Article

Describing selected populations of the rice black bugs in the Philippines using Correlation Analysis Based on Distances (CORIANDIS)

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Abstract

Morphological shape variations of biological structures have been considered an important factor that affects the survival pattern of an organism. This study focuses to gain information on intraspecific variation among different populations of the rice black bugs (RBBs) using Correlation Analysis Based on Distances (CORIANDIS) from the generated relative warp scores of landmark-based geometric morphometrics. Results revealed that the shapes of genital plates have largely contributed a high observable disparity in all morphological characters. The shapes of the head and forewings also contributed noticeable variations while minimal variances were observed in the shapes of the scutellum and pronotum, respectively. The species/group projected plot among populations are clustered together except Bohol and Leyte populations which show a departure from other populations based from the centroid in the compromise space. The quality of the compromise is 52.99% for males and 51.40% for females. It was noticeable that the trait variance is unproportioned to the area occupied by datasets indicating population differentiation from other populations. These results indicate that geographic variations among populations of RBBs were contributed by high species' divergence of the combined characters. This study suggests that CORIANDIS is a useful tool in describing population variability as this has the capacity to integrate all available morphological characters of populations to be able to visualize the underlying relationships among populations.

Keywords geometric morphometrics, phenotypic plasticity; species divergence, CORIANDIS; centroid.

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1 Introduction

The highly cryptic invasive insect pest of rice *Scotinophara coarctata* has become an extremely destructive pest in several parts of the Philippines, particularly in the province of Palawan. RBB outbreaks are argued to be attributed to the utilization of alternate crops grown together with or after rice (Ferrer and Shepard, 1987; Cuaterno, 2007; Barrion et al., 2008). This is further aggravated by the misuse and abuse of broad-spectrum non-selective synthetic pesticides by farmers (Domingo et al., 1985). To achieve effective and ecologically sound management of the pest in the rice agroecosystem, the availability of options such as the applications of biological control methods and host plant resistance as alternatives to synthetic pesticides were suggested (Losos, 1990; Ricklefs and Miles, 1994). However, these different management strategies applied for RBB control were not successful and outbreaks of RBBs were still found to happen in many areas in the Philippines. While RBBs were observed in different geographical areas, their pest status were not consistent. Some were in outbreak level while others were not. It is argued that these populations of the pest may vary and some studies have shown the identification of several Philippine species (Barrion et al., 2008). Succeeding studies in the Philippine RBBs also yielded population variability (Dela Cruz et al., 2011; Torres et al., 2013; Demayo et al., 2007). Whether these are due to phenotypic plasticity or genetically differentiated populations or different species has been the focus of so many debates. We further investigate this group of insects by looking into the morphometric variations observable in the different populations. Since the phenotype of an individual species is believed to be the result of an interaction between its genotype and its environment, the variations could be correlated to different geographic, altitude and climatological conditions (Khiaban et al., 2010) and thus, the phenotypic variation of an insect species like RBBs might be stochastic, environmentally induced or hereditary (Bolnick et al., 2011). The use of Geometric Morphometrics (GM), a quantitative tool to analyze shape morphology, an approach that compares and determines morphological shape variations of biological structures (Sansom, 2009) was applied in the current study. Unlike traditional approaches in morphometric studies, the data in GM is obtained from the coordinates of landmark points (Rohlf and Marcus, 1993; Adams et al., 2004), which are morphological points of specimens that are of biological interest (Richtsmeier et al., 2002) like stock identification and discrimination (Cadrin and Friedland, 1999; Heidari et al., 2013; Mousavi-Sabet and Anvarifar, 2013; Slice, 2007). Hence, the use of the tool to describe the variations in populations of RBBs may help clarify associations of variability in populations across geographical areas to the principle of genetic divergence towards fitness (Alibert et al., 2001).

To be able to examine variations in morphometric characters in various populations of RBBs, the methods of landmark-based geometric morphometrics and Correlation Analysis Based on Distances or CORIANDIS version 1.1 (Marquez and Knowles, 2007) was used in this study. Since understanding the diversification of organisms and understanding the diversity of biological life were primarily based on descriptions of morphological forms, the use of landmark-based GM to investigate variability in the pest is deemed appropriate. The morphological characters examined and are used to compare population variability are the shape of head, pronotum, scutellum, genital plates, and left- & right- forewings of the RBBs.

2 Materials and Methods

2.1 Study area

RBB samples were collected in several rice producing provinces in the Philippines (Fig. 1). The land areas of the study are covered with wide hectares of rice farms planted with irrigated-lowland rice varieties produced by PhilRice and these rice varieties have either succumbed to RBB infestation or not.

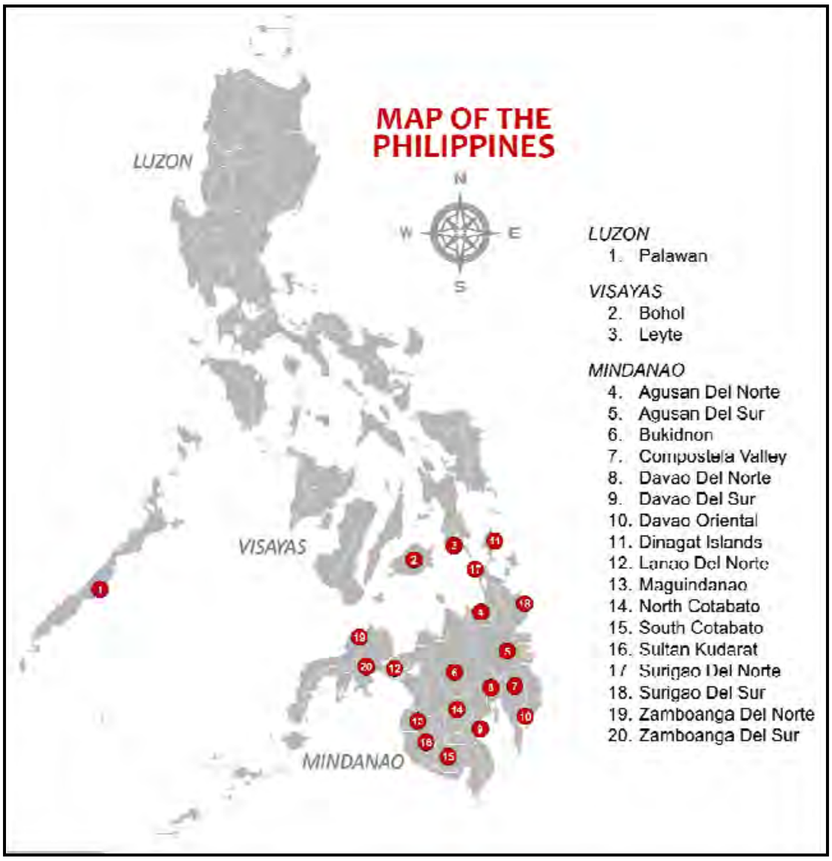


Fig. 1 Location map of the study areas.

