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Describing phenotypic diversity in an outbreak population of Rice Black Bugs from Balangao, Diplahan, Zamboanga Sibugay, Philippines, using principal component analysis and K-means clustering of morphological attributes

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Abstract. Rice black bugs (RBB) are believed to be a serious pest of rice infesting all growth stages of the plant. Different management approaches have already been applied to control and regulate populations of this pest. However, control efforts have been muddled by lack of understanding of the taxonomy of this insect resulting from immense intra- and inter-population diversity in phenotypic traits. Here, a total of thirty traits were scored from an outbreak population consisting of one hundred and twenty female RBB from Buug, Zamboanga Sibugay and analyzed using principal component analysis. Plots of the two principal components summarizing 68.8% of the total variation and subsequent K-means clustering showed that this population of RBB belong to at least four groups distributed as follows: group 1 – 49 individuals; group 2 – 46; group 3 - 14 and group 4 – 11. These individuals are polymorphic for eleven traits only, specifically on the relative lengths of the Tylus and the Jugum, presence of Cicatrices humps, number of antennal segments, shape and reach of the Scutellum, shape of the junction of vein R+M in the outer wing, number of closed marginal cells, number of longitudinal veins below discal cell, and Proboscis reach. The importance of these traits to intra-population divergence and life history of the RBB has yet to be determined. Thus, further studies should be conducted to determine the genetic and functional bases of the observed diversity. This information is necessary for the proper formulation of management strategies for the control and regulation of populations of this insect.

Keywords: Rice black bugs, RBB, phenotypic diversity, K-means clustering, principal component analysis, *Scotinophara molavica*.

Introduction. Rice black bugs (RBB) (Figure 1) are insect pests that attack rice at all stages causing the appearance of unfilled grains, decreased number of tillers and reduced number of grains per panicle (Carter et al 2004). Recently, there has been renewed interest in characterizing morphological diversity in populations of the rice insect pest *Scotinophara* spp. (rice black bug or RBB) owing to questions regarding its identity and exact taxonomic placement (Barrion et al 2007; Demayo et al 2007; Cruz et al 2011; Torres et al 2011). Aptly referred to as a 'problem species' with a 'species problem', the RBB species in our country has been shown to exhibit immense morphological diversity with regards to their general appearance, internal structures, wing morphology and reproductive traits. This paper is concerned with determining patterns of variability in morphological attributes within an outbreak population of *Scotinophara* spp. from Buug, Zamboanga Sibugay, Philippines (Figure 1).

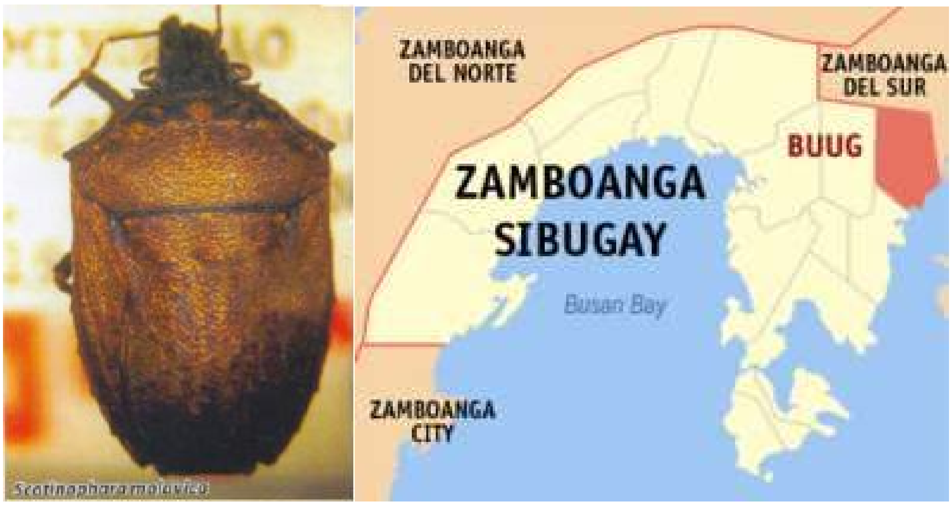

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Figure 1. (A) The Rice Black Bug *Scotinophara* spp. (Photo from Barrion et al 2007); (B) Map showing the location of Buug, Zamboanga Sibugay, Philippines.

