically significant. The result of the test is shown in Table 4.

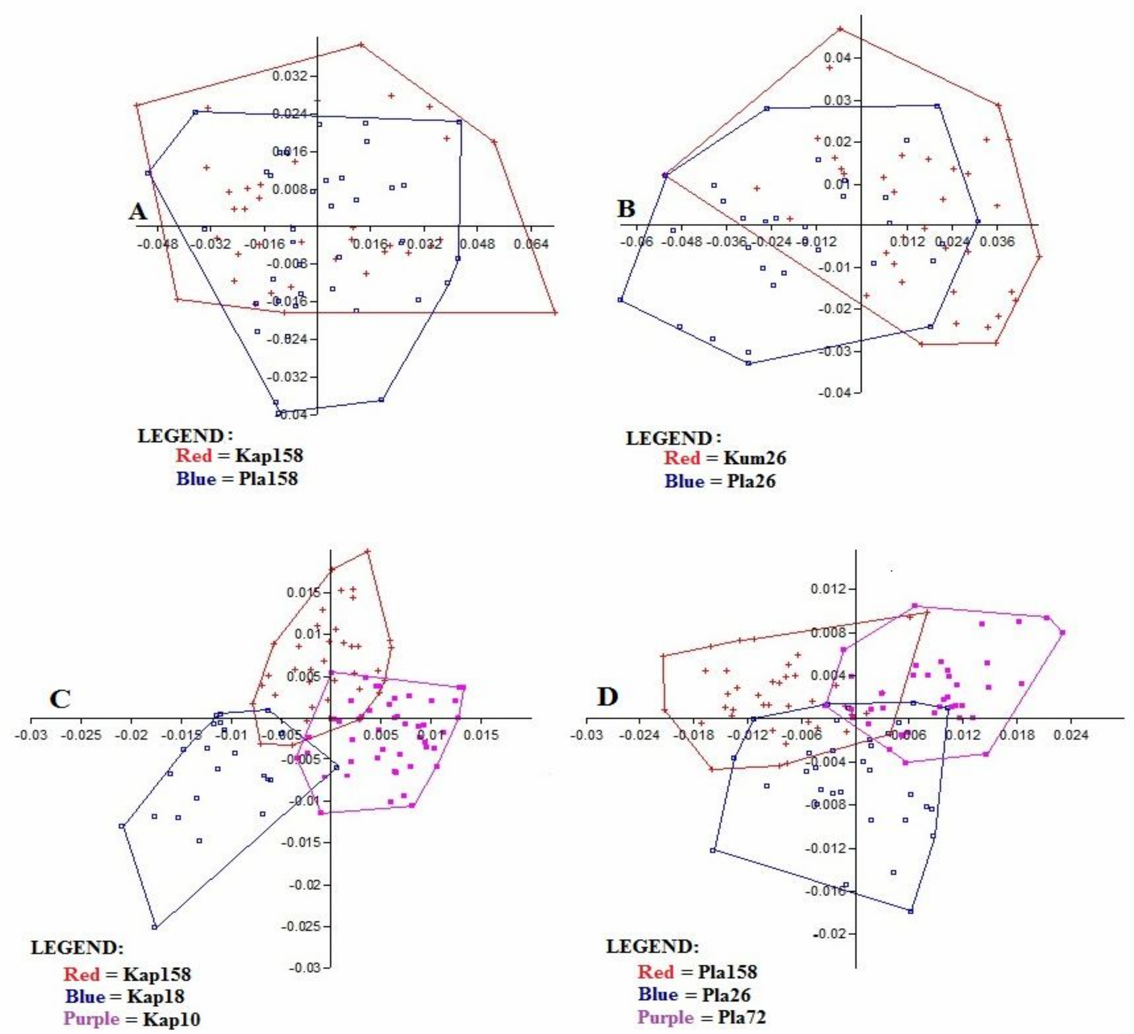

Figure 3. CVA scatter plot showing the head shape distribution of different white stem borer populations attacking the same varieties but different geographical location (A and B); and between different varieties of rice (C and D).

Table 2

Results of MANOVA test for significant variation in the head shape between different populations of white stem borer

	Wilk's Lambda	df1	df2	F	p(same)
Between Kap158 and Pla158 population	0.1018	60	15	2.794	0.05673
Between Kum26 and Pla26 population	0.1172	60	16	2.005	0.06187
Between Kap158, Kap18 and Kap10 population	0.05808	100	110	3.545	**1.265E-10**
Between Pla158, Pla26 and Pla72 population	0.09518	100	118	2.645	**2.543E-07**

Table 3

Variation in the head shape between white stem borer populations that host different rice varieties as explained by each of the significant relative warps and its corresponding percentage variance