2.2 Imaging and landmark points

A set of kit needles was needed for the dissection of RBB samples. In wing preparations, tweezers were used to carefully separate the forewings (left and right) from the body properly then mounted in a clear 1x3 inches glass slide by pairs of six immersed with glycerine. The head structure (dorsal view) and genital plate (ventral view) were arrested in white commercial clay separately. Images of the head, genital plate and forewings were taken using a SONY DSC-W830 digital camera under a stereomicroscope.

Both pronotum and scutellum in dorsal view was also arrested in white commercial clay yet images were taken using a DSLR Nikon D40 camera with 50mm 1:1 Macro lens prior for geometric morphometric (GM) analyses. Landmark points were assigned as an initial step for GM on shape variations.

The morphological characters chosen were digitized with landmark points by the following structures: head (14 landmark points), pronotum (20 landmark points), scutellum (22 landmark points), and genital plates (14 landmark points for males; 17 landmark points for females), and forewings (26 landmark points) as shown in Fig. 2. The description for each morphological characters were classified in Table 1. Landmarks were chosen based for their ease of identification, their homology and for the ability to capture the general shape of each morphological structure (Bookstein, 1991). These landmarks were chosen to provide some information such as the orientation, rotation and scale of the morphological specimen. These landmarks were chosen and the Cartesian coordinates were digitized by TpsDig program and used TpsUtil program to build Tps file and make links files (Rohlf, 2004). The analysis of superimposed specimens (Walker, 1997) 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).

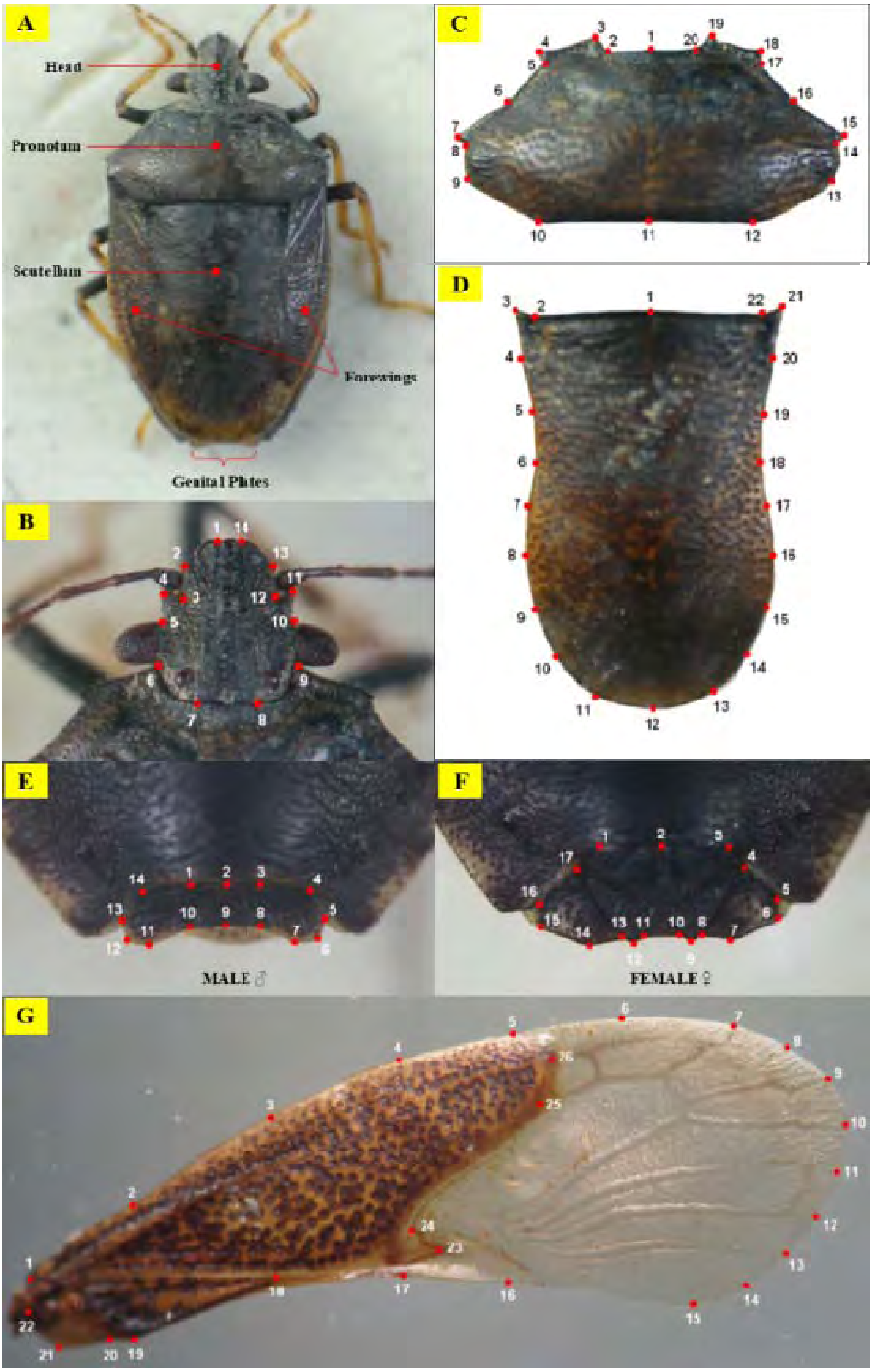


Fig. 2 The morphology of RBB (A) and the positions of landmark points in head (B), pronotum (C), scutellum (D), genital plates (E -male; F -female) and forewing (G).

Table 1 The classification and descriptions of the digitized landmarks of rice black bugs (RBBs).