Material and Method. The use of phenotypic markers in species delimitation is widespread in the scientific literature (Rojas 1992; Zink & McKitrick 1995; Duffy 1996; Turner 1999; de Queiroz 2005). Since anything observed on organisms is potentially useful in identifying species boundaries provided that the extent to which the features concerned is representative of the organism as a whole, phenotypic observations of natural populations of the RBB known to be a complex insect group is important. We conducted the evaluation of an outbreak population of this insect group to understand the extent of within-population variability. A total of 120 female *Scotinophara* spp. samples were collected from rice fields in Balangao, Diplahan, Zamboanga, Philippines. These samples were dissected, examined and described under a stereomicroscope. Then, a total of 30 morphological attributes (Table 1) of the RBBs were scored and summarized in a matrix prior to data analysis. The data was then subjected to Principal Component Analysis and K-means Clustering to determine patterns of morphological diversity.

Table 1

List of characters used in the analyses

1	Size	(0) small; (1) medium; (2) medium-large; (3) large.
2	Color	(0) brown; (1) dark brown to light reddish brown; (2) brownish yellow; (3) red; (4) grayish black.
3	Serration on lateral margins of pronotum	(0) serrated, minute; (1) serrated, large; (2) not serrated.
4	Direction of anterior lateral margins of pronotum	(0) absent; (1) oblique-upward; (2) lateroupward; (3) oblique-posterad.
5	Length of anterior lateral margins of pronotum	(0) absent; (1) very short, slightly beyond eye; (2) distinctly long, approximately 2x eye length.
6	Shape of anterior lateral margins of pronotum	(0) absent; (1) slightly concave; (2) concave; (3) straight.
7	Shape of lateral margin of pronotum	(0) slightly sinuate to sinuate; (1) concave to concave straight; (2) slightly concave; (3) straight concave; (4) doubly convex and oblique.
8	Head notch	(0) distinct; (1) absent.
9	Tylus vs. Jugum	(0) Tylus = Jugum; (1) Tylus > Jugum; (2) Tylus < Jugum; (3) Tylus ≤ Jugum; (4) Tylus ≥ Jugum

10	Direction of anterior lateral spine:	(0) absent; (1) obliquely upward; (2) laterad; (3) posterad; (4) posterolaterally.
11	Size of anterior lateral spine:	(0) absent; (1) small; (2) largely triangular.
12	Anterior lateral spine (ALS) vs. prehumeral spine (PHS)	(0) PHS = ALS; (1) PHS < ALS; (2) PLS > ALS; (3) absent.
13	Length of antennal segment I vs. II in females	(0) I = II or subequal; (1) I > II; (2) I < II.
14	Number of antennal segments	(0) four; (1) five.
15	Cicatrices humps	(0) absent; (1) present.
16	Tip of scutellum	(0) rounded; (1) slightly cleft; (2) truncate; (3) truncate to slightly emarginated; (4) emarginated; (5) pointed.
17	Reach of scutellum	(0) near midabdomen; (1) near abdominal tip.
18	Antennifers	(0) not distinct; (1) slightly cleft to cleft; (2) oblique-pointed to pointed; (3) not cleft or blunt.
19	Inner spine in tibia II	(0) 0, 0-1, 1; (1) 1-2, 2; (2) 3; (3) 4.
20	Femur 1 ventral spine	(0) present; (1) absent.
21	Lateral spine in Coxa 1	(0) present; (1) absent.
22	Ocelli	(0) absent; (1) present
23	R+M - Cu1 triangle	(0) absent; (1) large; (2) moderate.
24	Junction of vein R+M	(0) absent; (1) square; (2) rectangular.
25	Number of closed marginal cells	(0) 0; (1) 1-2; (2) 2-3 (this is the range in the group); (3) 3-5 (this is the range in the group); (4) 4-5.
26	Longitudinal veins below discal cell	(0) 3; (1) 4; (2) 5; (3) 6; (4) 7.
27	Proboscis reach	(0) abdominal segment I; (1) coxae III; (2) before coxae III; (3) between coax I and II; (4) midcoxa III.
28	Proboscis segment IV vs. III in females	(0) subequal; (1) IV > III; (2) IV < III.
29	Pronotal lobes	(0) distinct; (1) indistinct.
30	Feeding status	(0) non-pest; (1) pest.

