Systematic relationships of rice black bugs, Scotinophara spp. inferred using nonmetric multidimensional scaling technique and parsimony analysis

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ABSTRACT

The systematic relationships of Philippine rice black bugs (RBBs), *Scotinophara* spp. was inferred using nonmetric multidimensional scaling technique (MDSCALE) and parsimony analysis to determine patterns of variation among the species and species groups. The MDSCALE result revealed a very distinct structure with clusters representing species groups visually apparent in the map. The distinct gaps in the phenetic spaces between species groups reflect the ease by which the species can be classified using numerical phenetics. On the contrary, parsimony analysis showed that the species groups did not form monophyletic groups. The results of the study are discussed in the light of patterns of differentiation among the species. It is suggested that more parsimony informative characters should be included to shed light into our basic understanding of the various evolutionary processes involved in the differentiation of these species of black bugs.

Keywords: *Scotinophara* spp., black bugs, Nonmetric multidimensional scaling, Parsimony analysis, autapomorphies, minimum spanning tree, species complex, systematics.

INTRODUCTION

Throughout the distributional range of the genus *Scotinophara* Stal, 1867, a total of 42 species of the sap-feeding black bugs have been reported (Miyamoto *et al.*1983). Of these, 69% (29 species) are representatives of the taxon in the Oriental and Pacific Regions. Six oriental species, i.e., *S. cinerea* (Le Guillou), *S. coarctata* (Fabricius), *S. latiuscula* (Breddin), *S. lurida* (Burmeister), *S. serrata* (Vollenhoven), and *S. tarsalis* (Vollenhoven) occur in the Philippines (Barrion and Litsinger, 1994; Heinrichs, 1994; Reissig *et al.*1986). To date only *S. coarctata* and *S. latiuscula* have been documented to feed on rice (Barrion and Litsinger, 1987).

As early as 1982 to the present date, the rice black bugs (RBB) had been reported to destroy rice farming communities and many of the local government units in the entire Philippine Archipelago. Widespread yet difficult to observe, this highly invasive species has been found in the Philippines in four major islands to date: Palawan (1982), Mindanao (1992-1997), the Visayas (1998-1999) and Luzon (2005-

2006) (Miyamoto *et. al.*, 1983, Barrion *et al.*, 2008). Attraction of large numbers of bugs to lights is often mistakenly called outbreaks and result in anticipated infestation and yield loss. Rice black bugs rarely cause damage to the plant except in non-irrigated areas where no supplemental water is available for the rice plant. Hence, drought stress killed the plants hastened by the additional sap-sucking behavior of the rice black bugs (Heinrich, 1994).

Although only *S. coarctata* is commonly reported on rice throughout the Philippines, we suspected that a complex of related species exists because bugs from different localities behaved differently in feeding trials (Heinrichs *et al.*, 1987). In addition, many of the bugs were found only on older plants and abandoned stubbles.

The most recent and comprehensive study of RBB in the Philippines placed *Scotinophara* species into four taxonomic groups: (a) *tarsalis* with a single species, (b) *serrata* with two species, (c) *lurida* with five species, and (d) the *coarctata* with 16 species (Barrion *et al.* 2007). Of the total taxa reported, 19 are newly described species. The new species belong to the *serrata* group. Two previously reported taxa, *S. lurida* (Burmeister) from Mindanao Island and *S. ochracea* (Distant) from Luzon Island were not examined due to lack of specimens from the current and loaned collections.

This study aimed to investigate the systematic relationships of the Philippine RBBs species belonging to the four taxonomic groups to determine patterns of morphological variation using the tools of phenetics and cladistics. The method of non-metric multidimensional scaling will be applied on the MDSCALE distance matrix to produce an unrooted tree, which will depict the phenetic relationships of the species.

MATERIALS AND METHODS

A total of 19 newly described species of black bugs from the Philippines were examined along with five previously known species: *S. tarsalis, S. serrata, S. latiuscula, S. cinerea* and *S. coarctata* (Table 1, Fig. 1) (Barrion *et al.*, 2007). Central to problems with the determination of the phylogeny of organisms is the appropriate choice and application of outgroup data. For the purpose of this study, *Antilochos nigripes* of family Pyrrhocoridae which is a sister taxon to the Pentatomidae was chosen.

