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Paramere Morphology of Two Colormorphs of the Brown Planthopper *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae)

Adam Roy V. Galolo
Biology and Environmental Division
Natural Science Department
College of Arts and Sciences
Caraga State University
Ampayon, Butuan City 8600
Philippines

Mark Anthony J. Torres and Cesar G. Demayo
Department of Biological Sciences
College of Science and Mathematics
MSU-Iligan Institute of Technology
Iligan City 9200
Philippines
For Correspondence email: cgdemayo@gmail.com

***Abstract* - A major rice-infesting insect pest, the brown planthopper (*Nilaparvata lugens* (Stål), consists of populations or morphs exhibiting range in body coloration, namely: black, brown, red, and their combinations. To determine the possible correlation of such color polymorphism with reproduction of *N. lugens*, this study investigated the paramere morphology of the black and brown morphs. The paramere is a part of the male genitalia directly involved in the bisexual mating of N. lugens. The paramere shape was examined through the truss network geometric morphometric method on 27 brown and 26 black individuals from two populations. A total of 24 points were defined manually on the left and right parts of the structure as landmarks using an image processing software. These landmarks were used to establish the internal outlines of the paramere. The Cartesian coordinates digitized from the landmarks were then transformed into shape residuals as Procrustes coordinates. Results revealed clear-cut morphological differences in the shape of the paramere between the two color morphs. Specimens of the brown and black morphs were classified correctly 100% of the time. A statistically significant difference was also found between the two groups when the shape data was used as morphometric variables and subjected to analysis of similarity and nonparametric multivariate analysis of variance. Differences in the shapes of the paramere between the two morphs were also visualized using Relative Warp Analysis (RWA) and thin-plate spline transformation grids.**

Keywords: Nilaparvata lugens (Stål), truss network, geometric morphometrics, landmarks

I. INTRODUCTION

Insect pests like the brown planthopper *Nilaparvata lugens* Stål (Homoptera: Delphacidae) (Fig. 1), are considered the main constraints limiting rice yields in tropical environments. It is a serious pest of rice crops throughout Asia, damaging plants both through its feeding behavior and by acting as a virus vector [1]. Both the nymphs and adults of this rice pest insert their sucking mouthparts into the plant tissue to remove plant sap from phloem cells. Uncontrolled use of insecticides and monoculture are considered the main causes of BPH outbreaks in many rice growing countries [2]. For the past several years improving a durable and broadspectrum resistance in cultivated rice were done but only a few studies have been made on the genetic control of the durable and

Figure 1. *Nilaparvata lugens* (Homoptera: Delphacidae) brown planthopper and the damage to the rice plants.

broad-spectrum BPH resistance. Fourteen (14) major effective BPH resistance genes assigned to rice chromosomes were identified in *indica* cultivars [3-11] and four wild relatives, *Oryza australiensis, O. officinalis, O. latifolia* and *O. eichingeri* [12-15]. Deployment of resistant cultivars however still suffers from BPH outbreaks and new biotypes/races were identified. A closer look at the insect pest showed not only they differ in their response to the resistance factors in the rice plant but differences in morphological appearance were also observed [19-24]. Individuals of this phloem-feeder insect pest exhibit genetically determined discontinuous variation in body color varying dramatically with some populations having black, red and brown morphs and their combinations. Selection studies of more than 25 successive generations for specific wing form and body color under low-density rearing conditions in N. lugesn failed to produce pure-bred lines with those characters [16]. However selections over 12 to 30 successive generationa under high-density rearing conditions were successful in obtaining various lines predominantly producing specific wing form and body color over a broad range of nymphal densities in both sexes; totally brachypterous lines with yellowish brown or highly melanized body color and totally macropterous lines with highly melanized or yellowish brown body color. Thus, it was concluded that the wing form and body coloration are controlled by different genes. Breeding experiments show that monogene for colormorphism exist in three allelic forms >-b^{+} (brown or wild type), b^{o} (orange) and b^{b}(black). The dominance relationship was b^{b}>b^{+}>b^{o}. The relationship between phenotype and genotype in *N. lugens* was not fixed. The six possible genotypes produced eight phenotypes. Intermediates between the extreme parents in the F1 and F2 of all the crosses were manifestations of certain variabilities that can be attributed to (1) residual heredity or filial regression due to incomplete dominance, environment, and epistasis, or (2) modifier genes that change the phenotypic effects of other genes in quantitative fashion through dilution or enhancement of major gene effects [17]. To further evaluate whether colormorphs also vary morphometrically, we investigate the morphology of the paramere, of pure strains of black and brown morphs of the hopper. A number of methods have been used to explain variations in this highly dynamic insect but less has been accounted using geometric morphometric (GM) approach. The field of geometric morphometrics is relatively new and has shown very rapid progress over the last few years. Defined as the fusion of geometry and biology [18], geometric morphometrics deals with the study of form in two- and three- dimensional space. The field flourished from a desire to analyze biological forms in ways that preserve the physical integrity of form in two dimensions, and to avoid collapsing the form into a series of linear or angular measures that do not include information pertaining to geometric relationships of the whole. Geometric morphometrics (GM) has recently become a popular tool in many fields of biology including systematics, ecology or anthropology, as it affords new possibilities for the interpretation of shape and shape change in organisms. It is, therefore, the main objective of this study to use the tools of GM to be able to describe and understand if there exist variations in in the pest especially in one of its reproductive structures, the paramere (Fig. 2). The parameres are the principal genital claspers of the adult. The term paramere refers to the lateral genital lobes shown to be derived from the lateral branches of the primary phallic rudiments. In taxonomy, the male showing distinctive genitalic characters such as the paramere establish beyond any doubt the identity of this species as examples of intraspecific variation in differentiated populations.

