Egypt. Acad. J. biolog. Sci., 4 (1): 21-31 (2011) **A. Entomology**
Email: egyptianacademic@yahoo.com **ISSN: 1687–8809**
Received: 2/2/2010 **www.eajbs.eg.net**

Geometric Morphometric Analysis of the Head, Pronotum and Genitalia of the Rice Black Bug Associated with Selected Rice Types

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ABSTRACT

The Rice black bug, *Scotinophora coarctata* (Fabricius, 1798) is one of the major rice pests causing high yield loss in terms of rice production in the Philippines. Resistant varieties developed to reduce yield has never been successful as there are populations of the pest that are able to survive and utilize the different rice types. It was argued that population differentiation occurs in deployed rice varieties thus this study was conducted. Landmark - based and outline morphometric analysis and correlation analysis based on distances (CORIANDIS) were used to compare the shape and sizes of the head, pronotum, abdominal segment, genital plate and scutellum of rice black bug individuals associated with rice host varieties. Results of the analysis showed that RBB populations differ in the shapes and sizes of the scutellum indicating a significant rice host associated shape differences. It can be concluded from this study that rice types or genotypes affect population structures of the pest.
Keywords: Geometric Morphometric Analysis, Pronotum , Genitalia, Rice Black Bug

INTRODUCTION

Rice is one of the dominant and most important food crops of the world based on cultivated area (Heinrichs, 1986). About 80% of the world's population depend on rice as a major source of calories (Zohary and Hopf, 2000 as cited by Genil, 2007). However, rice production has been decreasing all these years due to pest outbreaks. Increase or decrease for about 10% in food grains production on a global scale can make a difference between a glut and acute scarcity (Swaminathan, 1983; Heinrich, 1986). Since rice is the staple crop of practically all nations in Asia, a tremendous effort has been exerted in the attempt to meet the demand for rice of the rapidly growing populations. Therefore, there is a continuous effort in understanding all the factors and conditions affecting the growth and production of rice (Wongsiri, 1975).

Chemical control, use of natural enemies, breeding of rice varieties containing different genes for resistance and other control measures have been, for years, continuously developed to reduce populations to reach economic threshold levels. Breeding and planting of resistant varieties for example are considered to be very important components for integrated control of rice insect pests. Evidences show that plant host can fulfil functions for an insect to improve fitness (Bernays and Graham, 1988) or it lowers the percentage of insects' species population (Litsinger, 1991). However, evolution of pest populations that are able to counteract the effects of resistance factors continuously becomes a hindrance to high yields. One insect pest that has recently been considered a major problem of rice producing regions in the Philippines is the rice black bug, *Scotinophora coarctata* (Fabricius, 1798). This insect is one of the serious insect pests of rice in Mindanao. This pest has been reported to be of considerable importance to rice plants in many parts of Asia (Wongsiri, 1975) and particularly a dreadful pest in the Philippines (Estoy, 1999). It was first reported as a serious pest of rice in Palawan in 1979. In Mindanao, RBB was first reported to infest rice in Curuan, Zamboanga City in late June 1992 and damaged about 2,070 hectares of rice fields towards the end of the year (PhilRice, 1995). RBB attacks rice plants at almost all stages of its growth and could result in severe to complete crop loss during heavy infestation (PhilRice, 2000). Torres (2008) observed differences among black bug populations from different parts of the Philippines and also found out that some features were absent in the Malaysian RBB samples arguing that rice black bugs from the Philippines is comprised of many cryptic species and suspected insect-host interactions facilitated evolutionary diversification. Further studies therefore are needed to understand the nature of the populations of this pest. It is argued that a successful control of any pest is based on correct identification, and inability to recognize distinct populations can have drastic and costly consequences for pest management (Menken and Ulenberg, 1987).

A better understanding of the host populations' differences of polyphagous pest like *S. coarctata* can be very useful to understand the structure, population dynamics, their behaviour and response to various selection pressures for pest management and control. Thus, this study is conducted to gain information on intraspecific variation in rice black bug and searched for significant differences among populations from different rice host varieties. Since understanding the diversification of organisms and understanding the diversity of biological life were both historically based on descriptions of morphological forms, we used the landmark and outline-based analysis in geometric morphometrics (GM) to investigate variability in the pest. These methods combined with powerful and flexible tools of multivariate statistics make it possible to study morphological variation with direct reference to the anatomical context of the structure under study (e.g. Bookstein, 1991, 1996a, b; Dryden and Mardia, 1998).