The 60 characters (16 binary and 44 multistate) identified from both sexes (Figs 2-4) were all unordered and equally weighted to refrain from any hypothesis about their transformation (Appendix A). In particular, coding does not imply whether a state is ancestral or derived. Unknown characters were coded as "?". The data matrix was subjected to non-metric multidimensional scaling analysis (MDSCALE, Kruskal, 1964). A minimum spanning tree (MST) was fitted to the data to determine the phenetic relationships of the species (Rohlf, 1970). This method was used to indicate the near neighbor distance relation among the points in the dimensional space. The graph was drawn using the program PAST v. 1.70 (Hammer *et. al.*, 2001). The outgroup was excluded from the analysis because of its extreme separation from the in-group species based on the plot of the first two coordinates.

The data matrix was constructed and edited with WinClada as a shell program (Nixon, 1999) (Table 2). Parsimony analyses were conducted with Nona using the Parsimony ratchet (Goloboff, 1993). Two hundred iterations were performed (one tree per iteration), with the search repeated ten times. Tree characters were sampled for reweighing during the parsimony ratchet. All searches were done under the collapsing option "ambiguous" which collapsed every node with a minimum length of 0. Per character consistency (ci) and retention

indices (*ri*) were calculated to determine reliable and informative characters. Characters with consistency indices of 100 show no homoplasy.

| GROUP | SPECIES | | | | |
|----------------------|--------------------------------------|--|--|--|--|
| Tarsalis [1 species] | S. tarsalis (Vollenhoven) | | | | |
| Serrata [2 spp.] | S. serrata (Vollenhoven) | | | | |
| | S. pseudoserrata (Barrion et al.) | | | | |
| Lurida [5 spp.] | S. luzonica (Barrion et al.) | | | | |
| | S. molavica (Barrion et al.) | | | | |
| | S. kalinga (Barrion et al.) | | | | |
| | S. arkwata (Barrion et al.) | | | | |
| | S. latiuscula (Breddin) | | | | |
| Coarctata [16 spp] | S. cinerea (Le Guillou) | | | | |
| | S. sorsogonensis (Barrion et al.) | | | | |
| | S. pirurotonga (Barrion et al.) | | | | |
| | S. agusanortica (Barrion et al.) | | | | |
| | S. tantanganica (Barrion et al.) | | | | |
| | S. midsayapensis (Barrion et al.) | | | | |
| | S. putikanika (Barrion et al.) | | | | |
| | S. coarctata (Fabricius) | | | | |
| | S. alegria (Barrion et al.) | | | | |
| | S. kabangkalanensis (Barrion et al.) | | | | |
| | S. zamboanga (Barrion et al.) | | | | |
| | S. trifurcata (Barrion et al.) | | | | |
| | S. landangica (Barrion et al.) | | | | |
| | S. maguindanaoana (Barrion et al.) | | | | |
| | S. mlanga (Barrion et al.) | | | | |
| | S. ilonga (Barrion et al.) | | | | |

Table1: Taxonomic groupings of the 24 species of previously (5) and newly (19) recorded rice black bugs in the Philippines.

Table 2: Data matrix.