Results and Discussion. All of the female *Scotinophara* spp. samples were found to be consistent in 19 out of 30 characters examined in this study. All samples are described as follows: general body color brownish yellow; lateral margins of pronotum not serrated; anterior lateral margins of pronotum lateroupward in direction; with very short anterior lateral margins of pronotum; anterior lateral margins of pronotum concave in shape; shape of lateral margin of pronotum slightly sinuate to sinuate; head notch absent; direction of anterior lateral spine obliquely upward; with small anterior lateral spine; Anterior lateral spine (ALS) shorter than the prehumeral spine (PHS); antennal segment I shorter than the second segment in females; with five antennal segments; Inner spine in tibia II 0, 0-1, 1; Femur 1 ventral spine absent; Lateral spine in Coxa 1 present; Ocelli absent; R+M - Cu1 triangle moderate; Pronotal lobes distinct; Feeding status pest.

Plots of the first two principal components divide the population into four groups (Figure 2). Cluster membership is reflected in Table 2 and Figure 3. The first axis separates the RBBs based on the shapes of the tip of the Scutellum – those that have either rounded or emarginated tips. The second axis further divides the population into four groups based on the length of the proboscis. There are RBBs whose proboscis reach only the either the first abdominal segment, the third coxae or before the third coxae. There are also RBBs whose proboscis can reach the third midcoxa.

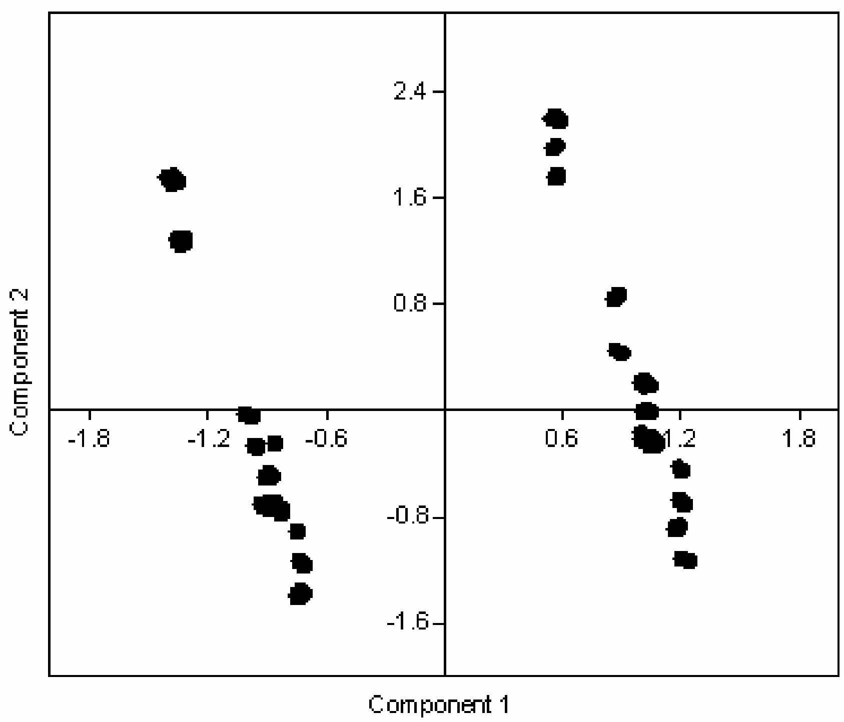

Figure 2. Plots of the two principal component axes showing four groups of Rice Black Bugs from Buug, Zamboanga Sibugay, Philippines.