MATERIALS AND METHODS

Collecting of Specimens and Samples Preparation. Adult insects were collected from different rice host varieties - PSB Rc18 (Ala), PSB Rc26H (Magat), PSB Rc82 (Penaranda), NSIC Rc122 (Angelica), NSIC Rc124H (Mestizo4) and an unknown variety but is commonly referred to as "B1" by the local farmers. All rice types were irrigated-lowland varieties produced by the International Rice Research Institute (IRRI) except for NSIC Rc124H of which Bayer is the breeding institution. The collected insects were placed in plastic containers containing a prepared fixative (70% ethyl alcohol + 30% glacial acetic acid) for preservation. The images of the head (dorsal and ventral view), pronotum, scutellum, abdominal segment and genital plates (dorsal view, undissected) (Fig. 1) were captured using a MacronCam attached to a research stereomicroscope and connected to a laptop computer. A minimum of fifty male and fifty female adults that were collected from PSB Rc18 (Ala), PSB Rc26 (Magat), PSB Rc82 (Penaranda), NSIC Rc122 (Angelica), B1, and NSIC Rc124 (Mestizo 4) hosts were used in the study.

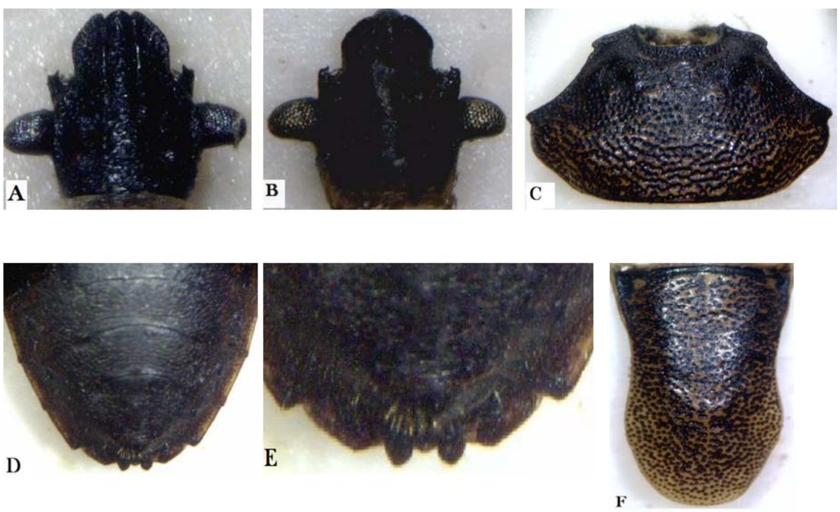

Fig. 1: Photograph of six body parts of Rice Black bug: A. Head, dorsal; B. Head, ventral; C. Pronotum; D. Abdominal segment; E. Genital plate; F. Scutellum.

Landmark Selection and Digitization. Only the head of the insect was used for the landmark-based analysis of morphological variations in shape. In landmark-based morphometric analyses, the morphology of an object is represented by coordinates of sets of landmarks points (Bookstein, 1991). Landmarks were chosen for their ease of identification, their homology and for the ability to capture the general shape of each morphological structure. The landmark data will provide some information such as the orientation, rotation and scale of the specimen. Only 12 landmarks of the head (ventral and dorsal) were chosen (Fig. 2) and Cartesian coordinates were digitized by TpsDig ver.2 (Rohlf, 2004). Analysis of superimposed specimens (e.g. Walker, 1993) describes shape variation by comparing individual specimen with a consensus configuration, representing the average Cartesian coordinates for each landmark across all specimens (Bookstein, 1991; Rohlf, 2004; Adams and Funk, 1997). The raw coordinate data were aligned prior to analysis using tpsRelw (version 5.0) to remove size and arbitrary positioning effects of the specimens relative to the reference axis (Khiaban *et al.*, 2010). Generalized Procrustean superimpositions make data standardise size of the landmark, thus removing differences due to translation and rotation.