II. MATERIALS AND METHODS

Samples. Prepared slides of the parameres of pure strains from a total of twenty six brown and twenty seven black color morphs of the brown planthopper were photographed using a microncam connected to a microscope (400x magnification). A scale was used to standardize the sizes of the photographed images.

Data collection. The two-dimensional Cartesian coordinates of a total of 24 landmarks were recorded with a digitizinsoftware. Figure 2 shows the position of the landmarks taken from the left and right parameres.

Geometric Morphometric Analyses. Raw coordinates were superimposed using a Procrustes generalized least-squares (GLS) superimposition algorithm. This is an important procedure as it removes variation in digitizing location, orientation and scale allowing for the analysis of the shape of the parameres only. The superimposed coordinates were subjected to a thin-plate spline-based relative warps analysis. Thin-plate spline parameters allow the reconstruction of shapes, permitting direct visualization of shape differences. Relative warp analysis, on the other hand, corresponds to a Principal Component Analysis (PCA) of the shape variables. Tests for significant differences were carried out using Analysis of Similarity (ANOSIM) and the nonparametric Multivariate Analysis of Variance (MANOVA).

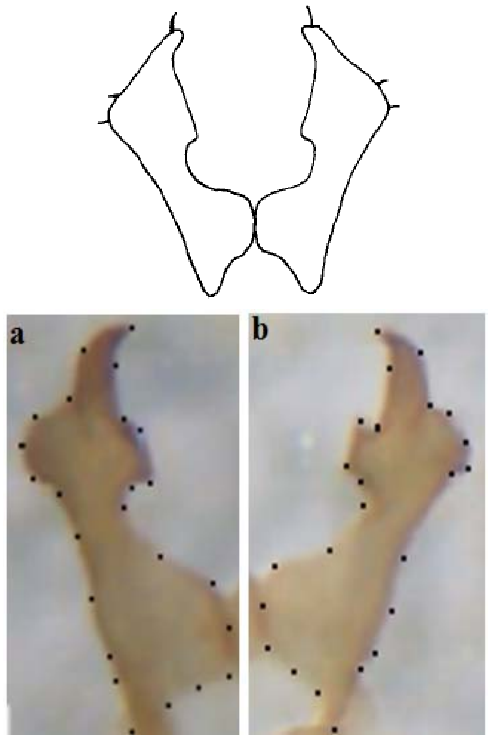


Figure 2. Landmark points around the (a) left and (b) right parts of the paramere.

TABLE 1. RESULTS OF THE TEST FOR SIGNIFICANT DIFFERENCES IN THE SHAPES OF THE PARAMERES OF THE BLACK AND BROWN MORPHOTYPES.

