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Inter- and intra-population variation in genital shapes of Rice Black Bugs, *Scotinophara* spp.

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Abstract

It is much harder and less effective to manage pest if their identities are unknown. The rice black bug (RBB), *Scotinophara* spp., is an epitome of such pest. It is said to attack rice at all stages of the plant's development causing reduced yield in many farms in the Philippines. However, questions abound regarding its identity because of the immense morphological diversity its population's exhibit, even in traits that are traditionally believed to be stable such as the genitalia. As a case study, samples of RBB from two locations in Lanao del Norte, Philippines were examined and tested for variability in genital shapes using the method of Geometric Morphometric. The findings show that among females, 55% of the variation is related to asymmetrical differences the left and right sides of the genital plates. While among males, 32.5% is acrued to differences in the concavity of the gentalia. Data show continuous shape variation within each sex of RBB, which open up a lot of questions regarding the utility of the genitalia as diagnostic character in species delimitation. Traditionally, reproductive characters are used as bases in splitting or lumping species. However, due to its continuous nature and the wide extent of variation it manifests even within species, the genitalia may be used with caution in delimiting species boundaries.

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Introduction

The Rice black bug (RBB), *Scotinophara* spp., is of recent interest to entomologists and rice researchers in the Philippines as it appears to be a complex species whose component taxonomic groups may or may not actually be pest of rice. Close examination of these organisms reveal various morphotypes, which were describerd as either color morphs, varieties, ecotypes, or separate species depending on the tools used to delineate taxonomic boundaries. There are various hypotheses regarding the origin of RBB in the country, however, they were not known to the local scientific community until their first discovery in Bonobono, Bataraza Southern Palawan (Miyamoto *et al.,* 1983). Fears abound regarding its ability to infest vast fields of rice farms (de Sagun *et al., 1991*).

There are various ways to establish the taxonomic placements of populations of RBB that differ in certain morphological characters. One of it is to test for variations in the shape of the genitalia, which traditionally are believed to be stable for a particular species. There is however a caveat to this claim of genital stability as morphological variations in this trait has been documented especially among animals (Eberhard, 1985; Hosken and Stockley, 2004).

In 2007 for example, by observing external morphological characteristics, de Frietas and Hermanson showed the existence of wide intra- and inter-population variation in the genitalia among animals (de Frietas and Hermanson, 2007).

While variation in the genital shapes is widespread even within species, it is nonetheless still important to document such. In the recent years, quantitative analyses of shapes of the genitalia and other biological structures have been possible because of the advent of Geometric Morphometrics (Rolly, 2012). The advantage of using GM is that aside from quantitatively describing shape differences, it also permits visual interpretration of reconstructed images of the biological structure through Thin-plate spline grids (Adams *et al.,* 2013).

Mindful of the above, the approach of GM was used in this study to describe differences in the shapes of the genital plates within and between two populations of rice black bugs from Tominobo, Iligan City and Matampay, Lanao del Norte.

This study is part of many years of research in an attempt to mine and map data on RBB characteristics in the hope of resolving ecological and taxonomic questions regarding the organism's real identity and nature. Using GM, this study aimed at describing patterns of genital shape variation in two populations of RBB from Iligan City and Matampay, both from Lanao del Norte, Philippines. This study specifically aims to: (1) Utilize landmark-based GM to harvest Cartesian coordinates of landmark points in an attempt to capture the shapes of the RBB genitalia; (2) Employ multivariate methods of analyses on harvested Cartesian coordinates to determin intra- and inter-population variation in genital shapes; (3) and reconstruct images of the genital plates using Thin-plate spline grids to allow visual interpretation of shape differences.

Materials and methods

The structure and content of this paper follows the general outline in geometric morphometric studies, starting from the collection of RBB and preparation of genitalia, harvesting of landmark data and extracting Cartesian coordinates, multivariate analyses and visual reconstruction via Thin plate spline grids.

Collection and sample preparation.

A total of 114 samples were examined in this study, 67 of which were collected from Matampay in Lanao del Norte and 47 from Tominobo, Iligan City (Fig. 1). Sex disaggregated data shows that among the RBB from Matampay, 42 were females while 24 were males. For Iligan samples, the ratios between sexes were roughly equal at 24 females and 23 males. All these samples were handpicked from the bases of the rice plants, were the RBBs were seen to cluster together. In the interest of preserving the samples, the RBBs were placed in containers with fixed in 70% ethanol.