<table>
<tr><th colspan="2">LANDMARK NUMBER</th><th colspan="2">DESCRIPTION</th></tr>
<tr><th colspan="4">Head (Dorsal View)</th></tr>
<tr><td colspan="2">1, 14</td><td colspan="2">Sides of the tylus at the tip of the head</td></tr>
<tr><td colspan="2">2, 13</td><td colspan="2">Shape of the jugum</td></tr>
<tr><td colspan="2">3, 4, 11, 12</td><td colspan="2">The antennifer on the lateral sides of the head</td></tr>
<tr><td colspan="2">5, 6, 9, 10</td><td colspan="2">Position of the compound eyes</td></tr>
<tr><td colspan="2">7, 8</td><td colspan="2">Posterior margin of the head & junction to prosternum</td></tr>
<tr><th colspan="4">Pronotum (Dorsal View)</th></tr>
<tr><td colspan="2">1, 2, 3, 19, 20</td><td colspan="2">Anterior margin of pronotum</td></tr>
<tr><td colspan="2">4, 5, 17, 18</td><td colspan="2">Anterolateral spine</td></tr>
<tr><td colspan="2">6, 16</td><td colspan="2">Lateral margin of pronotum</td></tr>
<tr><td colspan="2">7, 8, 14, 15</td><td colspan="2">Prehumeral spine</td></tr>
<tr><td colspan="2">9, 13</td><td colspan="2">Posterolateral margin of pronotum</td></tr>
<tr><td colspan="2">10, 11, 12, 13</td><td colspan="2">Posterior margin of pronotum</td></tr>
<tr><th colspan="4">Scutellum (Dorsal View)</th></tr>
<tr><td colspan="2">1</td><td colspan="2">Mid-anterior segment of scutellum</td></tr>
<tr><td colspan="2">2, 3, 21, 22</td><td colspan="2">Scutellar pit</td></tr>
<tr><td colspan="2">4, 5, 6, 18, 19, 20</td><td colspan="2">Anterolateral sides of scutellum</td></tr>
<tr><td colspan="2">7, 17</td><td colspan="2">Lateral sides of scutellum</td></tr>
<tr><td colspan="2">8, 9, 10, 14, 15, 16</td><td colspan="2">Posterolateral sides of scutellum</td></tr>
<tr><td colspan="2">11, 12, 13</td><td colspan="2">Posterior margin of scutellum</td></tr>
<tr><th colspan="4">Genital Plates (Ventral View)</th></tr>
<tr><td colspan="2"></td><th colspan="2">Male ♂</th></tr>
<tr><td colspan="2">1, 2, 3</td><td colspan="2">Anterior margins of genital plate</td></tr>
<tr><td colspan="2">4, 5, 13, 14</td><td colspan="2">Lateral margins of genital plate</td></tr>
<tr><td colspan="2">6, 7, 11, 12</td><td colspan="2">Posterolateral margins of genital plate</td></tr>
<tr><td colspan="2">8, 9, 10</td><td colspan="2">Genital segments (with saddle shape)</td></tr>
<tr><td colspan="2"></td><th colspan="2">Female ♀</th></tr>
<tr><td colspan="2">1, 2, 3</td><td colspan="2">Anterior margins of genital plate</td></tr>
<tr><td colspan="2">4, 5, 16, 17</td><td colspan="2">Lateral margins of genital plate</td></tr>
<tr><td colspan="2">6, 7, 14, 15</td><td colspan="2">Posterolateral margins of genital plate</td></tr>
<tr><td colspan="2">8, 9, 10, 11, 12, 13</td><td colspan="2">Genital segments (with two triangular warts)</td></tr>
<tr><th colspan="4">Forewings</th></tr>
<tr><th>Landmark</th><th>Description</th><th>Landmark</th><th>Description</th></tr>
<tr><td>1</td><td>Proximal end of Subcosta (Sc)</td><td>14</td><td>Anterior margin of Membrane (Mb)</td></tr>
<tr><td>2</td><td>Posterior of Corium (Co)</td><td>15</td><td>Posterior margin of Membrane (Mb)</td></tr>
<tr><td>3</td><td>Midpoint of Corium (Co)</td><td>16</td><td>Curve of Wingfold (W)</td></tr>
<tr><td>4</td><td>Anterior of Corium (Co)</td><td>17</td><td>Distal end of Wingfold (W)</td></tr>
<tr><td>5</td><td>Curve of Corium (Co)</td><td>18</td><td>Nodus (N)</td></tr>
<tr><td>6</td><td>Distal end of Radius (R)</td><td>19</td><td>Anterior margin of Clavus (Cl)</td></tr>
<tr><td>7</td><td>Radial branch 1 (R_1)</td><td>20</td><td>Posterior margin of Clavus (Cl)</td></tr>
<tr><td>8</td><td>Midpoint of R_1 and R_2</td><td>21</td><td>Anal margin of Clavus (Cl)</td></tr>
<tr><td>9</td><td>Radial branch 2 (R_2)</td><td>22</td><td>Proximal end of Costa (C)</td></tr>
<tr><td>10</td><td>Radial branch 3 (R_3)</td><td>23</td><td>Proximal end of Wing fold (W)</td></tr>
<tr><td>11</td><td>Cubitus vein 1 (Cu_1)</td><td>24</td><td>Proximal end of Media (M)</td></tr>
<tr><td>12</td><td>Cubitus vein 2 (Cu_2)</td><td>25</td><td>Curve of Media (M)</td></tr>
<tr><td>13</td><td>Cubitus vein 3 (Cu_3)</td><td>26</td><td>Distal end of Media (M)</td></tr>
</table>

2.3 Relative Warp Analysis

The raw landmark coordinates were first superimposed using a generalized procrustes super position algorithm, whereby the sum of squared distances between each object and a reference configuration (consensus) were iteratively minimized by translations and rigid rotation (Khiaban et al., 2010). The partial warp (Generalized procrustes super position algorithm) scores of these super imposed data were used as shape variables. Finally, the Thin-Plate Spline (TPS) interpolation function derived from the mean of the superimposed data was applied to a squared grid overlaying the mean landmark configuration to provide a direct and quantitative implementation. The consensus shape data of each separate group were measured by relative warps ordinations plots using tpsRelw 1.36 (Rohlf, 2004). Relative warps are computed from the partial warps and characterized by its singular value and explains a given variation in shape among specimens summarizing shape differences. The relative warp scores were saved as matlab files that can be open in worksheets and analyzing shape variation using PAST version 3.0 software prior to multivariate analysis (Hammer et al., 2001). The relative warp scores were used to generate values that constitute the multivariate shape data sets.

