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Quantitative Description of Head Shape Dimorphism in the Rice Black Bug *Scotinophara* sp. using Landmark-based Geometric Morphometric Analysis

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ABSTRACT

Rice black bug (RBB), Scotinophara coarctata, is small and cryptic pest species that is believed to have caused significant yield loss of rice production in the Philippines. The study focuses on the geometric morphometric variability by landmark-based approach to determine discontinuities of dorsal head shape for both male and female Scotinophara sp. population from Kabacan, North Cotabato. Results revealed that males has 77.72% variation and females has 74.31% variation for quantitative analysis. The values depicted on MANOVA shows significant differences existing between both sexes with p = 1.68 x 10-60. CVA plots also had a highly significant differences in the morphological attributes existing between the sexes showing no overlaps and having a higher DFA corrected classified value of 99.72% than the cut off score/value (70%). Implying that there is a significance on the morphological structures between the male and female Scotinophara sp. which can be attributed to sexual dimorphism.

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INTRODUCTION

The rice black bug (RBB), *Scotinophara* sp. is an emerging, highly cryptic and predominantly invasive insect pest in rice agricultural systems (Ferrer and Shepard, 1987, Cuaterno, 2007, Barrion *et al.*, 2008). Early studies on this insect show these bugs were found only on older plants and abandoned stubbles (Heinrichs *et al.*, 1987). The pest has been known as serious pest of rice in Malaysia as early as 1920s (Corbett and Yusope, 1920; South, 1026; Dammerman, 1929; Miller and Pogden, 1930). It attacks rice plants in irrigated area from early vegetative to maturity. The most susceptible stage is from maximum tillering to ripening stage. After years of uncontrolled infestation, it was considered to be a major threat to rice production in the Philippines. First infestation occurred in Palawan in 1979 and followed in 1982 covering 4,500 hectares of rice fields. It was first observed in Mindanao Island in 1992 damaging about 2,070 hectares and later invaded the whole of Region 9 including the Autonomous Region of Muslim Mindanao (ARMM) and in Region 12. At present, the pest is already a part of the ecology of the whole Mindanao Island. The pest was spotted in the Visayas in late 1998 particularly in Negros Occidental. It then spread to Siquijor Island then to Bohol and Iloilo which extends to the southernmost part of Luzon. Many attempts at controlling this pest using insecticides failed, challenging the authorities to investigate the problem further. Host plant resistance was explored but early screening of 300 rice breeding lines for resistance to the pest in the field in the Philippines only 2 entries survived to 60 days after infestation (Domingo *et al.*, 1985). The problem is compounded as major behavioral differences were also observed in feeding trials leading to the assumption of the existence of more variant forms of the species. Morphological examination of different populations by Barrion *et al*. (2007) show some chaetotaxic differences which led to the naming of more than 20 species of *Scotinophara*. Other studies conducted in this species show variations within, between and among outbreak and non-outbreak populations suggesting that this species is morphologically plastic and genetically variable (Torres *et al*., 2013). In the current study, we explored more on the existence of variations in the insect by focusing on dimorphism of the head of the insect. While studies on sexual dimorphism used qualitative measures, we explore the application of quantitative methods in describing variability in the morphological shape of the head as a phenotypic marker. It is argued that this character is triggered by a fundamental genetic variation and not due to plastic changes in response to environmental flux. For the purpose of this study, 'head morphology' is one of the most commonly used form of phenotypic marker,

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although other characters are also used as well (Rojas, 1992; Zink and McKitrick, 1995; Duffy, 1996; Turner, 1999; de Queiroz, 2005). Understanding variability in head shape of rice black bug *Scotinophara* sp. is of great importance in measuring the extent of variability of this insect pest of rice as this character plays an important role in allowing the insect feeding structures to penetrate the rice plant and feed on the plant juices. We used the tools of geometric morphometrics (GM) in quantifying the head shape of the insect. There are two methods in describing the form of an organism through GM: (1) by landmark based analysis that uses a set of landmarks to describe the object or specimen and (2) by outline based analysis which extract the margin around the specimen. GM analyzes biological form in such a way that will sustain the physical integrity of the form (Richtsmeier *et al*., 2002).