Fig. 1. Geographic positions of the two sampling sites, namely Tominobo, Iligan City and Matampay, Philippines. Map sourced online from www.google.com.ph.

Image acquisition and selection of landmark points

Images of the genital plates from both sexes were taken using a digital camera. Close examination of the genitalia reveals a degree of sexual dimorphism in shape. Among female RBBs, the genital plates possess triangular protruding warts, which are located in the interior tip of the abdomen. For male RBBs, the samples have saddle-shaped abdominal tips located anterior of the body. Because of the shape differences in genital shapes, more landmarks were collected for females (17). For males, only 16 landmarks were assigned around the biological structure (Fig. 2).

Digitization of landmarks and harvesting of Cartesian Coordinates

Manual dissections of the genital plates were done to separate the structure from the rest of the body parts. Then, photographs of the genitalia were taken using a DSLR camera. These images were then loaded to tpsUtil ver.1.74 to produce tps files.

The tpsDig version 2 was then used to process the resultant tps images, and subsequently utilized as platform to manually locate landmarks and harvest the Cartesian coordinates (Bookstein, 1991; Rohf 2004).

Data analyses

Relative warp analysis was applied to the Cartesian coordinate using the tps.Relw software (Rohlf and Slice, 1990). With the aid of this software, it was possible to generate relative warp scores (RW scores) through a generalized least squares Procrustes fitting. These RW scores were then used as morphometric variables for descriptive and multivariate analysis using the PAST software (Hammer *et al.,* 2009). For the descriptive analysis, the RW scores were summarized using box-and-whisker plots constructed to visually compare the shape parameters between the two populations. Thin plate spline grids were then crafted to aid in explaining shape differences. For multivariate analyses, the Canonical Variate Analysis (CVA) was employed to to test for significant differences in the relative warp scores between the two populations (Adams *et al.,* 2004).

Results and discussion

Visual inspection of the thin-plate spline grids show that the patterns of shape variation between the two populations differ between sexes (Fig. 3 & 4).

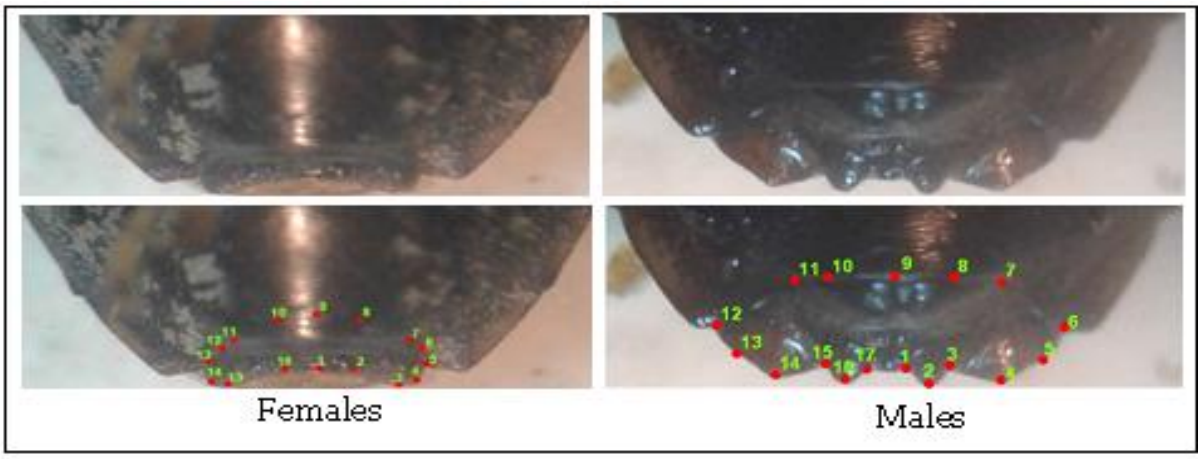

Fig. 2. The figure shows the male and female genital plates, a male showing a saddle-shaped abdominal tip and triangular warts for female and their land marking in which female has 17 landmark points and male has 16 landmark points.