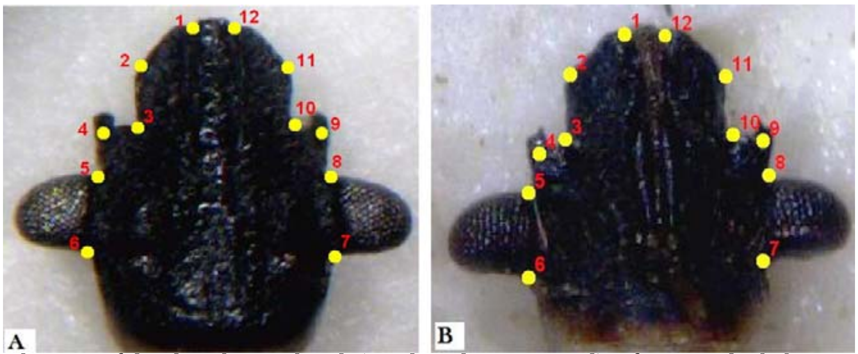

Fig. 2: Distribution of landmarks on head (A. dorsal; B. ventral) of Rice Black bug: Landmarks' description: 1 and 12 are located at the sides of the tylus at the tip of the head; 2 and 11 define the shape of the jugum; 3, 4, 9 and 10 describe the position of the antennifer on the lateral sides of the head; 5, 6, 7 and 8 locate the positions of the compound eyes of the RBB.

Outline Data Acquisition. Outline-based analysis was used to analyze the shape of pronotum, scutellum, abdominal segment and genital plate of the insects (Fig. 3). The tpsDig ver. 2 software (Rohlf, 2004) allows placing landmark on the images and recording the scale factors, saving the data into a thin plate spline (TPS) file. One hundred (100) points along the shape of the images of the specimen were made. The non-shape information was held constant to removed non-shape variation.

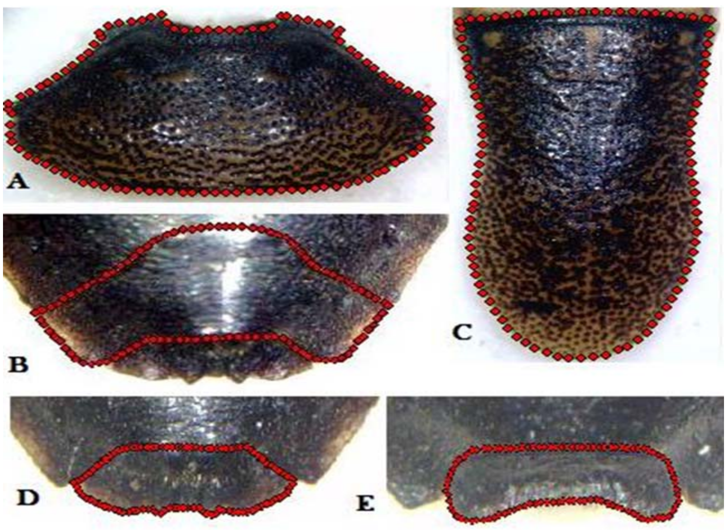

Fig. 3: Coordinates of the outlines of the different morphological structures of the Rice black bug. (A. Pronotum; B. Abdominal segment; C. Scutellum; D. Female genital plate; E. Male genital plate).

The landmarks digitized from each image specimen were optimally aligned using a general procrustes analysis (GPA) using the TpsRelw, ver. 141 software to remove the non-shape effects of translation, rotation, and scale (Rohlf, 2004; Pizzo *et al.*, 2008) and a TPS analysis was then done (Bookstein, 1991). TPS translates in a mathematically rigorous way Thompson's idea of transformation grids, where object is "warped into another and will generate shape variables that allow mapping deformation in shape of an object to another. Variation among specimens is expressed as variation in the parameters of an interpolation function that compares a given specimen to the consensus configuration (Adams and Funk, 1997). Each type of analysis and for each structure under consideration was conducted separately for males and females.

Correlation Analysis Based on Distances (CORIANDIS). The Correlation Analysis Based on Distances or CORIANDIS ver. 1.1 Beta (Marquez and Knowles, 2008) was used to determine if the overall differences and/or similarities of the different body parts of RBB populations is associated with rice types. It is interpreted as a decomposition of group distinctiveness from other groups in terms of specific traits or characters (Abdi *et al*., 2005). The software implements most of the methods for a broad spectrum of data types, including 2-D landmark and distance data (Marquez and Knowles, 2008). Non-metric multidimensional scaling on the matrix of Euclidean distances obtained from each dataset where intergroup distances in the matrix was used to obtain a configuration of points corresponding to groups dimensions. A compromise was built and plotted to interpret the structure of intergroup similarity averaged among the RBB populations over multiple multivariate traits and interpreted in terms of the congruence among traits. Finally, squared distances to centroid for individual sets were computed for each 6 datasets (head (dorsal and ventral views), pronotum, abdominal segment, genital plate and scutellum) showing how much each population differs from the other population in terms of the different body parts.