RW	% Variance	Kap158 vs. Kap18 vs. Kap10	% Variance	Pla158 vs. Pla26 vs. Pla72
1	26.36%	Variation in the base of the vertex and location of the frons of the head. Among the three populations, Kap18 has the most varied vertex and frons. Kap18 population has head characterized with less define curvature in the base of the vertex and with frons shifted toward the center.	26.95%	Variation in the shape of anterior region and base of the vertex. Pla158 population has head characterized with pointed anterior region and more defined curvature in the base of the vertex compared to Pla26 and Pla72 with slightly rounded anterior region and less defined curvature in the base of the vertex.
2	14.90%	Variation in the shape of anterior region and width of the head. Kap18 population has slender head with more pointed anterior region compared to Kap158 and Ka10 populations.	13.66%	Variation in the shape of the anterior region and position of the frons. The Pla158 and Pla26 populations share the same head feature with slightly blunt anterior region and frons shifted towards the anterior region. Pla72 population is slightly different with head having pointed anterior region and frons shifted near the center of the head.
3	9.47%	Variation in the width of the head. Kap10 population showed a wider head compared to Kap158 and Kap18 populations.	9.21%	Variation in the dimension of frons and width of the head. Pla158 population is characterized with slender head compared to Pla26 and Pla72 populations.
4	6.85%	Variation in the dimension of the frons and width of the head. Kap10 population has slender head with larger frons compared to Kap18 populations with larger frons and broader head. Kap158 population has the average head shape.	7.51%	Variation in width of the head. There is no considerable variation in the width of the head between populations. The presence of outliers in Pla26 and Pla72 populations made the populations varied in this relative warp.
5	6.28%	Variation in the shape of anterior region and width of the head. Kap10 and Kap158 populations have slender head with pointed anterior margin compared to Kap18 populations that have wider head with slightly blunt anterior region.	5.26%	Variation in the shape of the anterior region and base of the vertex. Pla158 population has head characterized with more pointed anterior region and less defined curvature in the base of the vertex compared to Pla26 and Pla72 populations.
6	6.16%	Variation in the dimension of frons and base of the vertex. Kap18 population has head characterized with large frons and less defined curvature in the base of the vertex compared to Kap158 and Kap10 populations with smaller frons and more defined curvature in the base of the vertex.		

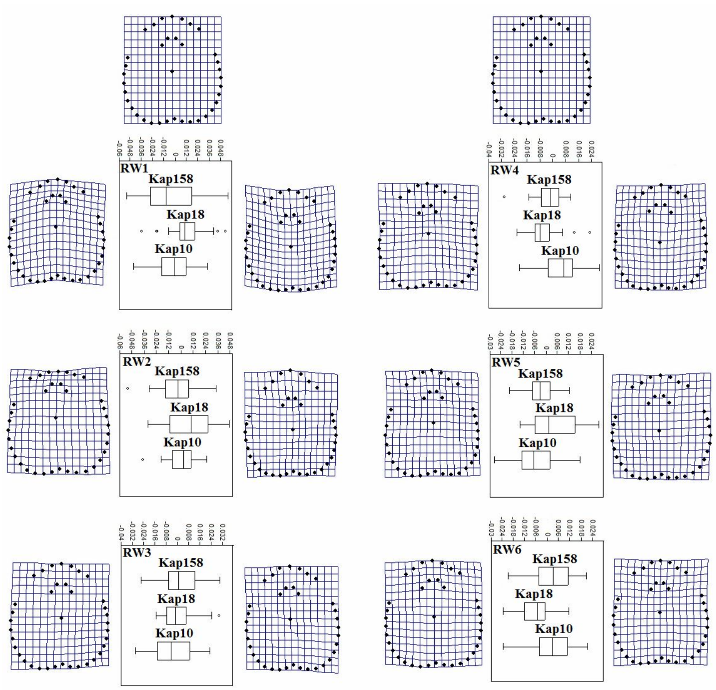

Figure 4. Summary of Landmark based geometric morphometric analysis showing the consensus morphology (uppermost panels) and the variation in the shape of head between Kap158, Kap18 and Kap10 population of white stem borer as explained by each of the significant relative warps.

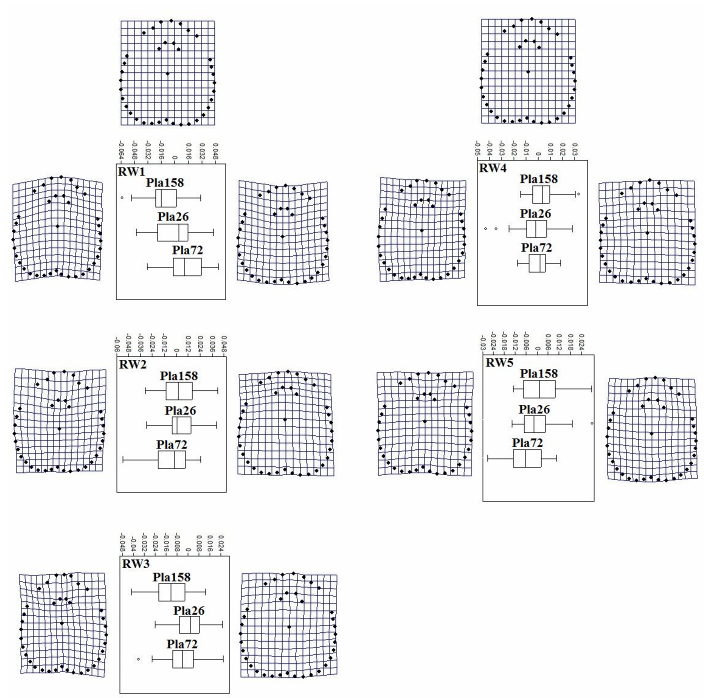

Figure 5. Summary of Landmark based geometric morphometric analysis showing the consensus morphology (uppermost panels) and the variation in the shape of head between Pla158, Pla26 and Pla72 population of white stem borer as explained by each of the significant relative warps.

