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Research Article

Quantitative Description of the Hindwings of the different populations of the Rice Black Bug *Scotinophara coarctata* using Landmark-based Geometric Morphometrics

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Abstract. Due to pest outbreaks, rice production has been decreasing all these years. The rice black bug (RBB), *Scotinophara coarctata* has recently been considered to be a major problem of rice producing regions in the Philippines especially in Mindanao. Since there were observations that the patterns of infestations of rice plants vary between rice fields in different geographical areas, it was hypothesized that populations of the pest vary. To be able to understand these differences observed, morphological shape variations of hindwings in RBB were quantified and determined using landmark-based geometric morphometrics. Results revealed within population variations due to sexual dimorphism. Significant differences between non-outbreak and outbreak populations and between geographically different populations were also observed although the differences were not based on distances between populations. The results indicate that the differences could be attributed to the distinctness of each of the populations examined and could be due to selection of selected genotypes that are able to survive on the rice genotypes planted on the different rice fields. The study also shows that quantitative analysis of populations through geometric morphometric analysis of shape variations is helpful in understanding the nature of variability among populations.

Keywords. Hindwings; Geometric morphometrics; *S.coarctata*

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1. Introduction

Among all insect pests of rice, the rice black bug (RBB) *Scotinophara coarctata* has recently been considered a major problem of rice producing regions in the Philippines [1], [2]. RBB strikes rice plants at almost all stages of its growth and could result in severe to complete crop loss or bug burn during heavy infestation [2]. Since many of the reported cases of pest outbreaks vary from locations to locations, it was hypothesized that the pest's population structure also varies between locations and also in response to the different genotypes of the rice plant. To be able to understand the nature of those populations attacking the rice plant, the study was conducted on the different populations of the pest using the tools of *geometric morphometrics* (GM).

GM is an approach that investigates variability in shape morphology of biological structures. One type of method is the 'landmark-based' analysis that uses a set of landmarks to describe the object or specimen. This method allows the study of shape and size that offers powerful analytical and graphical tools for the quantification and visualization of morphological variation among organisms [3]. Also it combines with powerful and flexible tools of multivariate statistics make it possible to study morphological variation with direct reference to the anatomical context [4].

In the current study, the hindwings of the insect pest were studied since wings of insects are considered an important organ that can adapt to various environmental conditions [5]. Wing traits evolve rapidly in respond to geographic clines and artificial selection [6]. Wing venation pattern and shape are species-specific thus are used in taxonomic identification [7]. It is argued that the wings have contributed to the unparalleled success and diversity of this insect thus the study of the RBB flight's evolutionary origin based on its structural design is of general interest. Flight represents a major innovation and the theory that RBB is a species complex based on observed geographical differences can be due to variation in their wing design. The current study focused on the patterns of morphological shape variations of hindwings of the rice black bugs *S. coarctata* that were quantified and determined using landmark-based geometric morphometrics. Variation within and between outbreak and non-outbreak populations and among different geographical locations were described.

2. Materials and Methods

Insect samples were collected in several rice producing municipalities of Carmen, Kabacan, Matalam, M'lang, and Tulunan in North Cotabato, Philippines (Figure 1) covering of more than 30,000 hectares of rice farms planted with irrigated-lowland rice varieties produced by PhilRice and distributed by the Department of Agriculture in North Cotabato Province. These varieties have either succumbed to RBB infestation or not.

Outbreak populations of RBB was classified as a large, sporadic population of the insect pest rising significantly above economic threshold level while a non-outbreak population is a small dispersed group of the insect pest occurring at the minimum than the overall farm areas [8]. Based on the annual record from PhilRice (Philippine Rice Research Institute) and BAS (Bureau of Agricultural Statistics), RBB's heavily damaged rice crops are in Kabacan, M'lang, and Tulunan except in Carmen and Matalam [9].