| | 0123456789 | 1111111111 0123456789 | 22222222222 0123456789 | 33333333333 0123456789 | 444444444 0123456789 | 5555555555 0123456789 |
|---------------------------------|------------|--------------------------|---------------------------|---------------------------|-------------------------|--------------------------|
| Antilochos nigripes | 3320000110 | 0300005000 | 030100???? | ??????????? | ?????????0 | 000244221? |
| S. tarsalis | 0001110021 | 2111110102 | 0011110000 | 1000100001 | 0100002001 | 0??1320010 |
| S. serrata | 3112110022 | 1221111101 | 0211110000 | 1110111102 | 2211020020 | 0112010010 |
| S. pseudoserrata | 1212132131 | 1222110103 | 0011111010 | 1110012113 | 101??????? | 01?1111110 |
| S. luzonica | 1222120111 | 1000102111 | 1111111020 | 1011111200 | 3011001020 | 1121202210 |
| S. molavica | 1222120101 | 1222104113 | 1211110010 | 1011111110 | 112??????? | 1??2312210 |
| S. kalinga | 1022130111 | 1200102111 | 1111110020 | 1010111204 | 100?????10 | 1??1112210 |
| S. arkwata | 1222130111 | 1200103111 | 1011110000 | 1011111210 | 101??????? | 0??1210010 |
| <i>S. latiuscula</i> (Breddin) | 1023130113 | 1211102112 | 1011111020 | 0201111220 | 1013011010 | 0222202211 |
| <i>S. cinerea (</i> Le Guillou) | 1023232104 | 1222100111 | 1011111000 | 1310111214 | 1011011000 | 0122311211 |
| S. sorsogonensis | 1123231104 | 1222100121 | 1111112121 | 0311110023 | 1103110130 | 1112112210 |
| S. pirurotonga | 1223231133 | 1222100121 | 1011112211 | 0311100020 | 1103120220 | 2124201210 |
| S. agusanortica | 1123233113 | 1222100121 | 1211112121 | 0310000025 | 0102120120 | 1114311210 |
| S. tantanganica | 1123232133 | 1222100121 | 1111113111 | 0311100003 | 1103140200 | 1114421110 |
| S. midsayapensis | 1123231103 | 1222100121 | 1011113111 | 0311110025 | 1104140200 | 1124210010 |
| S. putikanica | 1123232103 | 1012100123 | 1011112101 | 0311010003 | 1113140130 | 2122202210 |
| S. coarctata (F.) | 1123232134 | 1011100111 | 1011113121 | 0311000000 | 1103110110 | 1112112211 |
| S. alegria | 2123233113 | 1022100111 | 1011113121 | 0310102020 | 1100120120 | 1124121110 |
| S. kabangkalensis | 1223231103 | 1200100111 | 1011113311 | 0310102020 | 1104120220 | 1114211110 |
| S. zamboanga | 1123231134 | 1222100111 | 1011113111 | 0310000002 | 3123140200 | 1124111110 |
| S. trifurcata | 1123231134 | 1221100111 | 1011112121 | 0310000025 | 1103140220 | 1122211110 |
| S. landangica | 1223232104 | 1022100121 | 1011112122 | 0310100002 | 1104120210 | 0124212210 |
| S. maguindanaoana | 1123230114 | 1222100122 | 1011112122 | 0310100020 | 1104120210 | 1113112210 |
| S. mlanga | 1123231144 | 1222100112 | 1011113121 | 0310100024 | 4103210100 | 1223111110 |
| S. ilonga | 1123232144 | 1022100112 | 1011113110 | 0310100003 | 1003230100 | 2212112210 |



Fig.1: Rice blackbugs used in this study. (1) S. kalinga (Barrion et al.) (2) S. (Barrion et al.) (3) S. latiuscula (Breddin) (4) S. cinerea (Le Guillou) (5) S. trifurcata (Barrion et al.) (6) S. pseudoserrata (Barrion et al.) (7) S. luzonica (Barrion et al.) (8) S. molavica (Barrion et al.) (9) S. ilonga (Barrion et al.) (10) S. alegria (Barrion et al.) (11) S. kabangkalanensis (Barrion et al.) (12) S. zamboanga (Barrion et al.).



Fig. 1cont. : Rice blackbugs used in this study (cont.) (13) S. landangica (Barrion et al.) (14) S. maguindanaoana (Barrion et al.) (15) S. mlanga (Barrion et al.) (16) S. serrata (Vollenhoven) (17) S. tantanganica (Barrion et al.) (18) S. midsayapensis (Barrion et al.) (19) S. putikanika (Barrion et al.) (20) S. coarctata (Fabricius) (21) S. tarsalis (Vollenhoven) (22) S. sorsogonensis (Barrion et al.) (23) S. Pirurotonga (Barrion et al.) (24) S. agusanortica (Barrion et al.).