2.4 Correlation Analysis based on Distances (CORIANDIS)

The Correlation Analysis Based on Distances or CORIANDIS ver. 1.1 Beta (Marquez and Knowles, 2007) was used to determine the overall differences and/or similarities of the different morphological shapes of RBB. It is interpreted as a decomposition of group distinctiveness from other groups in terms of specific traits or characters (Abdi et al., 2005; Zhang, 2015, 2018). The software implements most of the methods for a broad spectrum of data types, including 2-D landmark and distance data (Marquez and Knowles, 2007). 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 group 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 datasets (head, pronotum, scutellum, genital plates, and left- & right- forewings) showing how much each population differs from the other population in terms of the different structures.

3 Results

The consensus configuration obtained using relative warp analysis was used as the reference configuration by which variations in other shape transformations. Significant disparities were observed in the mean shapes of head, pronotum, scutellum, genital plates, and forewings of RBBs (Fig. 3 to Fig. 7, respectively). The shape of head are characterized by major differences in the distances of tylus, jugum, lateral sides, insertions of antennifer, and position of compound eyes; and minor head variations are associated with the inclinations on junctions to prosternum (Fig. 3). Male head is smaller compared to larger head of female RBBs. In Fig. 4, the shape of pronotum of RBBs are characterized by major differences in the concavity of the anterior, lateral, and posterolateral margins; and minor pronotum variations are associated with the bluntness of anterolateral and prehumeral spines. Male pronotum is narrower and tightly concave compared to wider and freely concave pronotum of female RBBs. In Fig. 5, both male and female scutellum respectively are characterized by major differences in the concavity of anterolateral and posterolateral sides, inclinations of lateral sides, and roundness of posterior margin; and minor scutellum variations are associated with the bluntness of scutellar pit. Male scutellum is narrower and pointed compared to slender and rounded scutellum of female RBBs. In Fig. 6, the shape of genital plates are characterized by major differences in the concavity of anterior margins and size of genital segments; and minor variations in the inclinations of lateral and posterolateral margins. Male genital plates is concave and narrower compared to convex and wider genital plates of female RBBs. Fig. 7 show both male and female forewings are characterized by major differences in the distances of Corium, Clavus, and membrane and the size of wingfold; minor variations in the compressions of proximal ends of Costa & Subcosta and the compressions of Radius, Radial branches, and Cubitus veins. Male forewings has longer left- but shorter right- compared to female forewings that are both longer in left- and right- with slight variations in compression.

Results of the Correlation Analysis based on Distances (CORIANDIS) which integrated all available character datasets for both sexes are shown in Fig. 8. The visualized results show the underlying relationships and sources of variability among the groups in terms of relative contribution and congruence among characters. As shown in the Figure, the resulting clusters that represent the location of the male and female morphs on the ‘compromise’ space reflected the overall similarity based on all investigated morphological characters of RBBs from the different populations. This procedure allows both looking into similarities among specimens/groups, and interpreting such similarities in terms of congruence among traits. For the male RBBs, BKN and CPV populations are clustered together due to the variations of pronotum attributed in the concavity of the anterior, lateral, and posterolateral margins. For LDN, MGD, NCT, SCT populations, these are clustered together due to the variations of forewings attributed in the distances of Corium, Clavus, and Membrane and the size of Wingfold. The BHL and LYT populations are clustered together due to the variations of scutellum attributed in the concavity of anterolateral and posterolateral sides, inclinations of lateral sides, and roundness of posterior margin. The AGN, AGS, DVN, DVS, SRS populations are clustered together due to the variations of head attributed in the distances of tylus, jugum, lateral sides, insertions of antennifer, and position of compound eyes. The PLW, DNG, DOR, SKD, SRN, ZBN, ZBS populations are clustered together due to the variations of genital plates attributed in the concavity of anterior margins and size of genital segments. For females populations, clustering of PLW, DNG, SKD, SRN, ZBN, ZBS and AGN, AGS, LDN, DVN, DVS, DOR, SRS populations are due to the major variations in pronotum; BKN, CPV, MGD populations are due to the major variations in genital plates; NCT and SCT populations due to the major variations in forewings; BHL and LYT departs considerably from other populations due to the major variations in head and scutellum, respectively. The quality of the compromise is 52.99% for males and 51.40% for females.

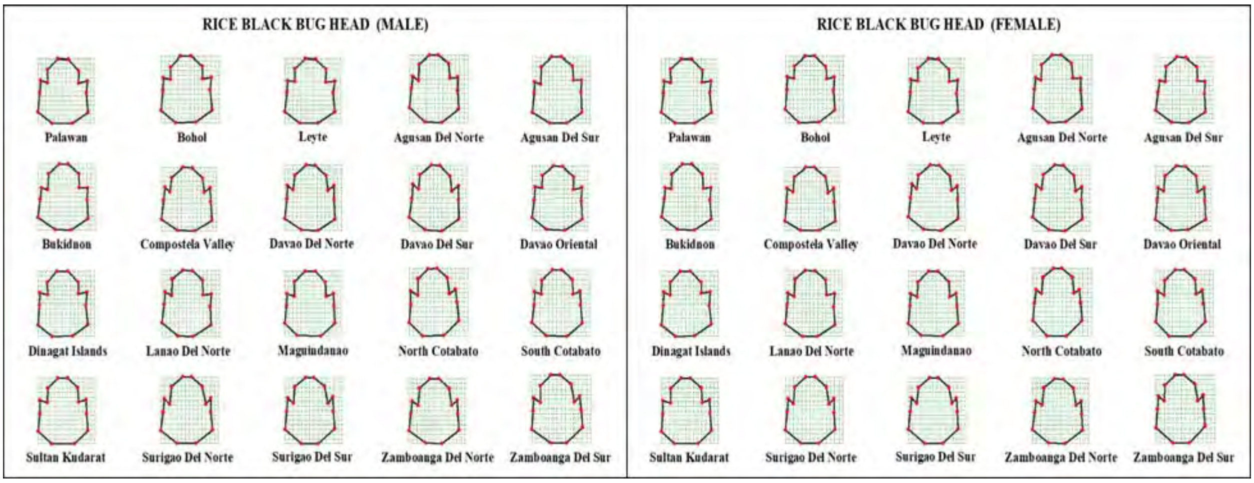


Fig. 3 Mean shapes of the head of male and female RBBs from different populations in the Philippines.