Unlike analytical approaches, the geometric method is aimed at a comparison of shapes themselves. By enumerating morphological variation, it is easier to identify the relationship between morphology and ecology (Losos, 1990; Ricklefs and Miles, 1994) and thus create additional informed inferences on the evolution of pest organisms (Adams, 1999). Since the head is associated with feeding preferences, this study was therefore conducted in this structure to determine whether variability still exist within a population. This study will provide information as to the extent of morphological variability in the pest by identifying discontinuities in the shape of the dorsal head. We specifically used the landmark-based method of geometric morphometrics. The tool is useful in allocating a better comparison of the shapes and would no longer depend on word descriptions that mostly encounter problem of being interpreted differently by scientists (Adams *et al*., 2004).

Methodology:

Study Area:

The study area chosen in this study is North Cotabato, simply 'Cotabato', a landlocked province of the Philippines that lies on the eastern part of Region XII (SOCCSKSARGEN) and is centrally located in Mindanao and is considered one of the rice-producing area of Mindanao. It is subdivided into seventeen municipalities and one capital city. The town of Kabacan was specifically identified (Fig. 1). The town is predominantly composed of rice farms of more than 30,000 hectares. Rice production in this area is threatened because there is an abrupt reduction of rice yield per year due to the primary infestation of *Scotinophara* sp.

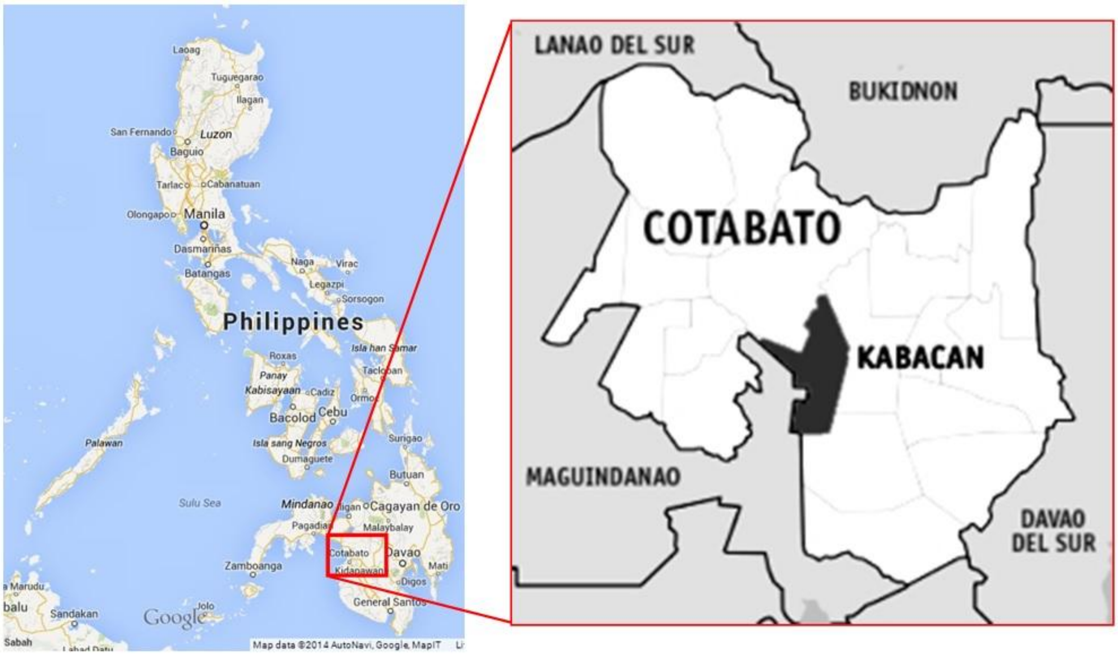

Fig. 1: Sampling area located in the town of Kabacan at Cotabato Province with coordinates of 7°07' north latitude and 124°49' longitude.

Collection of Samples:

Opportunistic sampling method of Torres *et al*. (2013) was adapted with some modifications. Rice black bugs were collected randomly from a rice field through hand-picked as RBBs were generally located at the base of the infected rice plant. The samples consisted of 120 specimens (60 males and 60 females). Samples were placed in a container soaked with 70% ethanol and sealed properly.