Fig. 2: Rice Black Bug (RBB) morphology – general habitus. (a) antiniffer (b) antenna (1-5 segments)
(c) tylus (d) jugum (e) vertex (f) ocellus (g) anterior angle (h) anterolateral spine (i) cicatrice
(j) tubercle (k) lateral margin (l) prehumeral spine (m) humeral angle (n) pronotum (o) posterolateral margin (p) posterolateral angle (q) femur (r) tibia (s) tarsus (1-3 tarsal segments)
(t) scuttellar pit (u) scuttellum (v) clavus (w) claval suture (x) corium (y) membranal suture (z) membrane (a') abdomen (2-6 sternites) (b') genital segments (c') punctures



Fig. 3: Morphology of selected external and internal structures of the male rice black bug I. Lateral view of the head (a) antinnifer (b) gena (c) buccula (d) rostrum or proboscis. II. Ventral view of abdominal tip (a) midanterior margin of sternite VII. III. Antennifer. IV. Cross section of Gonophore (a) aedeagal cap (b) clasper (c) posterior lope of gonophore. V. Right clasper (a) blade (b) median posterior surface (c) teeth (d) inner arm (e) stem. VI. Aedeagus (a) anterior phallotheca (b) posterior phallotheca (c) pivot (d) lateral arm (e) basal plate (f) ejaculatory duct (g) penial plate (h) membranous conjunctiva. VII. Tergite X (a) dorsolateral lobe.



Fig. 4: Wing morphology and female reproductive structures of the rice black bugs. I. Forewing (a) corium (b) vein R+M (c) closed marginal cells (d) membrane (e) longitudinal cells (f) clavus; II. Hindwing (a) vein R+M (b) knob-like process (c) R+M junction (d) vein R (e) vein M (f) vein CuA (g) secondary veins (h) vein1A (i) vein2A (j) vein3A (k) R+M-CuA triangle; III. Spermatheca (a) proximal spermathecal duct (b) median dilation (c) spermathecal bulb (d) sclerotized median duct (e) pump (f) proximal flange; IV. (a) proctiger (b) 9th paratergite (c) 8th paratergite (d) 2nd gonocoxae (e) 1st gonocoxae (f) arcus.

RESULTS AND DISCUSSION

Figure (5) shows the results of the MDSCALE analysis applied to the data matrix. The final stress value was S = 0.169. The 24 species are identified with a system of code numbers indicated in the caption. The near neighbor relations are shown by the MST superimposed on the ordination map and shown as line segments interconnecting the points.



Fig. 5: Ordination map produced by non-metric multidimensional scaling analysis of the data matrix. *Tarsalis* group: 1. S. tarsalis (Vollenhoven); Serrata group: 2. S. serrata (Vollenhoven), 3. S. pseudoserrata (Barrion et al.); Lurida group 4. S. luzonica (Barrion et al.) 5. S. molavica (Barrion et al.), 6. S. kalinga (Barrion et al.), 7. S. arkwata (Barrion et al.), 8. S. latiuscula (Breddin); Coarctata group: 9. S. cinerea (Le Guillou), 10. S. sorsogonensis (Barrion et al.), 11. S. pirurotonga (Barrion et al.), 12. S. agusanortica (Barrion et al.), 13. S. tantanganica (Barrion et al.), 14. S. midsayapensis (Barrion et al.), 15. S. putikanica (Barrion et al.), 16. S. coarctata (Fabricius), 17. S. alegria (Barrion et al.), 18. S. kabangkalensis (Barrion et al.), 19. S. zamboanga (Barrion et al.), 20. S. trifurcata (Barrion et al.), 21. S. landangica (Barrion et al.), 22. S. maguindanaoana (Barrion et al.), 23. S. mlanga (Barrion et al.), and S. ilonga (Barrion et al.).

Overall, the MDSCALE result revealed a very distinct structure with clusters representing species groups visually apparent in the map. Also, species in the same group are found near one another and linked through the MST. The distinct gaps in the phenetic spaces between species groups reflect the ease by which *Scotinophara* spp. can be classified using numerical methods. The species belonging to the *Coarctata* group are in the same region and are near neighbors of one another. These species have characteristically long anterior lateral margins in the pronotum the length of which is found to be approximately twice the entire length of the eye. Members of the *Lurida* group formed a connected set with members of this set shown as near neighbors of the *Serrata* group. The plot shows *S. tarsalis* as an isolated species and support the contention that this taxon be placed on its own monotypic species group. This species differs from the others in having a small body size, minute serrations on the lateral margins of the pronotum, anterior lateral margins in the pronotum which is oriented obliquely-upward, large and triangular anterior lateral spine, prehumeral spine shorter in length than the anterior lateral spine, and a widely or shallowly concave tip of the

membranous conjunctiva. Moreover, *S. pseudoserrata* and *S. serrata* were the only members of *Serrata* species group. A distinct characteristic of these species is the presence of a spine in the pygophore (cross-section).