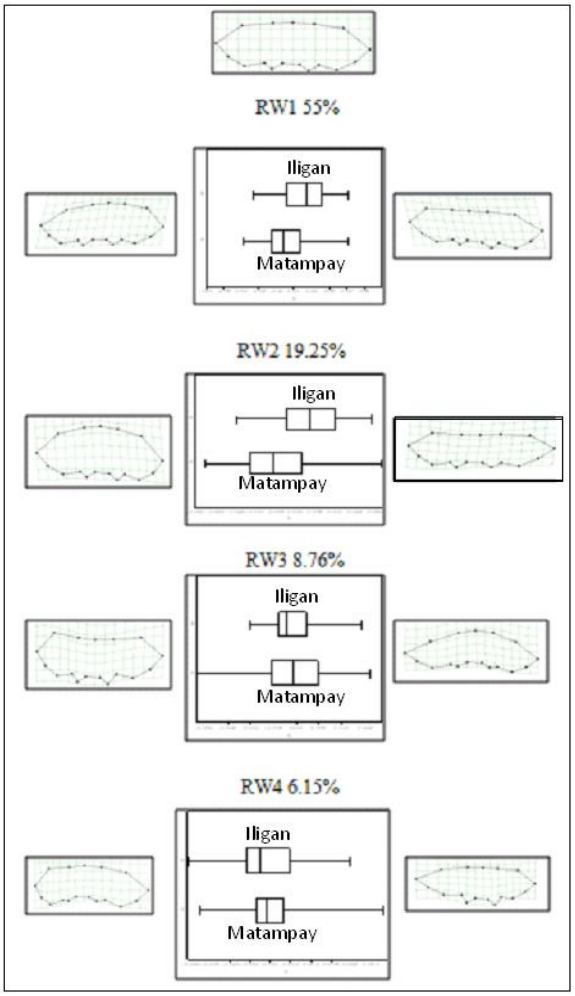

Fig. 3. Box-and-whisker plots of relative warp scores showing differences in shape parameters between two populations of female Rice Black Bugs from Iligan and Matampay.

Among females, the genital plates appear to be asymmetrical. In fact, asymmetry in this character contributes to a little more than 50% of the shape variance. Aside from this, the Iligan and Matampay populations also seem to differ in the direction of the genitalic plate's asymmetry (RW1 of Fig. 3).

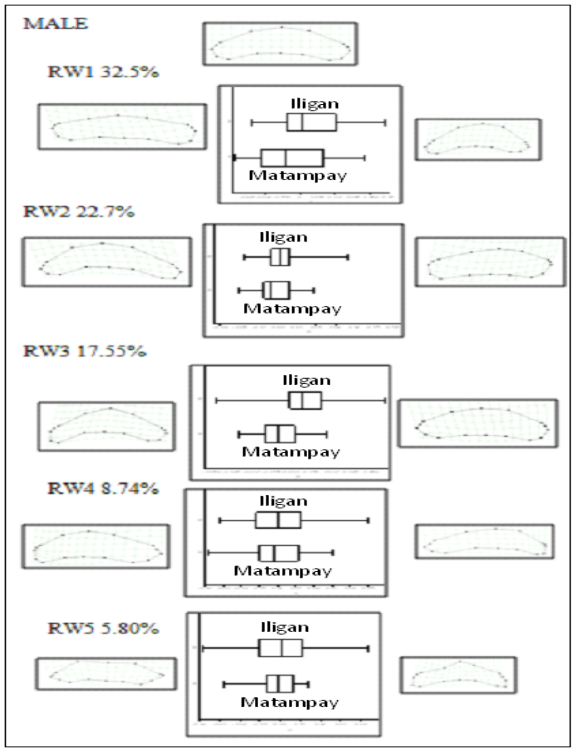

Fig. 4. Box-and-whisker plots of relative warp scores showing differences in shape parameters between two populations of male Rice Black Bugs from Iligan and Matampay.

For females, what is more evident is the shape differences in the anterior margins rather than the posterior one (19.25%), which also scores low among males (8.76%). Further asymmetries in the shapes of the lateral margins can also be seen among females and males which contributes to 6.15% and 8.74% to the total variance respectively (RW4 of Fig.3; RW4 of Fig. 4).