RESULTS AND DISCUSSION

Head

Thin-plate spline reconstructions of the shape of the head (dorsal and ventral view) of the rice black bugs are shown in Figure (4). A comparison of the mean shapes of the dorsal view of the heads of female RBB associated with different rice types show minor differences among populations specifically in the shape of the antennifer on the sides of the head (Fig. 4a). RBB associated with PSB Rc18 showed a more tilt formation between landmarks 3, 4, 9 and 10 when compared to the other varieties. Results showed that the antennifers are curved outwards and pointed apically and that the inner part is slightly bigger and higher than the lower part. Deformation of grids was visible in landmarks 4, 5, 8 and 9 in PSB Rc18 which is the position of the lower part of the antennifer and the upper part of the eyes indicating that the compound eyes of RBB associated with PSB Rc18 rice type is highly close to the antennifer. In terms of the posterior end of the head, the grid shows that Rc18 has a narrower base creating a convex shape while B1 and NSIC Rc 124H have a broader posterior end. RBBs associated with PSB Rc82 show a concave shape of the head while NSIC Rc122 has a slightly concave shape of the head.

By visual inspection of the shapes of the dorsal view of the head of the male RBB generated by TPS, adult RBB associated with PSB Rc18 showed a minor difference when compared to other populations. Deformation of grids along the antennifer part of the head was observed. Compression of the grids along the antennifer indicates that adult RBBs associated with PSB Rc18 possess a head that is more convex compared to the other populations.

When shapes of the ventral view of the head were compared for both female and male adults, results showed that RBBs associated with PSB Rc18 differ in head shape from other populations (Fig. 4). Compressions of the grid along landmarks 3, 4, 9 and 10 of which located along the antennifer on the lateral view of the head was observed similar to the dorsal view of the head result.

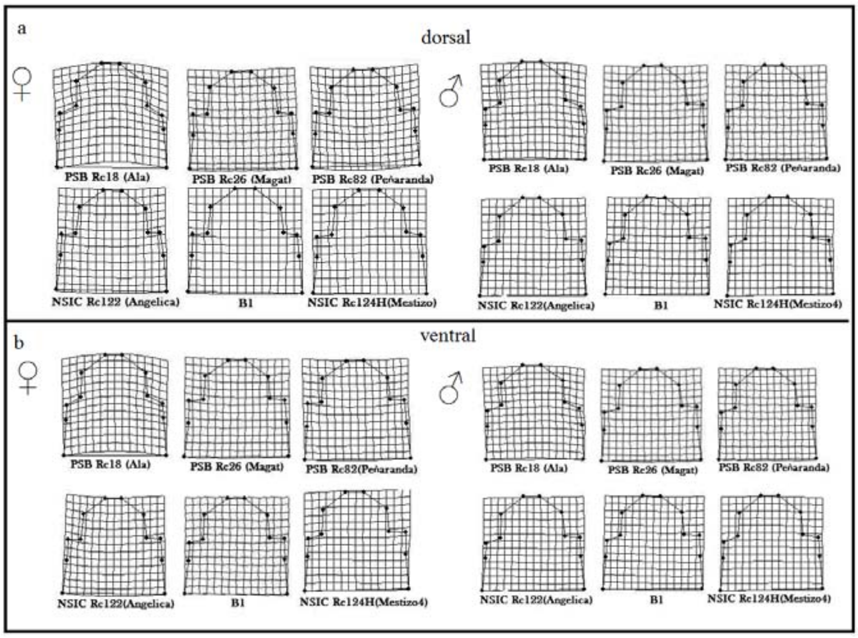

Fig. 4: Mean shapes of the head of RBBs associated with different rice types.