SIDE	TEST	STAT	P-VALUE	REMARKS
Right	ANOSIM	**R: 0.1326**	0.0002	Extremely significant
	NPMANOVA	**F: 3.961**	0.0004	Extremely significant
Left	ANOSIM	**R: 0.1034**	0.0006	Extremely significant
	NPMANOVA	**F: 3.378**	0.0004	Extremely significant

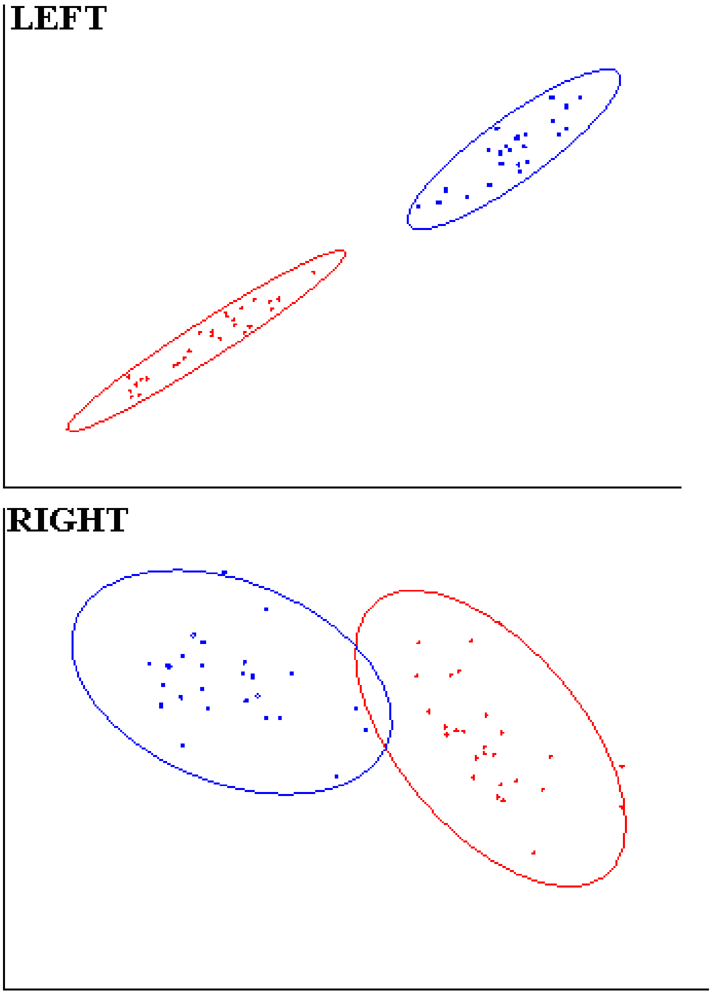
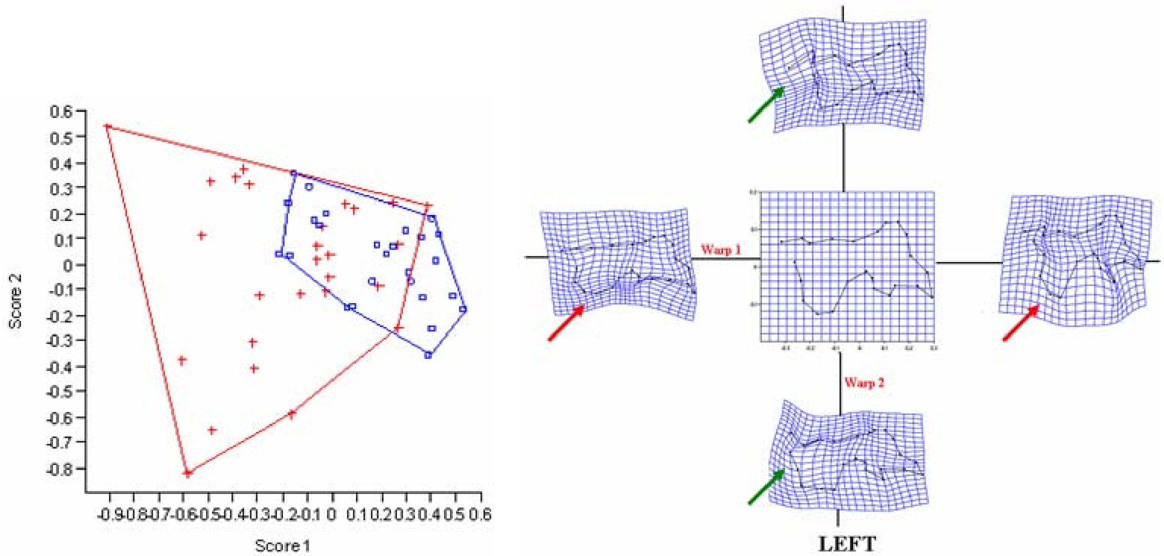


Figure 4. Projections of the brown plant hoppers along the first two canonical axes.