Segregation of Sexes:

Each of the captured samples were segregated by their sex through observation under a stereo microscope. Sex of rice black bag is distinguishable by means of their genital plates found at the tip of the anterior of the body as shown in Figure 2. Male RBB's are distinguishable by means of a saddle shaped abdominal tip of the anterior of the body. Female RBB's are distinguishable by means of two triangular protruding warts located in the inferior tip of the abdomen. Each RBB specimen was placed in a separate container and soaked with 70% ethanol and properly labelled by sex and specimen number (Torres *et al*., 2013).

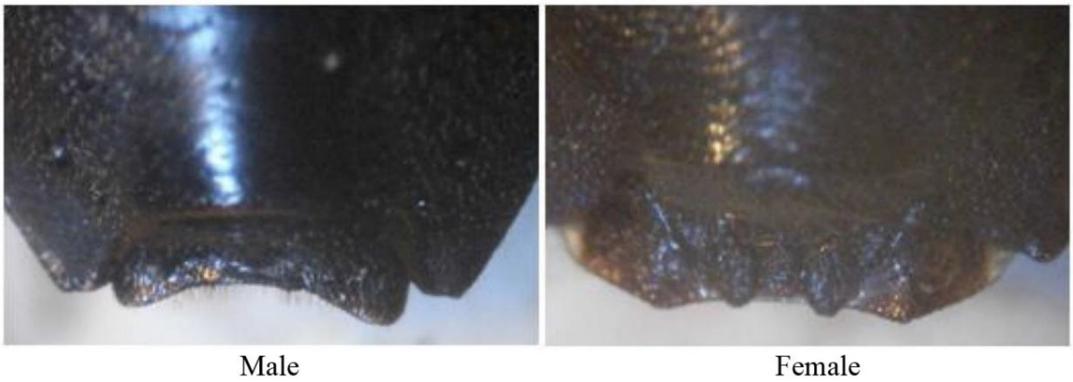

Fig. 2: Determination of male and female RBB through genital plates showing a saddle shaped abdominal tip for male and triangular warts for female (Torres *et al*., 2013).

After segregation of male and female RBB's, the head sections were observed for 2-dimensional geometric morphometric analyses. The images of the head were taken using a digital camera, SONY-W690 with 25mm wide-angle 16.1 megapixels G-lens, under a stereo microscope with 200X magnification.

Landmark Points:

The two-dimensional Cartesian coordinates of 14 landmarks for the head (dorsal view) was based on the defined landmarks of Torres *et al*. (2010) as shown in Figure 3. Sample specimens were digitized by TpsDig version2 software (Rohlf, 2004). These landmark points were chosen as an initial observation for the analyses on the characters which have object type of symmetries and differences between the left and right sides, both male and female RBB, that can often be seen.

Fig. 3: Position of the 14 digitized landmarks on the head (dorsal view) of rice black bug *Scotinophora* sp. as follows; Landmarks 1 and 14 are located at the sides of the tylus at the tip of the head; Landmarks 2 and 13 define the shape of the jugum; Landmarks 3, 4, 11 and 12 describe the position of the antennifer on the lateral sides of the head; Landmarks 5, 6, 9 and 10 locate the position of the compound eyes of the insect; and Landmarks 7 and 8 are found at the posterior margin of the head defines the junction between the head and the prosternum.

Geometric Morphometric Analysis:

In order to graphically demonstrate patterns of shape variations, thin-plate splines were used based on the landmarks which denotes the transformation of the reference to each specimen (Bookstein, 1991). Male and female RBB digitized landmark head sections were run separately. All specimens, both 60 males and 60 females, were digitized with three replicates in order to reduce the measurement of error (Dvorak *et al*., 2005). With a software TpsDig version2, x and y coordinates of the landmark points which were the raw data used for the variation in the shape of the head sections obtained. The TpsUtil program were used to build Tps file and make links files. The relative warp analysis was performed using the TpsRelw program version 1.46 (Rohlf, 2004). The attained landmark configurations were then scaled, translated, and rotated against the consensus configuration by General Least Squares (GLS) Procrustes superimposition 2D method (Bookstein, 1991; Rohlf and Marcus, 1993; Dryden and Mardia, 1998). Multivariate analysis was done using the Palaeontological Statistics (PAST) software.