Pronotum

Outline analysis of the shapes of the pronotum of RBB associated with different rice types show that females associated with PSB Rc18 (Ala) differ from all other populations (Fig. 5). The posterolateral margin is more rounded, resulting to have a constricted and slightly convex shape of pronotum than the others. The male RBB populations have no remarkable differences except those associated with PSB Rc26H (Magat). Male RBBs associated with PSB Rc26H (Magat) have a convex and smaller pronotum than the other populations. The anterior margin is comparatively shallow and has a slightly concave lateral margin.

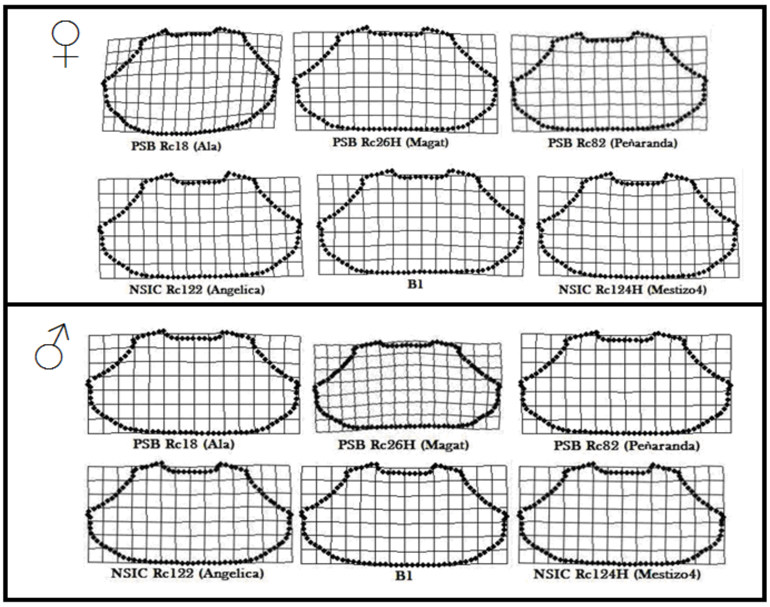

Fig. 5: Mean shapes of the pronotum of RBBs associated with different rice types.

Scutellum

Figure (6) shows evident differences in the shapes and sizes of the female scutellum. Deformation of grids revealed shape differences in a small geometric scale and it is manifested among the populations of female RBBs associated with NSIC Rc122, B1 and NSIC Rc124H. The three populations have more bended grids showing smaller scutellum than the other populations. The midbasal part is highly elevated forming a furrow towards the lateral margin and has a rounded to slightly cleft tip at the outer margin of abdomen. The other three populations, PSB Rc18, PSB Rc26 and PSB Rc82 have a scutellum with a broad flattened shape. Similar observations were also found in male populations. It was also observed that male RBBs associated with PSB Rc82 have a convex shape of scutellum similar to that of males associated with PSB Rc18, PSB Rc26 and PSB Rc82 rice types. Male RBBs associated with PSB Rc18 and PSB Rc26 have significantly bigger pronotum compared to other male populations.

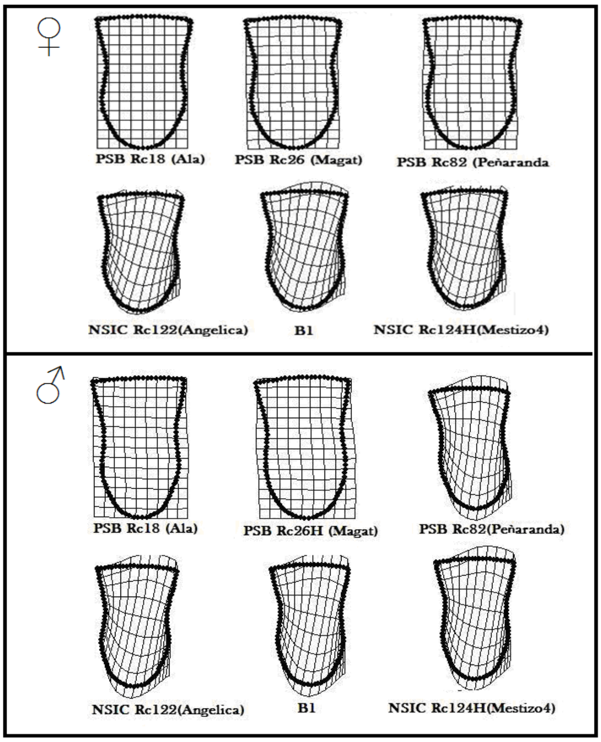

Fig. 6: Mean shapes of the scuttelum of RBBs associated with different rice types.