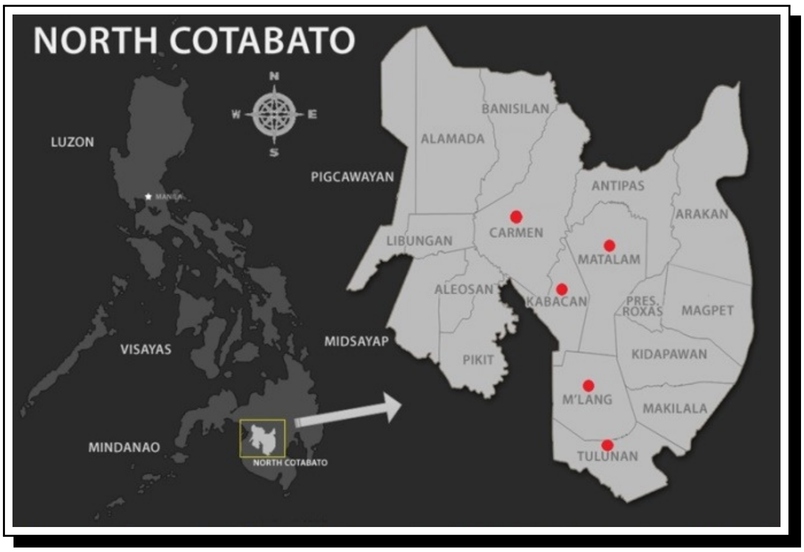

Figure 1. Collection areas of the rice black bugs

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 plants. Samples were placed in a container soaked with 70% ethanol + 30% glacial acetic acid and sealed properly. RBB's sex is distinguishable by means of their genital plates found at the tip of the anterior of the body [10].

For the wing preparations, tweezers are used to carefully separate the hindwings (left and right) from the body properly mounted in a clear 1×3 inches glass slide by pairs of six. Under a stereomicroscope, digital images were obtained using Sony Cybershot DSC-W690 with 16.1 megapixels prior for geometric morphometric analyses. Images of the hindwings (Figure 2) were digitized (Table 1) by tpsDig version1.40 software [11].

Table 1. Location and classification of 17 landmark points digitized on the 'hindwings' of RBB, *S. coarctata*

Landmark Number	Location
1, 2	Proximal end of the Costa (C)
3	Proximal end of the Cubitus (Cu)
4	Proximal end of Radius + Media (R + M)
5	Radial margin
6	Apical margin
7	Coastal margin
8, 9	Medial margin
10	Margins on the crossveins
11, 12	Distal end of the Cubitus (Cu)
13, 14, 15	Posterior margin
16	Anal margin
17	Proximal end of the Subcosta (Sc)

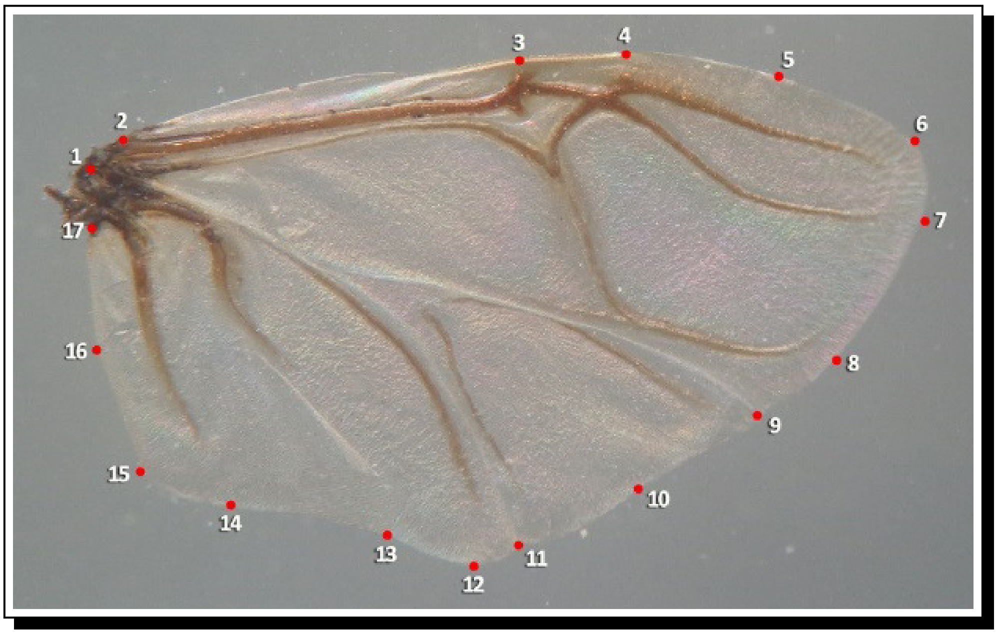

Figure 2. The position of the 17 landmark points of hindwings of RBB, *S. coarctata*