Analysis of the 60 characters identified 10 equally most-parsimonious trees (L = 12; CI = 58; RI = 78). Characters 10, 14, 17, 22, 23, 24, 25, 49, and 58 proved uninformative in the present dataset (See Appendix A for details). The strict consensus of these trees is presented in Figure 6 with areas of conflict in topology shown as polytomies. The presence of two polytomies was found in the basal as well as in the mid-apical nodes which may be the result of recurrent individual characters generated through different mechanisms (i.e. parallel evolution, homoplasy, convergent evolution). The results also show the lack of congruence between the results of the parsimony analysis and that of the MDSCALE presumably because of factors related to the choice of the outgroup, number of parsimony informative characters and the choice of the tree search algorithm.



Fig.6: Single cladogram found by Nona based on qualitative characters from species of black bugs from the Philippines. Closed circles represent synapomorphies, open circles represent reversals. Numbers above circles are character numbers (corresponding to Appendix 1); numbers below circles are states of the characters.

If character evolution for this group of species is to be founded solely on the strict consensus tree, then the observed similarities between species may have resulted from independent adaptations to a similar environment. Such recurrences in traits have been documented in groups in which most species are morphologically similar (i.e. sibling species) and especially when some of the species have invaded and have adapted to the same selective environment. Thus, the existence of recurrent traits in the morphological data used in this study (as evidenced by the low per character *ci* values in Table (3), makes intuitive sense because morphological characters are said to interact with the environment more directly and frequently that other forms of data such as molecular characters, which are often assumed to be selectively neutral or nearly so (Kimura, 1983; Hedges and Sibley, 1994; Gillespie, 1991).

| CHAR. | L | CI | RI | CHAR. | L | CI | RI | CHAR. | L | CI | RI |
|-------|---|-----|-----|-------|---|----|----|-------|---|----|----|
| 0 | 0 | 75 | 0 | 20 | 0 | 25 | 0 | 40 | 0 | 66 | 0 |
| 1 | 0 | 33 | 33 | 21 | 0 | 37 | 0 | 41 | 0 | 33 | 33 |
| 2 | 0 | 66 | 0 | 22 | 0 | NA | NA | 42 | 0 | 25 | 14 |
| 3 | 0 | 75 | 80 | 23 | 0 | NA | NA | 43 | 0 | 40 | 0 |
| 4 | 0 | 40 | 57 | 24 | 0 | NA | NA | 44 | 0 | 28 | 0 |
| 5 | 0 | 60 | 0 | 25 | 0 | NA | NA | 45 | 0 | 36 | 22 |
| 6 | 0 | 21 | 15 | 26 | 0 | 23 | 23 | 46 | 0 | 50 | 0 |
| 7 | 0 | 50 | 0 | 27 | 0 | 42 | 50 | 47 | 0 | 18 | 10 |
| 8 | 0 | 28 | 23 | 28 | 0 | 20 | 27 | 48 | 0 | 30 | 36 |
| 9 | 0 | 33 | 33 | 29 | 0 | 25 | 40 | 49 | 0 | NA | NA |
| 10 | 0 | NA | NA | 30 | 0 | 25 | 57 | 50 | 0 | 22 | 22 |
| 11 | 0 | 37 | 0 | 31 | 0 | 60 | 60 | 51 | 0 | 50 | 0 |
| 12 | 2 | 100 | 100 | 32 | 0 | 50 | 0 | 52 | 0 | 25 | 14 |
| 13 | 4 | 50 | 75 | 33 | 0 | 11 | 11 | 53 | 0 | 27 | 33 |
| 14 | 0 | NA | NA | 34 | 0 | 20 | 20 | 54 | 0 | 28 | 16 |
| 15 | 0 | 33 | 0 | 35 | 0 | 14 | 40 | 55 | 0 | 37 | 0 |
| 16 | 0 | 83 | 50 | 36 | 0 | 28 | 37 | 56 | 0 | 22 | 41 |
| 17 | 0 | NA | NA | 37 | 4 | 50 | 66 | 57 | 0 | 20 | 11 |
| 18 | 0 | 18 | 10 | 38 | 0 | 22 | 41 | 58 | 0 | NA | NA |
| 19 | 0 | 37 | 16 | 39 | 0 | 35 | 10 | 59 | 0 | 50 | 50 |

Table 3: Length, consistency and retention indices of the characters included in the study (NA – Uninformative characters).