Table 4

Results of Kruskal-Wallis test evaluating for significant variation in the head shape between populations of white stem borer that host different rice varieties based on each significant relative warps

Variation Between Kap158, Kap18 and Kap10				
Relative Warp	p-value	Mann-Whitney Pairwise Comparison		
		Kap158 vs. Kap18	Kap158 vs. Kap10	Kap18 vs. Kap10
1	**0.01774**	**0.02349**	0.3129	**0.008029**
2	0.09965	0.05737	0.1461	0.2324
3	0.5217	0.7902	0.3441	0.3483
4	**$7.931 x 10^{-5}$**	**0.04316**	**0.000944**	**0.000366**
5	**$8.79 x 10^{-5}$**	**$5.637 x 10^{-5}$**	0.736	**0.002981**
6	**0.001743**	**0.001648**	0.962	**0.001053**
Variation Between Pla158, Pla26 and Pla72				
Relative Warp	p-value	Mann-Whitney Pairwise Comparison		
		Pla158 vs. Pla26	Pla158 vs. Pla72	Pla26 vs. Pla72
1	**$4.084 x 10^{-5}$**	**0.0461**	**$5.244 x 10^{-6}$**	**0.03859**
2	0.07973	0.7639	0.07119	**0.04255**
3	**0.00719**	**0.00438**	**0.02569**	0.1569
4	0.1426	0.07804	0.131	0.5479
5	**0.00833**	0.5595	**0.01844**	**0.04435**

It can be seen from the results that differences in rice varieties were the major factors that determined variations in the head shape of white stem borers. Variation due to differences in the rice variety of plant host is associated with the varying resistance of the different rice varieties against the pest. Statistical and relative warp analysis showed significant variation in the head shape between the populations that host moderately resistant rice variety and susceptible rice variety e.g. between Kap10 and Kap18 populations. As shown in table 1, BPI Ri10 rice variety is more resistant against stem borer attacks compared to PSB Rc 18. The population of white stem borer that infest moderately resistant rice variety has an evidently slender head with pointed anterior region while those that infest susceptible rice variety has wider head with rounder anterior region.

According to Fordyce et al (2006), plant traits that confer resistance against herbivores can serve as strong selective agents on herbivore physiology, life history, behaviour, and morphology. Variation in plant resistance traits can lead to locally adapted herbivore phenotypes. Various morphological and anatomical plant characters were correlated with stem borer susceptibility. Tall plants with wide leaves and thick stems were more susceptible to stem borer damage. Whereas varieties with vascular bundles arranged closer than the width of the larval head offered resistance to larval boring (Chandler 1967). In a study on rice plants against striped stem borer (*Chilo suppressalis*), the interaction of leaf blade with a hairy upper surface, tight leaf sheath wrapping, small stem with rigid surface and thicker hypodermal layers were associated with resistant rice variety (Patanakamjorn 1965). Resistant rice varieties have thinner stem diameter compared to the susceptible varieties (Chandler 1967; Hosseini et al 2011). For the larvae to successfully penetrate its host plant with thinner stem diameter, it must press itself into the smaller hole causing its head to become slender and pointed. When an insect population has two or more host species, the possibility arises that gene flow is restricted between groups on different hosts that are subjected to divergent natural selection for host adaptations (Berlocher & Feder 2002) as shown in this study.

Conclusion. Landmark based geometric morphometric analysis in the head shape of white stem borer's (*Schirpophaga innotata* Walker) larvae showed variation between populations attacking rice varieties with different level of resistance. Relative warp analysis revealed that the variation in the head shape was mainly due to the variation in the anterior region and over-all width of the head. Head shape variation due to differences in the rice variety was associated with the varying resistance of the different rice varieties against stem borer attacks. Morphological characteristics of the variety and although not investigated in this study the biochemical characteristics of the plants were responsible for the variations observed in the head shape.

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Authors:
Queenilyn B. Albutra, Department of Biological Sciences, College of Science and Mathematics, MSU-Iligan Institute of Technology, Iligan City, Phillippines, e-mail: queenilyn_albutra@yahoo.com
Mark Anthony J. Torres, Department of Biological Sciences, College of Science and Mathematics, MSU-Iligan Institute of Technology, Iligan City, Phillippines.
Cesar G. Demayo, Department of Biological Sciences, College of Science and Mathematics, MSU-Iligan Institute of Technology, Iligan City, Phillippines, e-mail: cgdemayo@gmail.com
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