In order to graphically demonstrate patterns of shape variations, thin-plate splines were used based on the landmarks which denote the transformation of the reference to each specimen [12]. All specimens were digitized with three replicates in order to reduce the measurement of error [13]. With the software tpsDig version 1.40 [11], x and y coordinates of the landmark points, refer Table 1 which were the raw data used for the variation in the shape obtained. The tpsUtil program was used to build tps file and make links files. The relative warp analysis was performed using the tpsRelw program version 1.46 [14] that correspond to a covariance matrix of the partial warp scores [15] in which the most informative are the first and the second relative warps [16].

The attained landmark configurations were then scaled, translated, and rotated against the consensus configuration by *General Least Squares* (GLS) Procrustes superimposition 2D method [12], [17], [18]. The differences in spline shape were then used to describe the scale shape differences between sexes of each species. The relative warp scores recorded were converted in Excel Worksheet and were then used in analyzing shape variation using the *Paleontological Statistics* (PAST) version 3.0 software [16] prior to multivariate analysis. The relative warp scores were used to generate values that constitute multivariate shape data sets.

Using the result gathered from the relative warp analysis, histograms and box-and-whiskers-plots were generated by using the PAST software. The first and second relative warp scores, considered to be the most informative, were then subjected *Canonical Variance Analysis* (CVA) to determine variations among groups relative to the pooled within-groups variation and the Hotelling's pairwise comparison of the mean shape among populations. All statistical analyses used p-value of 0.05 for the level of significance.

3. Results

Disparity in the mean shape of the left and right hindwings within outbreak and non-outbreak populations of RBB is presented in Table 2. It shows that male and female sexes within outbreak and non-outbreak populations have significant differences in shapes in both left and right hindwings. Based on the CVA scatter plots, the distribution of relative warp scores within populations show that the non-outbreak populations have significant differences between sexes when compared to outbreak populations (Figure 3 and 4). However, when the geographically different populations were compared, differences were observed between all the populations compared regardless of the sexes of *S. coarctata* (Table 3).

Table 2. MANOVA/CVA of the left and right 'hindwing' shape variances within outbreak and non-outbreak populations of *S. coarctata*.

Hindwings	WITHIN	Wilk's lambda	Pillai trace	p(same)	Remarks
Left	Male and female Outbreak	0.9467	0.0533	**2.019E-11**	Significant
	Male and femaleNon-outbreak	0.9350	0.0650	**8.762E-10**	Significant
Right	Male and female Outbreak	0.9147	0.0853	**3.936E-19**	Significant
	Male and femaleNon-outbreak	0.8671	0.1329	**1.949E-20**	Significant

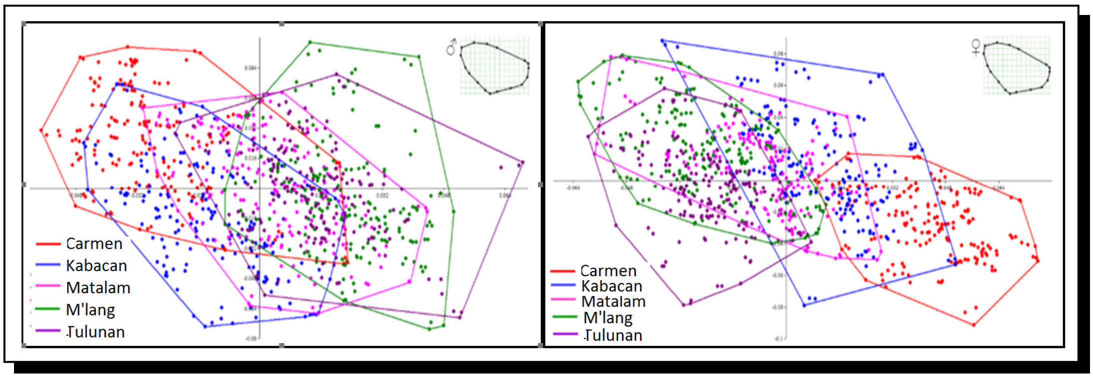

Figure 3. CVA scatter plots showing the distribution of male and female individuals of *S. coarctata* based on left hindwing shapes.