The method of Relative Warp (RW) analysis corresponds to a Principal Component analysis (PCA) of the covariance matrix of the partial warp scores (Frieß, 2003). As with PCA, the most informative are the first and the second relative warps (Hammer *et al*., 2001). In the present study, relative warp analysis was performed using the TpsRelw ver. 1.46 (Rohlf, 2008) following the algorithms developed by Bookstein (1991).

In order to graphically illustrate patterns of shape variations based on the landmarks which represents the transformation of the reference to each specimen, used the thin-plate splines. The principal warps are calculated from the reference configuration to define a set of coordinate axes for tangent space approximating the curved shaped space to which the shapes of specimens can be compared using standard linear statistical methods. The resulting x- and y- coordinates of the aligned specimens onto the principal warp axes are then projected (Bookstein, 1991).

Discriminant Function Analysis (DFA) was used to best discriminate (separate) scale shape difference between male and female RBB and equality of the means of the compared groups was tested using Hotelling's t-squarred showing a p value ($p < 0.05$) . Multivariate Analysis/Canonical Variate Analysis (MANOVA/CVA) was used for the analysis of these relative warp scores for the head shape of the male and female RBB.

Results:

Thin-plate spline reconstructions of the changes in the shape of the dorsal head were obtained from the overall consensus of the average configurations of the *Scotinophara* sp. Four significant RW for the male (77.72%) and 5RW (74.31%) for the females explain variations in the insect. These variations are graphically presented in in Fig. 4 and the descriptions shown in Table 1.

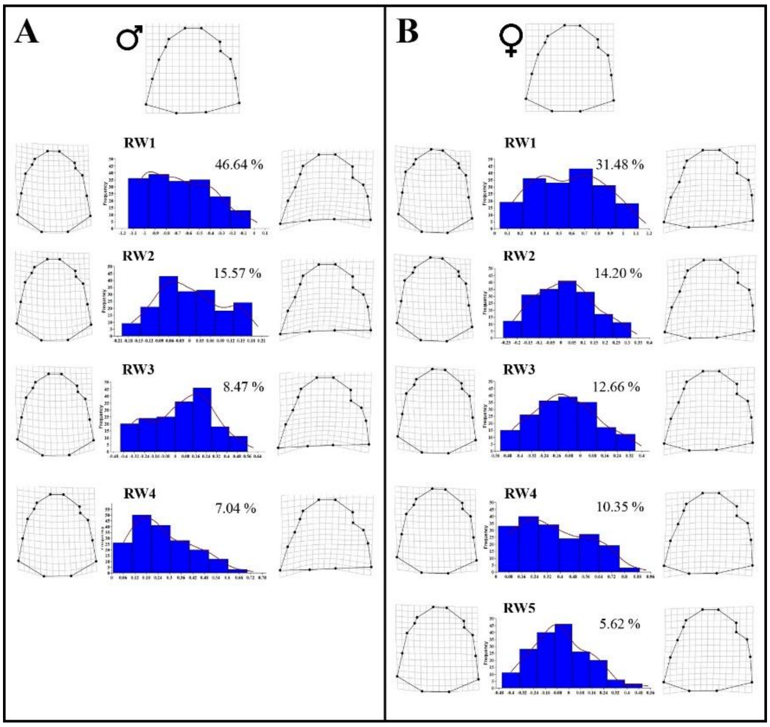

Fig. 4: Summary of the geometric morphometric analysis showing the head shape variations of (A) male and (B) female *Scotinophara* sp. from Kabacan, North Cotabato. It shows the consensus morphology (uppermost figure) and the extremes of their variation produced in male with five relative warps explaining more than 5% of the variance with 77.72% of the total variation (RW1= 46.64%, RW2= 15.57%, RW3= 8.47%, RW4= 7.04%). However in females, comprised of five relative warps with 74.31% of the total variation (RW1= 31.48%, RW2= 14.20%, RW3= 12.66%, RW4= 10.35%, RW5=5.62%).