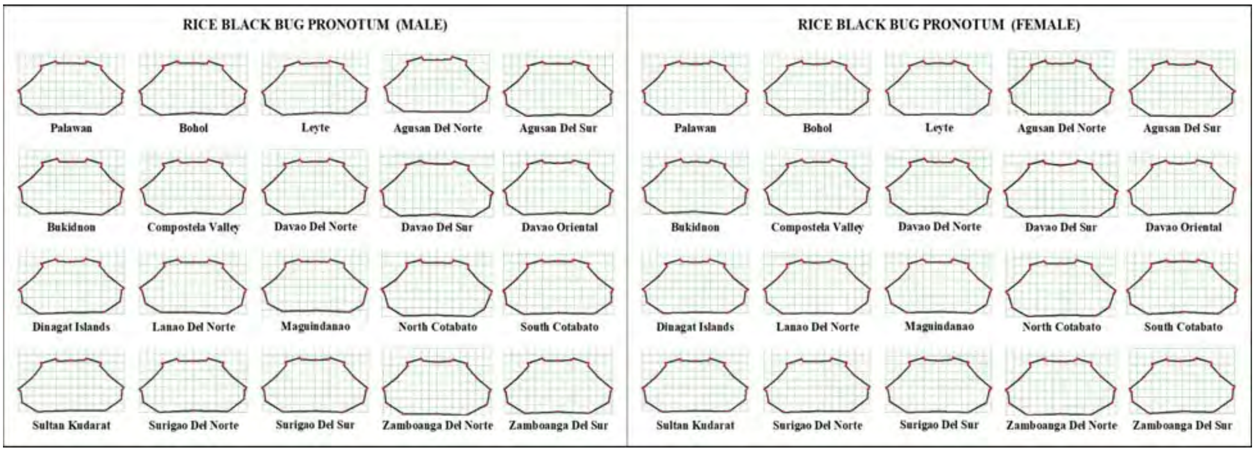


Fig. 4 Mean shapes of the pronotum of male and female RBBs from different populations in the Philippines.

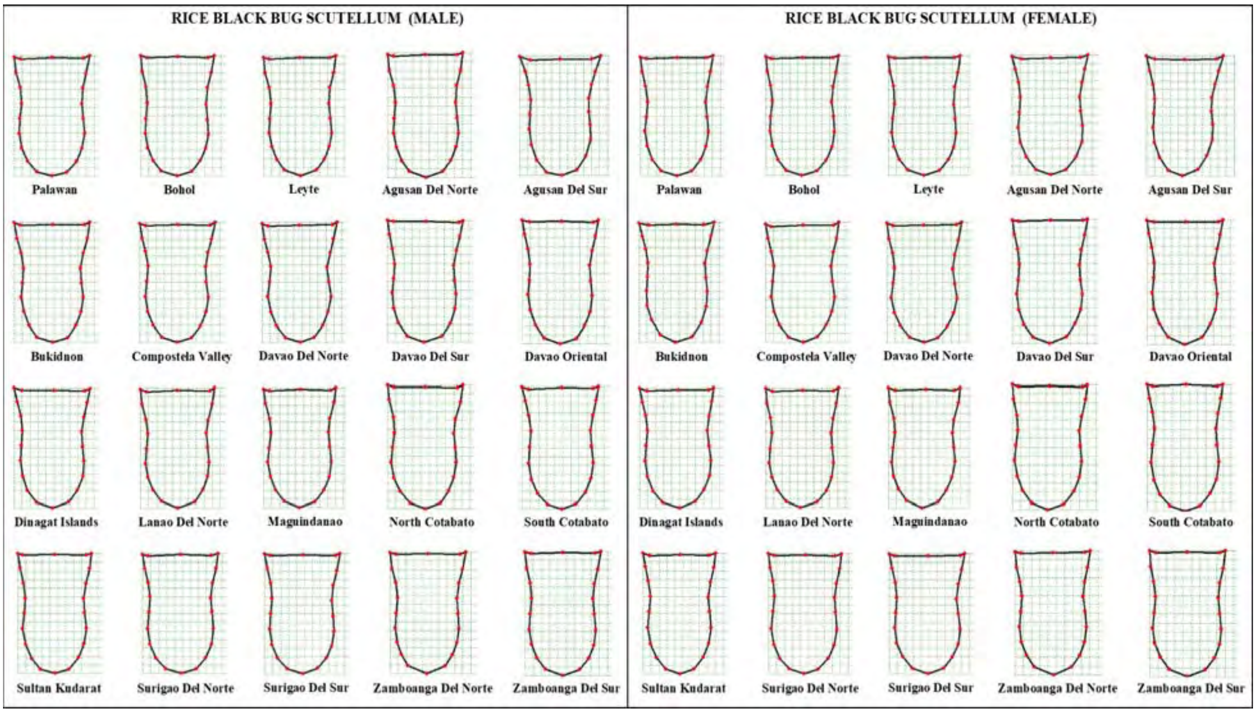


Fig. 5 Mean shapes of the scutellum of male and female RBBs from different populations in the Philippines.

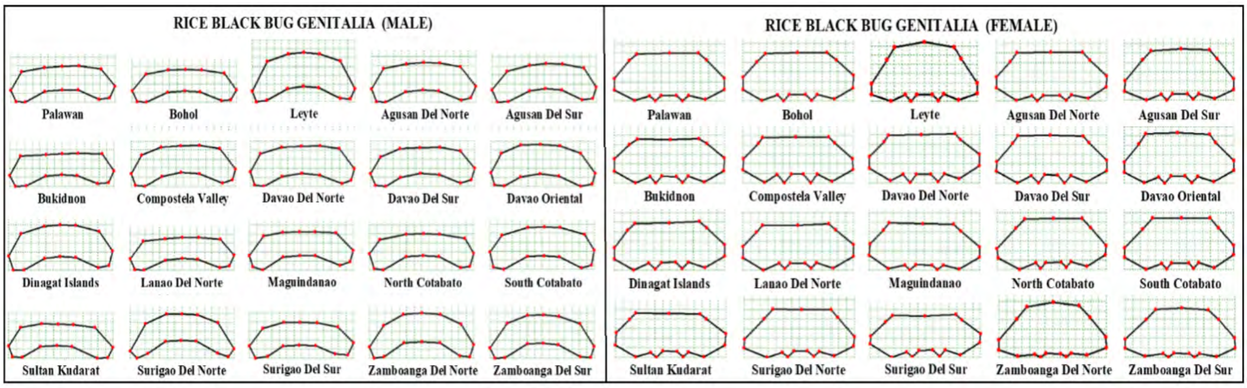


Fig. 6 Mean shapes of the genital plates of male and female RBBs from different populations in the Philippines.