Abdominal Segment

The shape of the sixth sternite of the abdomen was analyzed using the thin-plate splines analysis. Figure (7) shows asymmetrical shape of the abdomen of the RBBs associated with different rice types. Populations of female RBBs associated with PSB Rc18 and PSB Rc82 show deformations of grids along the midanterior margin of the sternite resulting to a more convex abdomen compared to other populations. Females have more convex anterior margin while males have widely inverted V-shaped sternite. Males associated with PSB Rc18 and PSB Rc82 showed a narrow dome-shaped sternite. NSIC Rc122 showed a smooth down margin compared to the other populations and has a blunt end along the right anterolateral margin the same with NSIC Rc124H.

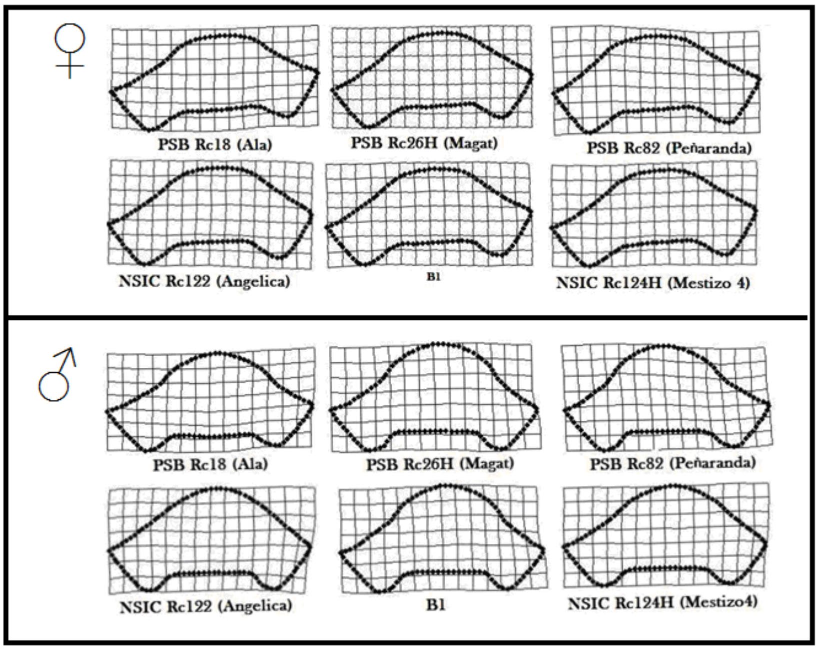

Fig. 7: Mean shapes of the abdominal segment of RBBs associated with different rice types.

Genital Plate

Geographic variation in genital characteristics is an issue of both evolutionary and systematic importance. Genital characteristics in taxonomy indicate their value in species delimitation and they show great divergence between species (Mutanen, 2006). Genital plates also plays important role in species speciation and reproduction. Thus, genital plates of female RBB types were also included for the analysis (Fig. 8). Asymmetry in the shapes of left and right sides in the genital plates were observed among female populations. Those associated with PSB Rc26H has a smaller genital plate than the other populations. Minor differences among male populations however were observed. RBBs associated with PSB Rc18 show more compressed medial region creating a thinner genital plate while those associated with NSIC Rc124H have shorter genital plate compared to the other populations. Males associated with PSB Rc82 also have minor compression along the medial region while PSB Rc26H, NSIC Rc122 and B1 show almost similar shapes among their genital plates.