This population of *Scotinophara* spp. is shown to be polymorphic for 11 traits, which includes among others the relative lengths of the Tylus and the Jugum, presence of Cicatrices humps, number of antennal segments, shape and reach of the Scutellum, Shape of the junction of vein R+M in the outer wing, number of closed marginal cells, number of longitudinal veins below discal cell, and Proboscis reach.

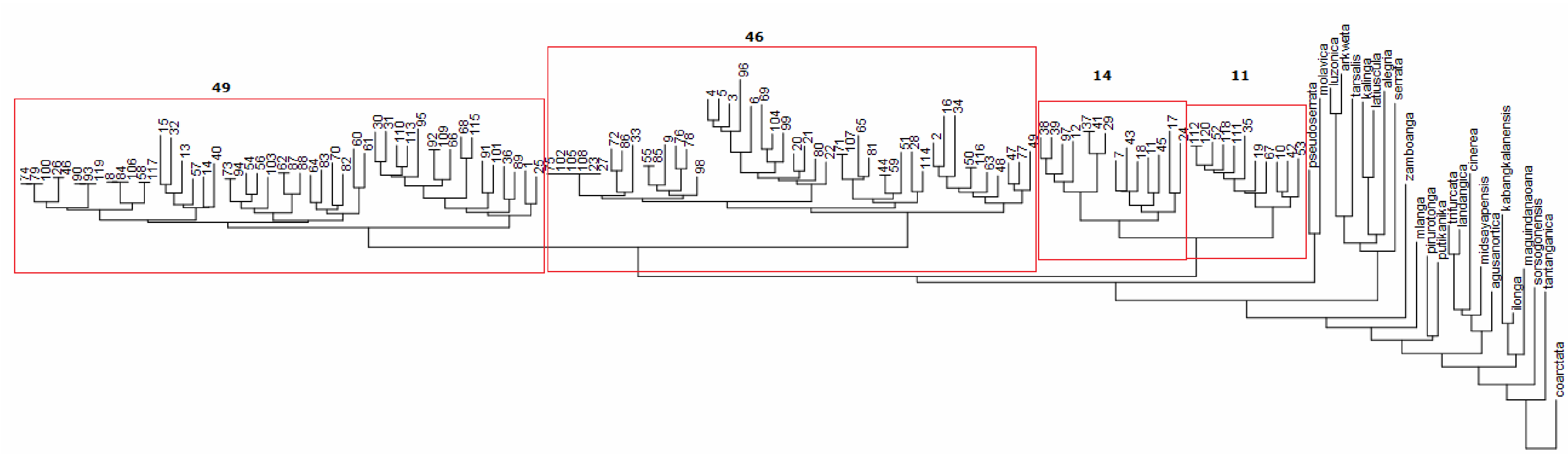

Figure 3. K-clustering of individuals in relation to the various identified species of RBB.

Table 2

Phenotypic diversity in ten morphological attributes of the rice black bug *Scotinophara* spp. from Buug, Zamboanga Sibugay, Philippines. Group I – RBBs with truncate to slightly emarginated tip of the Scutellum; Group II – RBBs with tip of the Scutellum rounded in shape

Character	*States*	*Group I*	*Group II*	*Pooled*
Size	Small	1.67% (1)	8.33% (5)	5.00% (6)
	Medium	98.33% (59)	91.67% (55)	95.00% (114)
Tylus vs. Jugum	Tylus = Jugum	43.33% (26)	48.33% (29)	45.83% (55)
	Tylus > Jugum	56.67% (34)	51.67% (31)	54.17% (65)
Cicatrices humps	Absent	41.67% (25)	55.00% (33)	48.33% (58)
	Present	58.33% (35)	45.00% (27)	51.67% (62)
Reach of Scutellum	near midabdomen	1.67% (1)	0.00% (0)	0.83% (1)
	near abdominal tip	98.33% (59)	100.00% (60)	99.17% (119)
Antennifers	slightly cleft to cleft	90.00% (54)	93.33% (56)	91.67% (110)
	oblique-pointed to pointed	10.00% (6)	6.67% (4)	8.33% (10)
Junction of vein R+M	Square	66.67% (40)	71.67% (43)	69.17% (83)
	Rectangular	33.33% (20)	28.33% (17)	30.83% (37)
Number of closed marginal cells:	2-3	1.67% (1)	10.00% (6)	5.83% (7)
	3-5	95.00% (57)	85.00% (51)	90.00% (108)
	4-5	3.33% (2)	5.00% (3)	4.17% (5)
Number of longitudinal veins below the discal cell	3	1.67% (1)	13.33% (8)	7.50% (9)
	4	70.00% (42)	56.67% (34)	63.33% (76)
	5	25.00% (15)	25.00% (15)	25.00% (30)
	6	3.33% (2)	5.00% (3)	4.17% (5)
Proboscis reach	Abdominal segment I	16.67% (10)	15.00% (9)	15.83% (19)
	Coxae III	58.33% (35)	58.33% (35)	58.33% (70)
	Before Coxae III	6.67% (4)	3.33% (2)	5.00% (6)
	midcoxa III	18.33% (11)	23.33% (14)	20.83% (25)