Table 1: Variations observed in the head shape of male and female *Scotinophara* sp. as outlined by each significant relative warps.

	Male	Female
RW1	Individuals tend to be mostly in positive deviation. Variation is attributed in length and width aspect ratio. Differences: In (-) RW1, has a shorter distance from jugum to antennifer and inclined position of the eye. The tylus has a shorter distance. In (+) RW1, has a downward position of the eye and wider. The tylus has a longer distance.	Individuals tend to be near to the mean deviation. Variation is attributed in anterior margin aspect ratio. Differences: In (-) RW1, has shorter distance from jugum to antennifer and has a slight inclination of tylus. In (+) RW1, eyes have a concave lining and inclined position between eyes and prosternum and a convex margin between jugum and antennifer.
RW2	Individuals apt to be in negative deviation. Variation can be seen in the anterior section for (-) RW2 and in posterior-lateral section for (+) RW2. Differences: In (-) RW2, tylus is slightly bend outward and a concave margin on antennifer. In (+) RW2, has shorter between jugum and antennifer and inward inclination on prosternum.	Individuals apt to be in negative deviation. Variation can be seen in the posterior margin for (-) RW2 and lateral margin of head for (+) RW2. Differences: In (-) RW2, tylus is shorter and outward inclination between eyes to prosternum and well defined. In (+) RW2, the lateral margins between jugum and antennifer is slightly outward.
RW3	Individuals move towards positive deviation. Variations mostly seen in length and width aspect ratio. Differences: In (-) RW3, distance between jugum to eye is longer and margins between eyes to prosternum bends out. In (+) RW3, tylus is shorter and the position between eyes to prosternum has outward inclination.	Individuals move towards positive deviation. Variations mostly seen in the lateral margins for both (-) and (+) on its length-width ratio. Differences: In (-) RW3, jugum bends outward and distance from antennifer to eye is longer. In (+) RW3, there is equal distance from boundaries between antennifer and eyes. Prosternum is slightly outward.
RW4	Individuals tend to be near the mean deviation. Variation is attributed to the concavity of its lateral position. Concave to those negatively correlated (-) RW4 and convex to those positively correlated (+) RW4. In (-) RW4, boundaries between antennifer and eye has concave form and wider and a shorter distance form eye to prosternum. In (+) RW4, the distance from jugum to eye is much shorter and has slight inclination from eye to prosternum. Inward inclination for prosternum.	Individuals tend to near the mean deviation. Variation is attributed in length and width aspect ratio. Differences: In (-) RW4, boundaries between antennifer and eye is shorter and boundaries from eyes to prosternum is wider. In (+) RW4, distance from antennifer to eye is equal and has longer distance on eye-prosternum boundaries.
RW5		Individuals apt to be in positive deviation. Variation is attributed to the concavity of its lateral portion. Concave to those negatively correlated (-) RW5 and convex to those positively correlated (+) RW5. Differences: In (-) RW5, a concave margin apt between jugum and antennifer and boundaries between eyes to prosternum is inclined outward. In (+) RW5, convex margin apt between jugum and antennifer and distance between eye to prosternum is shorter. Outward inclination for prosternum.

Multivariate analysis of variance, canonical variate analysis (MANOVA/CVA) discriminant function analysis (DFA) using the paleontological statistics software (Hammer *et al*., 2001) show significant differences between sexes in the shapes of their heads (Tables 2 and 3, Figures 4 and 5). Results of these statistical tests show sexual dimorphism in the head shapes of the pest.

Table 2: Results of MANOVA test for significant variation in the shape of the head in a population of male and female *Scotinophara* sp. from Kabacan, North Cotabato.

	value	df1	df2	F	p(same)
Wilk's Lambda	0.1626	4	355	457	1.68×10^{-60}
Pillai trace	0.8374	4	355	457	1.68×10^{-60}