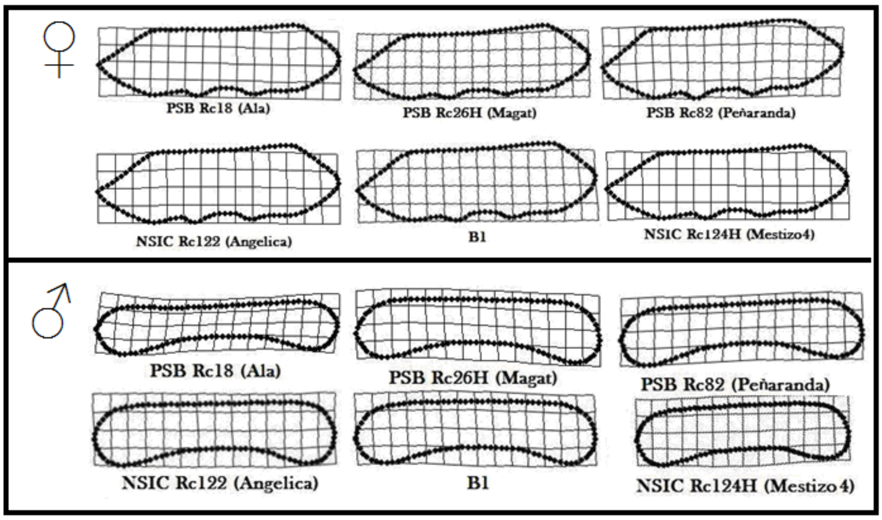

Fig. 8: Mean shapes of the genital plates of RBBs associated with different rice types.

Correlation Analysis Based on Distances (CORIANDIS)

To provide an idea about the association among all the multivariate traits examined in the rice black bugs associated with the different rice varieties, the method of correlation analysis based on distances or CORIANDIS (Marquez and Knowles, 2008) was used. The landmark/outline data were analyzed to evaluate the variation in the different body parts of RBBs in relation to their rice host varieties. Since distance among matrices with their compromises being projected in compromise space can be observed, congruence and disparity of multivariate traits were examined directly. Results of the analysis shown as stacked bar graphs revealed the total disparity among the six populations of rice black bugs based on the shapes of the head (dorsal and ventral), pronotum, abdominal segment, genital plate and scutellum (Fig. 9). In the chart, the total height of each segment of the bar indicates differences among each character by adding the squared distances of each trait interpreted as a decomposition of species distinctiveness from other species in terms of specific traits. As shown in the graph, female RBBs associated with PSB Rc18 showed disparity from all other populations of female RBB based on the shapes of the head (dorsal and ventral), pronotum and scutellum and only minor differences along the abdominal segment and genital plate. Females associated with PSB Rc82 rice type also showed disparity as compared to the other populations but not to all characters. Disparity can be only observed along the dorsal view of the head and the abdominal segment. PSB Rc26H, NSIC Rc122, B1 and NSIC Rc124H showed close similarities among all the characters examined.

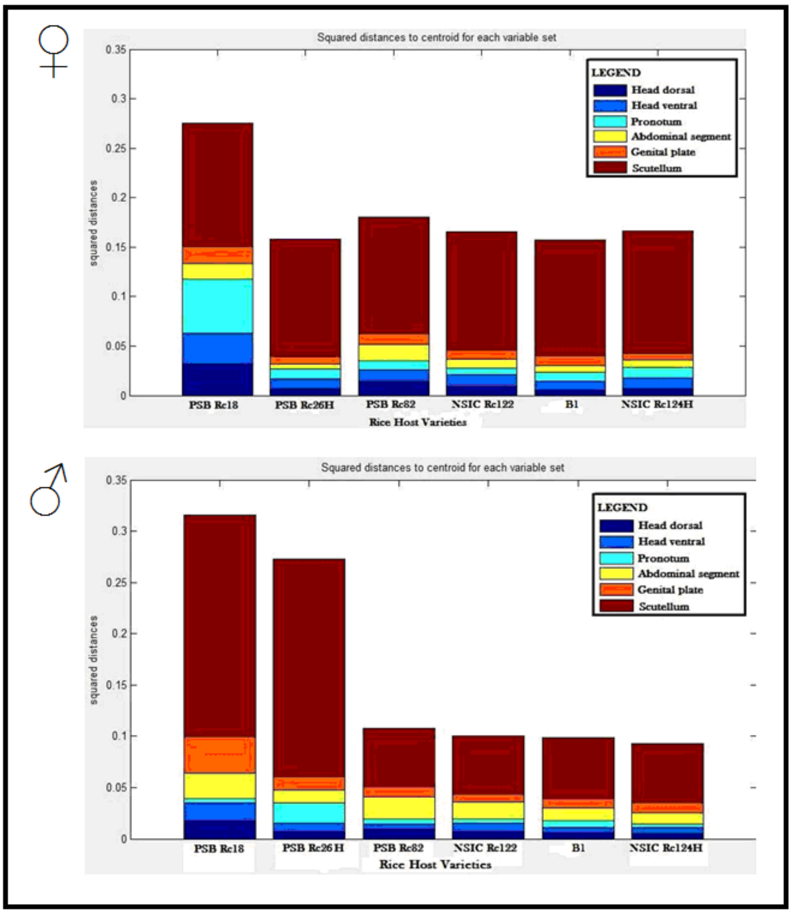

Fig. 9: Squared distances to centroid for each variable set of female rice black bug.