Character	States	Group I	Group II	Pooled
Proboscis segment IV vs. III in females	Subequal	26.67% (16)	21.67% (13)	24.17% (29)
	IV > III	13.33% (8)	10.00% (6)	11.67% (14)
	IV < III	60.00% (36)	68.33% (41)	64.17% (77)

The results of this study showed wide variation in this population of *Scotinophara* spp. from selected rice fields in the Municipality of Buug, Zamboanga del Norte. The implication of such variability to pest management cannot be understated. It is believed that population-level analyses such as this are deemed to contribute in the development of more effective control measures as members of the same taxonomic species may show as much ecological plasticity as they have in their morphology. Further studies on the genetic basis of the observed phenotypic diversity and possible intra-population variation in response to control strategies are deemed necessary.

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References

Barrion A. T., Joshi R. C., Barrion-Dupo A. L. A., Sebastian L. S., 2007 Systematics of the Philippine Rice Black Bug, *Scotinophara* Stål (Hemiptera: Pentatomidae). In: Rice Black Bugs: Taxonomy, ecology, and management of invasive Species. Joshi R. C., Barrion A. T., Sebastian L. S. (eds), pp. 3-180, Science City of Muñoz, Nueva Ecija: Philippine Rice Research Institute.

Carter C. A., Chalfant J. A., Goodhue R. E., 2004 Invasive species in agriculture: a rising concern. Western Economics Forum pp. 1-6.

Cruz L. M. L. D., Torres M. A. J., Barrion A. T., Joshi R. C., Sebastian L. S., Demayo, C. G., 2011 Geometric morphometric analysis of the head, pronotum and genitalia of the rice black bug associated with selected rice types. Egypt Acad J Biolog Sci A Entomology 4(1):21-31.

de Queiroz K., 2005 Ernst Mayr and the modern concept of species. Proc Natl Acad Sci U S A 102(1):6600-6607.

Demayo C. G., Torres M. A. J., Barrion A. T., Joshi R. C., Sebastian L. S., 2007 Geometric morphometrics in the Rice Blackbug, Scotinophara coarctata group. In: Rice Black Bugs: Taxonomy, ecology and management of invasive species. Joshi R. C., Barrion A. T., Sebastian L. S. (eds.), pp. 231-286, Philippine Rice Research Institute, Philippine.

Duffy J. E., 1996 Species boundaries, specialization, and the radiation of sponge-dwelling alpheid shrimp. Biol J Linn Soc Lond 58:307-324.

Rojas M., 1992 The species problem and conservation: What are we protecting? Conserv Biol 6(2):170-178.

Torres M. A. J., Yañez G. R. Q., Demayo C. G., 2011 Image analysis of intra-population variability in a non-outbreak population of the Rice Black Bug *Scotinophara* sp. from Tacurong, Sultan Kudarat, Philippines. Special Issue of the International Journal of the Computer, the Internet and Management 19(SP1):75.1-75.7.

Turner G. F., 1999 What is a fish species? Rev Fish Biol Fish 9:281-297.

Zink R. M., Mckitrick M. C., 1995 The debate over species concepts and its implications for ornithology. Auk 112(3):701-719.

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