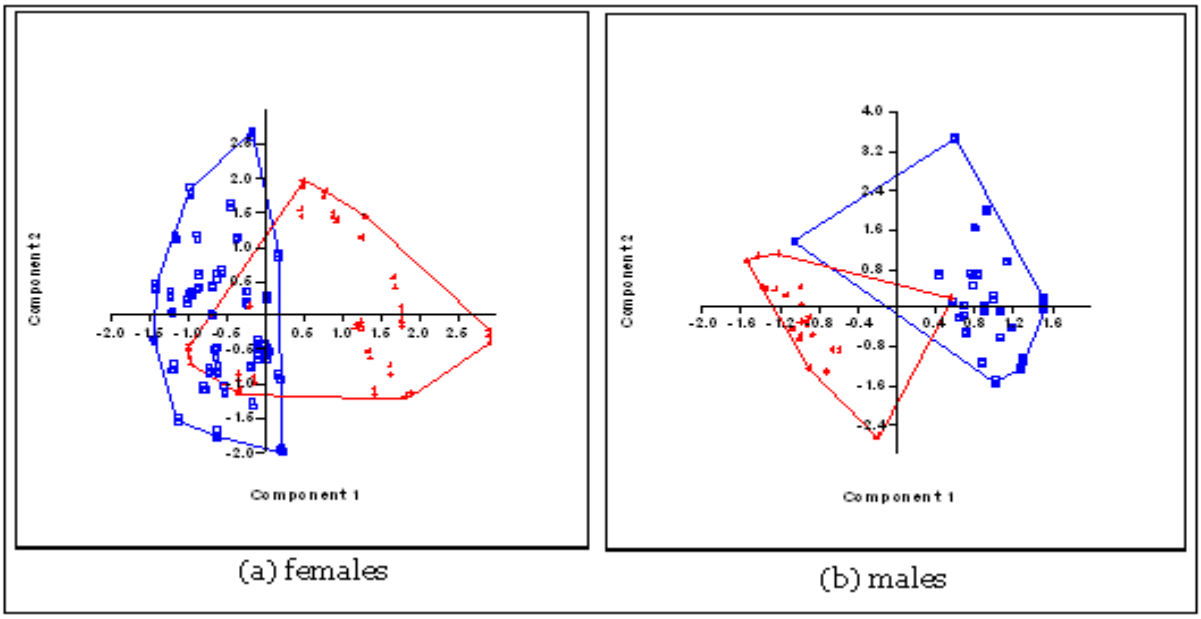

Fig. 5. Ordination plot produced through Canonical Variate Analysis of Relative Warp scores showing minimal overlap in the shape parameters between the two populations of Rice Black Bugs from Iligan (red) and Matampay (blue) in both sexes.

The ordination plot in Fig. 5 produced via CVA of the RW scores reveal that when all the relative warps were analyzed altogether, differences in the shape parameters between populations can be seen. This interpolation differences in not unique as this has also been documented in previous studies using similar multivariate methods that utilize techniques that mine patterns in data of high dimension (Smith, 2002).

The importance of this result is anchored in the notion that the genitalia is one of the most important character in the life cycle of sexually reproducing organisms. Aside from its function in procreation, the genitalia also serve as mechanical barrier to inter-species sex. Thus, it is vital to maintaining cohesion among members of a particular taxon. This school of thought of inflexible genitalia for a given species is however slowly being challenged as studies have come to show that even within species, the shapes and sizes of this structure can vary widely.

There are alternative explanations to the above observed variation. For some taxonomic groups, conspicuous differences in male and female genitalia may reveal episodes of rapid, divergent evolution within phylogenetic branches (Hedin, 1997). At the micro level, genetic variations accompany differences in the appearance of the genitalia among populations (Feuk *et al.,* 2006).

According to Eberhard (1985), the species-specific diagnosibility of male genitalia in most species reflects both the rate and extent to which organisms diverge. In this context, any structure as consistently useful as a taxonomic character such as the genitalia must evolve rapidly, as this divergence is inextricably coupled with speciation. For mating systems in which fertilization is internal, the male genitalia likely serve a stimulatory role during copulation, and thus undergo strong sexual selection through female choice (Eberhard 1985; Arnqvist*et al.* 1997; Markow 2002; Masta and Maddison 2002).

Conclusion

This study utilized the geometric morphometric method of landmark-based analysis to detect disparity in genitalic shapes between two populations of Rice Black Bugs, *Scotinophara* spp. from Iligan and Matampay in Lanao del Norte, Philippines. Results showed continuous variation in the shapes of the genitalia, be it from among the male or female samples. Notable findings include the fact that asymmetry in the genitalia among females constitute more than 50% of the variance. For males, the genitalia vary in terms of the extent of concavity of the outer margins, particularly of the posterior sides. The occurrence of variation in the shapes of the genitalia in RBB may open a lot of questions regarding the utility of reproductive characters in delineating species boundaries. While there is a dearth of research on RBBs in Mindanao, it is recommended that a total evidence approach be used to harvest and collate various kinds of data to have a comprehensive understanding of the true nature of this organism. Unless we be able to establish whether morphotypes represent only ecotypes or sibling species already, effective management of this RBB remains to be a remote possibility.

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