Although, recurrent characters are important in testing hypothesis of adaptation in these species of black bugs, the relative extent of such recurrences/homoplastic gains of derived states may obstruct the resolution of phylogenies (Sytsma and Gottlieb, 1986; Gottlieb, 1988; Givnish and Sytsma, 1997). Future studies based on morphology must be conducted on a matrix containing more characters with greater degree of character consistency or at least with per character consistency indices (*ci*) greater than 50 (parsimony informative characters; Farris, 1989).

Character evolution and evolutionary diversification of the black bugs are discussed based on the strict consensus tree. Some of the characters were found to be sexually dimorphic in only a few numbers of species. These includes characters 12 & 13 (lengths of antennal segment I vs. II) in *S. serrata*, *S. putikanica* and *S. trifurcata* and characters 56 & 57 (length of the proboscis segments IV vs. III) in *S. cinerea*, *S. pirurotonga*, *S. agusanortica*. This phenomenon might represent examples of character evolution via a sensory exploitation system and divergence in feeding ecology (Ryan *et al.* 1990 as cited by Penz and De Vries, 2002; Vincent, 2006).

A number of lineages are apparent in the strict consensus tree. Noteworthy is the group composed of the species *S. luzonica*, *S. kalinga* and *S. arkwata*, which forms one of the most basal lineages but is not supported by any synapomorphic characters. Few features may represent shared-derived characters within the in-group

taxa. One synapomorphic character (length of antennal segment I greater than that of II in males) unites the species *S. putikanica* with the already known species *S. tarsalis, S. latiuscula* (Breddin) and *S. coarctata* (F.). The results of the analysis also identified a close relationship between the previously known species *S. serrata* and the newly described *S. pseudoserrata* and *S. molavica*. Morphologically, these species share the following characters: cross sectional position of pygophore subglobose without spine and length of basal theca and anterior theca subequal.

Several autapomorphies were mapped in the preferred cladogram, noteworthy of which are the unique features in *S. tarsalis* which includes the following: a slightly sinuate to sinuate lateral margin of the pronotum (6:0); presence of minute serration on the lateral margins of the pronotum (2:0); anterior lateral margin pointed in an oblique-upward position (3:1); presence of a large triangular anterior lateral spine (10:2); length of the prehumeral spine less than that of the anterior lateral spine (11:1); presence of two spermathecal bulb processes (46:2) and presence of an exserted Paratergite IX (49:1). Almost all of these characters were also identified in the phenetic analysis.

S. serrata differs from the others species in having four autapomorphies, which includes: a slightly cleft tip of the scutellum, tapered separated tips of the penis, slightly exserted ejaculatory duct, and the presence of three longitudinal veins below the discal cell. Two other species are defined by a single autapomorphic character each. A truncate to slightly emarginate tip of the scutellum is autapomorphic for *S. arkwata* while an emarginate tip of the scutellum is unique for *S. molavica*.

Mapping of character state distributions across the preferred cladogram was done only on characters that exhibited a certain degree of homoplasy (*ci* values of 50 and above) (Fig. 7). The trees produced permit the study of character evolution in these species of bugs.

Among the characters that appeared twice in the preferred cladogram is the presence of a head notch in *S. tarsalis* and *S. serrata* (7:1), both of which belong to separate lineages. Only one character showed a high consistency index of 100 (character 12: length of antennal segment I vs. II in males), the character states of which grouped the species into three clusters: the first composed of *S. kabangkalanensis*, *S. luzonica*, *S. kalinga* and *S. arkwata*; the second composed of *S. putikanica*, *S. tarsalis*, *S. latiuscula* (Breddin) and *S. coarctata* (F.); and the last group comprised of all other species. Results showed that the three species are known to be pests of agricultural crops which include *S. latiuscula* (Breddin), *S. coarctata* (F.) and *S. cinerea* (Le Guillou) (59:1). The first two are shown to be more closely related in the cladogram produced. However, this study is taxonomic, and not designed to detect pest status.



Fig. 7. Character reconstructions over the preferred strict consensus tree. Character (0): size; (2) serration on lateral margins of pronotum; (3) direction of anterior lateral margins of pronotum;
(5) shape of anterior lateral margins of pronotum; (7) head notch; (12) length of antennal segment I vs. II in males.