For the male populations of RBB, significant size variation was observed. Similarity in the scutellum size between the populations of males associated with PSB Rc18 and PSB Rc26H was observed. Those associated with PSB Rc18 appeared to have a more distinct genital plate from other populations but have high similarity in the size of the genital plate with males associated with PSB Rc82. Males associated with PSB Rc26H show disparity among all populations in the size of pronotum when compared to all other male populations. Males associated with PSB Rc82, NSIC Rc122, B1 and NSIC Rc124H have high similarity in terms of their scutellum, genital plate, pronotum and the dorsal and ventral view of the head. However, minor variations were observed on their abdominal segments.

Cluster analysis of morphological distances in both male and female RBBs associated with different rice types show differentiation among all populations (Fig. 10). The 100% bootstrap values indicate that differences between populations are very significant and that rice types are very important factors in the differentiation of populations of RBB. Several explanations can be made on these results. Polyphagous species of herbivorous insects are sometimes morphologically variable, but with more specialized appetites (Adams and Funk, 1997; Mozaffarian *et al.*, 2007). It is argued that when an insect population has two or more host species, the possibility that gene flow will be restricted between groups on different hosts will be subjected to divergent selection for host adaptations (Khiaban *et al.*, 2010). The evolution and benefits of polyphagy have been studied in a number of insects (Sword and Dopman, 1999; Bezerra *et al.*, 2004; Mozaffarian *et al.*, 2007; Khiaban *et al.*, 2010). The ability of many insect species to survive on diverse plants is a useful strategy and adaptive advantage for their better survival in the ecosystem (Khiaban *et al.*, 2010). The selective use among diverse resources may lead to evolution of ecological specialization and adaptation (Khiaban *et al.*, 2010). It is argued that if host plant species constitute different selective regimes to herbivorous insects, genetic differentiation and host-associated local adaptation may occur. Likewise, to have a successful control of any pest, this should be based on correct identification of the organism since failure to recognize distinct populations can have drastic and costly consequences for pest management (Menken and Ulenberg, 1987). It is therefore important to achieve sound pest management to recognize the existence of host-associated populations.

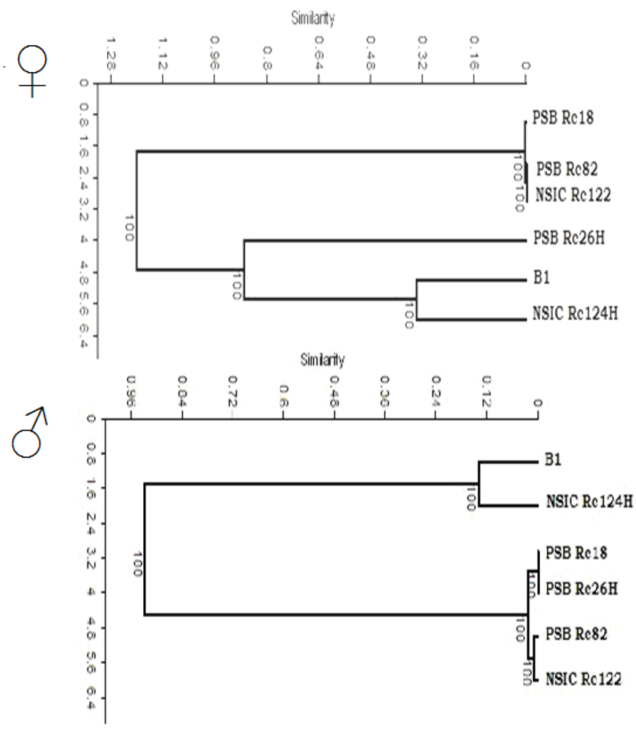

Fig. 10: Plot showing the degree of similarity of characters between six different populations of female rice black bug.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the Philippine Rice Research Institute (PhilRice) for the research grant and to Mr. Muhmin Michael Manting for the technical assistance.

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