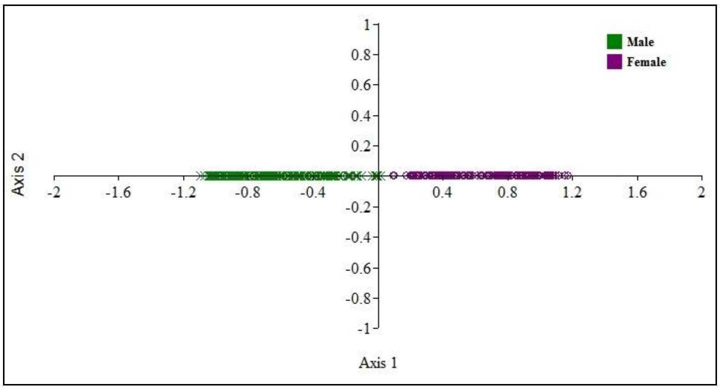

Fig. 5: CVA scatterplot of the landmark coordinate data of male and female head shape variation for the first four relative warp scores.

Table 3: Summary of DFA results for male and female *Scotinophara* sp. collected from Kabacan, North Cotabato.

	Number (N)	Male	Female	Corrected classified (%)
Male	180	180	—	100
Female	180	—	180	100

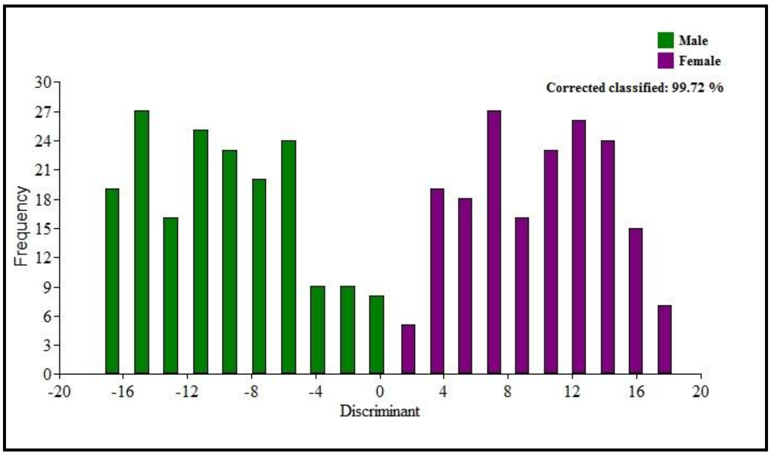

Fig. 6: Graph of Frequency Distribution of the Discriminant Scores (DFA) of the relative warp scores of highly significant scale variation between male and female with $p=1.68 \times 10^{-60}$.

Discussion:

It can be seen from the results that morphological variations between sexes exist in RBB. There were variations observed in symmetry. While morphological characters disproportion between sexes is a prevalent phenomenon (Benitez *et al*., 2010), structures with perfect symmetry are rare in nature. Klingenberg *et al.* (2002) argue that real organisms hardly ever are perfectly symmetric and that small asymmetries affect the structural features that lie in the midsagittal plane of the idealized body plan. In this current study for example, the tylus and other midline structures of the head can either project to the left or right to some extent. 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 within sexes. The extent of variations within sexes may explain the differences between sexes as shown by the outcome of MANOVA/CVA and DFA. Differences in development time between the sexes might be one of the major proximate mechanisms to produce sexual dimorphisms (Jarošík and Honek 2007). In a related study by Torres *et al.* (2010) comparing several populations of the insect show that the mean symmetric shapes of the heads of *Scotinophara* sp. for both sexes are different from that of the other populations. It therefore indicates that the pest is not only morphologically variable but genetically different as their results show geographic distance is not correlated with the differences observed. The dimorphism observed could also be associated with a sap-sucking adaptive character since female consumes more food and might be due to egg production (Heinrich, 1994). The differences observed between populations of the pest collected geographical locations could be attributed to differences in rice plant characters and the genotype of the insects that are able to feed on those rice types (Torres *et al*., 2010).

Conclusion:

Results of this study clearly show the importance of quantitative methods in describing variations in morphological characters of organisms especially in RBB which is considered one of the most problematic pest of rice. The tool of GM has helped detected the existence of sexual dimorphism in the shape of the head character of RBB. Geometric morphometric analysis showed that the differences can be quantitatively measured and evaluated if the differences observed were significant. GM however cannot be argued to be the only tool in quantitatively assessing variations in morphological characters, there are other existing tools that can be used to further explain the variations observable in morphological characters of organisms.

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