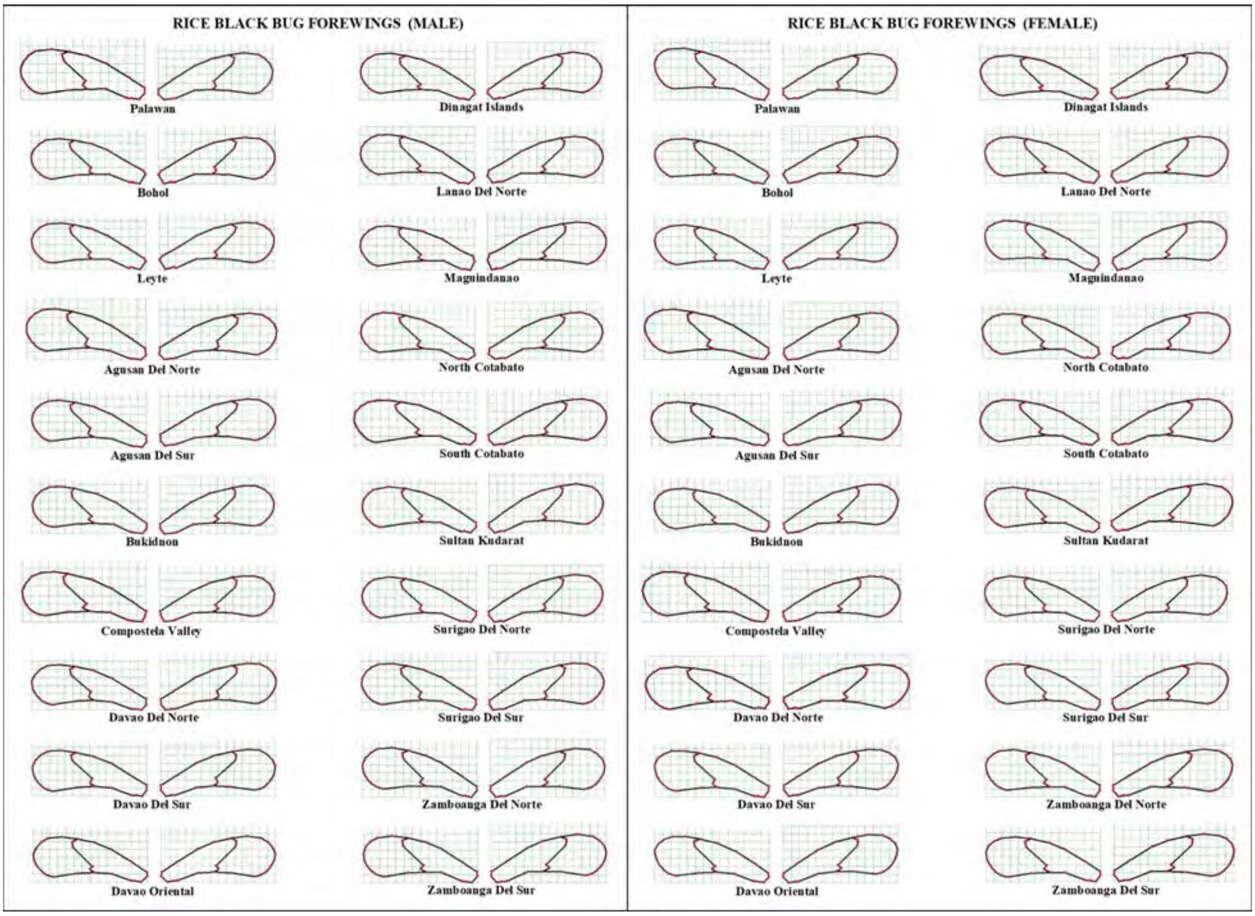
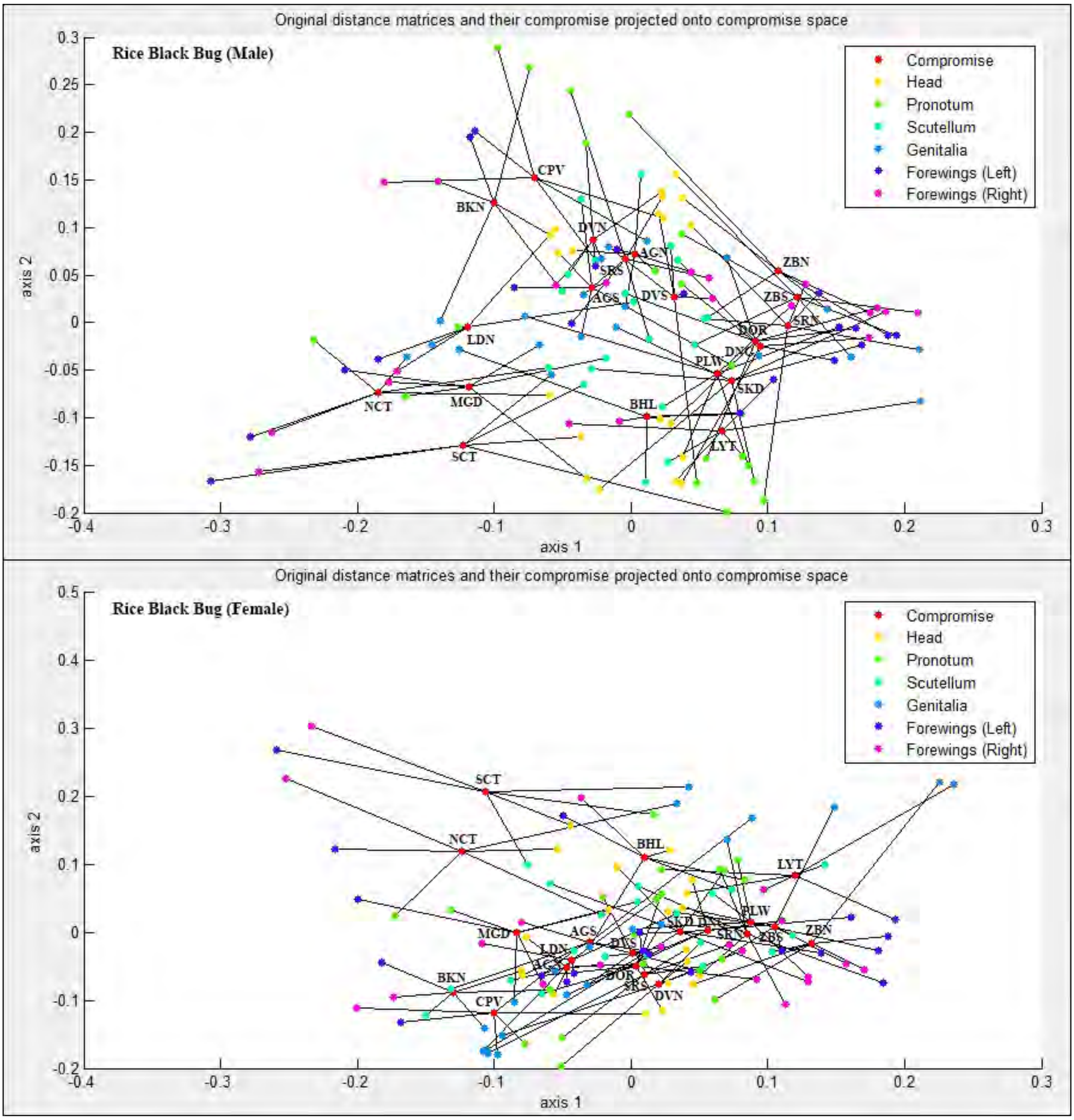