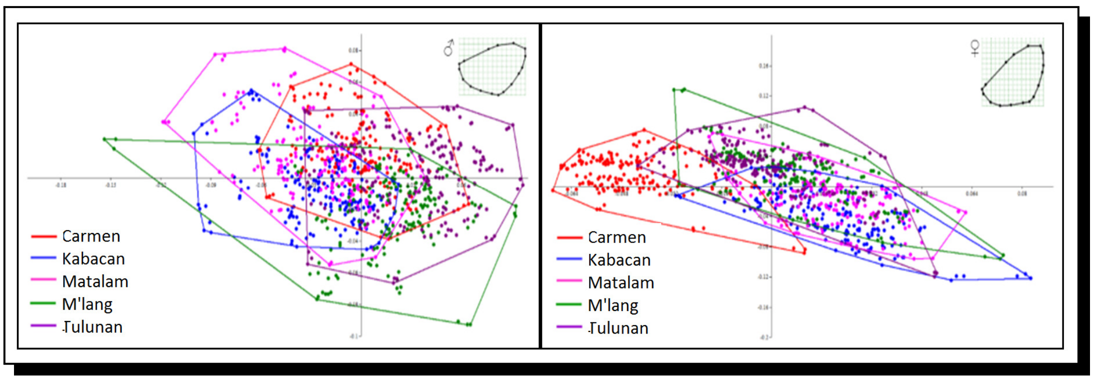

Figure 4. CVA scatter plots showing the distribution of male and female individuals of *S. coarctata* based on right hindwing shapes.

Table 3. Hotelling's pairwise comparison* in hindwing shape variances of the different populations of *S. coarctata*.

	NON-OUTBREAK		OUTBREAK		
	Left hindwing				
	Carmen	Matalam	Kabacan	M'lang	Tulunan
Carmen	-	2.089E-64	2.731E-33	1.347E-11	7.857E-92
Matalam	2.676E-10	-	9.028E-64	8.023E-30	1.865E-18
Kabacan	8.732E-75	9.433E-29	-	1.607E-82	2.449E-70
M'lang	2.123E-13	8.858E-19	6.549E-69	-	6.447E-14
Tulunan	1.072E-16	9.973E-50	8.688E-10	1.611E-20	-
	Right hindwing				
Carmen	-	5.185E-23	3.765E-23	2.254E-47	1.304E-30
Matalam	5.142E-12	-	6.686E-21	1.615E-43	2.638E-52
Kabacan	2.337E-11	3.666E-10	-	4.998E-37	1.440E-64
M'lang	1.508E-11	2.224E-19	2.671E-40	-	3.086E-24
Tulunan	5.617E-10	3.542E-36	9.700E-56	2.653E-07	-

*: Male-Above the diagonal; Female below the diagonal

The among-group differences in hindwings 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 [19]. Most of the time, aspects of these adaptive phenotypes are functionally connected with ecological performance. Disparities in morphology might be brought about by environmental stressors like habitat degradation and seasonal cropping which force the insect pest to change their location flying towards rice fields with favorable conditions, changing as well their food preference and feeding behavior, partitioning of resource, and even developing new competitions [20]. Furthermore, aside from flight ability of hindwings, it also functions as for protection. Factor that may influence variability in the shape of hindwings 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 [21]. Between sexes variation might be due to the fact that flight variation exists among RBB where it has been proving that females are strong fliers compared to the males [22]. According to [23], rice pest insects also differ 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 [24].

4. Conclusion

Results of this study show variability within and between and among populations of *S. coarctata*. While there are similarities that can be observed among the populations, the differences between them can be attributed to selected individual variations that are found to be distinct for each of the populations. This indicates that populations of the pest possess the potential to overcome the resistance factors present in the rice plants planted in the farms. This study also show that geometric morphometric methods are useful in detecting quantitative variations in shape and can be further used in describing other morphological parts of the RBB not only for taxonomic purpose but also population analysis.

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Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

All the authors contributed significantly in writing this article. The authors read and approved the final manuscript.

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