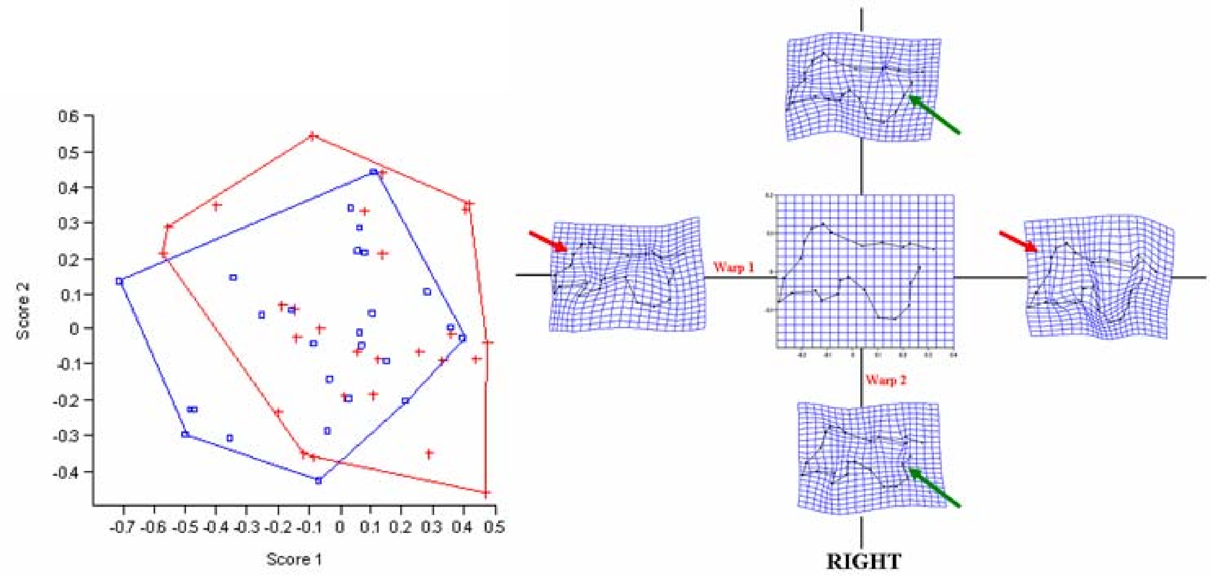


Figure 3. Projections on the first two relative warps. At the extremity of each axis are represented the shape transformations, represented as a grid. The mean consensus shape is represented at the intersection of the axes.

III. RESULTS AND DISCUSSION

The results from the analyses showed significant differences in the shapes of the parameres between the black and brown morphotypes of *Nilaparvata lugens*. Consequently, the present work allows one to hypothesize a possible evolutionary mechanism that is operating on these populations that contribute to the observed differentiation of the shapes of the paramere. Significant differences in paramere shapes of the two colormorphs of the brown planthopper can be seen based on the distribution of the samples along the first two canonical variate axes (Figs. 3).

The great diversity in structural detail of the external genital organs of male insects gives these organs a value for the identification of insect species which is considered almost equal to that of fingerprints for the identification of human individuals. In this study, the very structural diversity of the paramere makes us to understand the fundamental nature of the genital structure as one of the major structure of the genital organs that differentiates populations from each other. The colormorphs of the hoppers as differentiated by the paramere may lead us to conclude the importance of this structure in understanding population differentiation in this pest of rice [19-24]. This indicates that the differentiation in the paramere can be correlated to the differentiation of the insect based on colormorphs. The results also suggest that the existence of colormorphs is due to the selection of mates of the hoppers. The first two relative warps accounted for much of the variation observed. Figure 4 illustrates the projections onto the first two relative warps together with the transformation grids at each axis. Following the first axis, major differences between the black and brown morphotypes involve the shape of the part of the paramere indicated by the red arrows.

Further tests using Analysis of Similarities (ANOSIM) and Non-parametric Multivariate Analysis of Variance showed that the variations observed in the shapes of the parameres between the two morphotypes are statistically significant (Table 1).

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