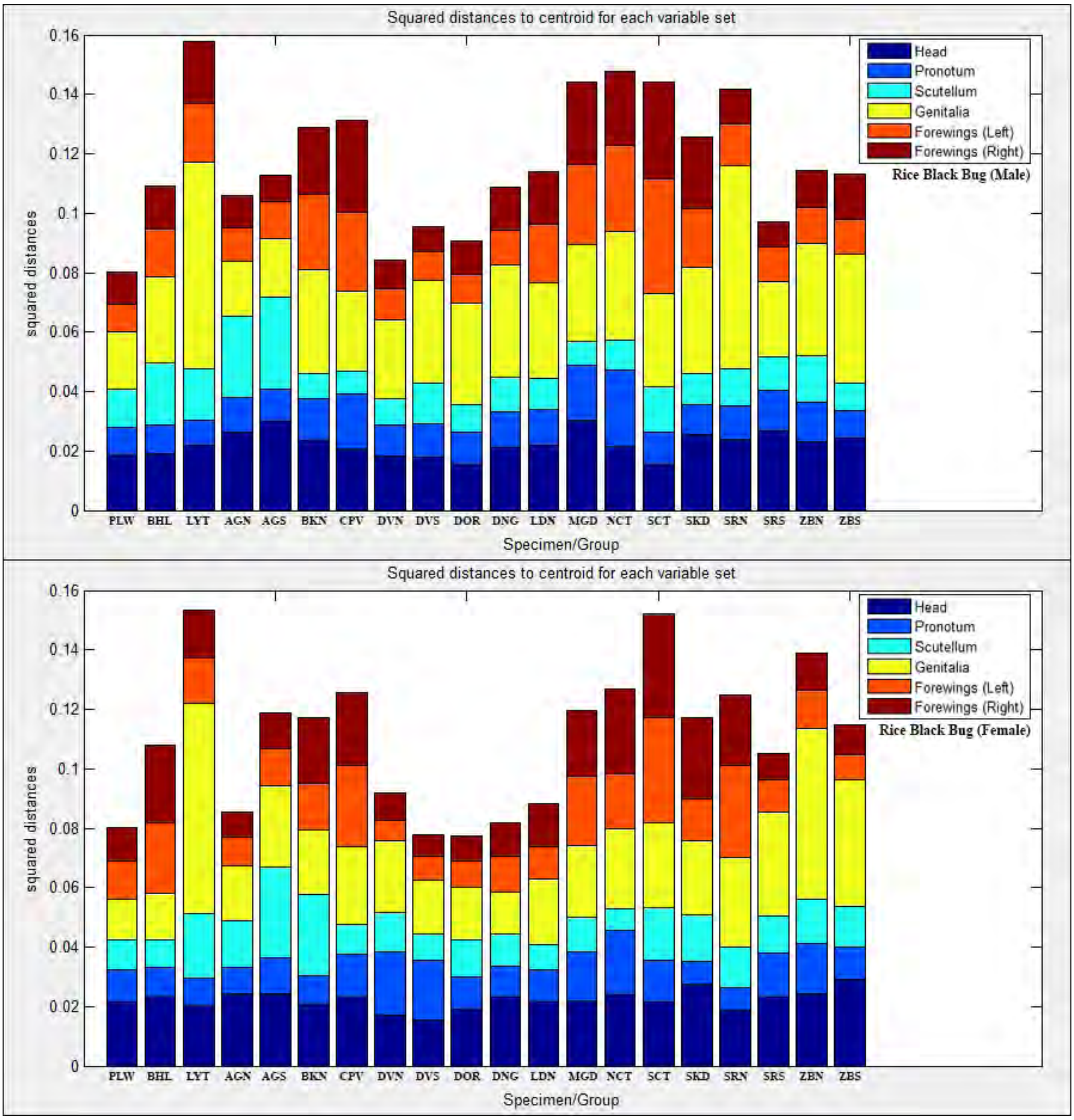
Fig. 7 Mean shapes of the forewings of male and female RBBs from different populations in the Philippines.

Fig. 9 illustrates a disparity chart for the morphs of both sexes of RBB populations showing the relative contribution of the combined sets of characters to species' divergence. Results show that among all populations for both sexes, the shape of the genitalia/genital plates have largely contributed a high observable disparity in all morphological characters. Correspondingly, the shapes of the head and forewings has also contributed noticeable variations. Minimal variances was observed in the shapes of the scutellum and pronotum, respectively. These observable disparities for RBB males, the populations of LYT, NCT, SCT, MGD, SRN, CPV, and BKN contributed a high combined morph variance. This is followed by SKD, ZBN, ZBS, LDN, AGS, DNG, BHL, and AGN populations, respectively. The populations of SRS, DVS, DOR, DVN, and PLW contributed minimal combined morph variance. For the RBB females, the populations of LYT, SCT, and ZBN contributed a high combined morph variance followed by the populations of NCT, CPV, SRN, MGD, AGN, AGS, SKD, ZBS, BHL, and SRS, respectively. The populations of DVN, LDN, AGN, DNG, PLW, DVS, and DOR contributed minimal combined morph variance.



Legend: PLW (Palawan), BHL (Bohol), LYT (Leyte), AGN (Agusan Del Norte), AGS (Agusan Del Sur), BKN (Bukidnon), CPV (Compostela Valley), DVN (Davao Del Norte), DVS (Davao Del Sur), DOR (Davao Oriental), DNG (Dinagat Islands), LDN (Lanao Del Norte), MGD (Maguindanao), NCT (North Cotabato), SCT (South Cotabato), SKD (Sultan Kudarat), SRN (Surigao Del Norte), SRS (Surigao Del Sur), ZBN (Zamboanga Del Norte), ZBS (Zamboanga Del Sur)

Fig. 8 Species/group plot of the principal components of "compromise" space axis of the male RBBs (above) and female RBBs (below) among different populations in the Philippines.



Legend: PLW (Palawan), BHL (Bohol), LYT (Leyte), AGN (Agusan Del Norte), AGS (Agusan Del Sur), BKN (Bukidnon), CPV (Compostela Valley), DVN (Davao Del Norte), DVS (Davao Del Sur), DOR (Davao Oriental), DNG (Dinagat Islands), LDN (Lanao Del Norte), MGD (Maguindanao), NCT (North Cotabato), SCT (South Cotabato), SKD (Sultan Kudarat), SRN (Surigao Del Norte), SRS (Surigao Del Sur), ZBN (Zamboanga Del Norte), ZBS (Zamboanga Del Sur)

Fig. 9 Stacked bar graph showing the disparity plot of squared distances to centroid for each morphological shape of the male RBBs (above) and female RBBs (below) among different populations in the Philippines.

4 Discussion

The congruence and disparity of multivariate traits as shown in the stacked bar graph and compromise space show the total height of each bar resulting from the addition of the squared distances of each trait separately which is a measure of trait disparity. This shows how much each population differs from the rest by interpreting such differences in terms of individual character (Márquez and Knowles, 2007). As can be seen from the results, the trait variance in the stacked-bar chart is unproportioned to the area occupied by datasets indicating species distinctness from other areas in terms of specific traits of RBBs. Overall, the results revealed that highly significant difference was distinguished between morphological characters and highly vary also among populations. The study show that while the head of RBB is inherently symmetric with an internal line or plane of symmetry so that the left and right halves are mirror images of each other (Klingenberg et al., 2002), variations were observable in all populations. The variations observed in the shapes of pronotum and scutellum of RBBs might be influenced by the insect's adaptation to changing environment. Ecological stresses such as habitat destruction and frequent eradication through application of pesticides and other traditional approaches are just few of the many factors that may impose changes in the shapes of pronotum and scutellum. Variations observed in the distances, curvature and concavity might be due to habitat preferences, competition for mating or survival, and could be linked with the flight biomechanics of the insect (Nugaliyadde et al., 1997). For the shape of genitalia/genital plates, morphological variations in the collected RBB populations may indicate possible genetic differentiation (Sasabe et al., 2007). A study conducted by Monti et al. (2008) using Elliptic Fourier analysis showed observed morphological differences among the genitalia of two closely related Noctuid moths *S. latifascia* and *S. descoinsi* showed the trait is controlled by polygenic system which maybe is true for the RBBs. In the Philippines, populations of RBB can be found at varying densities (Litsinger, 2007), where some populations can reach outbreak levels even at times when other populations are at low densities which may indicate density-dependent phase polyphenism resulting to high variability in a low density population. Since geographic variation in genital characteristics is an issue of both evolutionary and systematic importance, the observed variations may indicate their value not only in species delimitation but also in the divergence in the species (Mutanen, 2006).

The results of this study also indicate that forewings exhibit asymmetry between the left and the right forewing. In a wing study of an intraspecific geographical populations of two *Apis* species, it was argued that the variations in symmetry observed may probably have resulted from geographic isolation or due to the ecological differences of the location which restricted the gene flow between restrictive populations (Nunes et al., 2012; Barour et al., 2011). Aside from this, Barour et al. (2011) also observed a large variability at the colony and apiary levels involving mostly shape differences. Variability of the species in wing shapes could be associated with their foraging ranges. Aside from the flight ability of the forewings, these also functions for protection. Factor that may influence variability in the shape of forewings could be the necessity to guarantee shielding adaptations, making possible the wholeness of the delicate wing organs during inhabiting or visiting of the insect on whatever concealed habitats (Bax and Thresher, 2009). Rice black bugs migrate long distances after the rice crop is harvested especially in the absence of its alternative hosts in nearby areas (Nugaliyadde et al., 1997). According to Chakraborty (1990), rice pest insects also differs with varying geographical conditions. Variability of the rice complex in different areas is associated with differences in cropping intensity, irrigation, variety, fertilizer, and pesticide use. Genetic factor could possibly be a great influence of the observed variances due to phenotypic plasticity which the ability of its genotype to exhibit variable phenotypes in such variable environment (Pfennig et al., 2010).

5 Conclusion

The results of the study revealed that the geographic variation among populations of RBBs are shown by a high species' divergence of the combined characters. This may indicate phenotypic plasticity which allows the population to develop alternative phenotypes in response to altering environments and climate change. The application of correlation analysis based on distances (CORIANDIS) is a good alternative tool in understanding population structures of the RBBs since the tool involves the application of various characters such as head, pronotum, scutellum, genital plates, and forewings. Likewise, it is useful in detecting subtle differences among populations and understand the patterns of shape variation.

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