Fig. 7 (cont.): Character reconstructions over the preferred strict consensus tree (continued). Character (13) length of antennal segment I vs. II in females; (16) tip scutellum; (31) cross sectional position of pygophore; (32) ventral pygophore feature; (37) length of basal theca vs. anterior theca; (40) tip of penis.



Fig. 7 (cont.): Character reconstructions over the preferred strict consensus tree (continued). Character (46) spermathecal bulb processes; (51) R+M- Cu1 triangle; (59) feeding status.

APPENDIX A

List of Characters Used in the Analyses

- 0. Size: (0) small; (1) medium; (2) medium-large; (3) large.
- 1. Color: (0) brown; (1) dark brown to light reddish brown; (2) brownish yellow; (3) red; (4) grayish black.
- 2. Serration on lateral margins of pronotum: (0) serrated, minute; (1) serrated, large; (2) not serrated.
- 3. Direction of anterior lateral margins of pronotum: (0) absent; (1) oblique-upward; (2) lateroupward; (3) oblique-posterad.
- 4. Length of anterior lateral margins of pronotum: (0) absent; (1) very short, slightly beyond eye; (2) distinctly long, approximately 2x eye length.
- 5. Shape of anterior lateral margins of pronotum: (0) absent; (1) slightly concave; (2) concave; (3) straight.
- 6. Shape of lateral margin of pronotum: (0) slightly sinuate to sinuate; (1) concave to concavestraight; (2) slightly concave; (3) straight concave; (4) doubly convex and oblique.
- 7. Head notch: (0) distinct; (1) absent.
- Tylus vs. Jugum: (0) Tylus = Jugum; (1) Tylus > Jugum; (2) Tylus < Jugum; (3) Tylus ≤ Jugum;
 (4) Tylus ≥ Jugum
- 9. Direction of anterior lateral spine: (0) absent; (1) obliquely upward; (2) laterad; (3) posterad; (4) posterolaterally.
- 10. Size of anterior lateral spine: (0) absent; (1) small; (2) largely triangular.
- 11. Anterior lateral spine (ALS) vs. prehumeral spine (PHS): (0) PHS = ALS; (1) PHS < ALS; (2) PLS > ALS; (3) absent.
- 12. Length of antennal segment I vs. II in males: (0) I = II or subequal; (1) I > II; (2) I < II.
- 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. Scutellum ratio: (0) 1 to 1.49; (1) 1.5 to 1.59; (2) 1.6 to 1.7.
- 19. Antennifers: (0) not distinct; (1) slightly cleft to cleft; (2) oblique-pointed to pointed; (3) not cleft or blunt.
- 20. Transverse furrow: (0) distinct; (1) indistinct.
- 21. Inner spine in tibia II: (0) 0, 0-1, 1; (1) 1-2, 2; (2) 3; (3) 4.
- 22. Femur 1 ventral spine: (0) present; (1) absent.
- 23. Lateral spine in Coxa 1: (0) present; (1) absent.
- 24. Ocelli: (0) absent; (1) present
- 25. Compound eyes: (0) not pedunculate; (1) pedunculate.
- 26. Tergite X: (0) truncate to slightly cleft medially; (1) slightly emarginate to emarginate; (2) deeply concave; (3) shallowly concave, widely concave, concave.
- 27. Shape of clasper: (0) sicklelike; (1) slender clamp-like with long blade; (2) bolo-like with long blade and blunt tip; (3) bolo-like with short blade with blunt tip.
- 28. Reach of tip of clasper: (0) far from margin of T9; (1) close to margin of t9; (2) touching margin of T9.
- 29. Posterior median surface and setae character on clasper: (0) short blade; (1) long blade; (2) no setae
- 30. Inner tooth on clasper: (0) present; (1) absent.
- 31. Cross sectional position of pygophore: (0) subglobose without spine; (1) subglobose with spine;(2) globose without spine; (3) transverse without spine.
- 32. Ventral pygophore feature: (0) globose; (1) transverse.
- 33. Dorso-posterior margin of pygophore: (0) concave; (1) truncate or straight; (2) doubly convex.
- 34. Basal thecal scar: (0) present; (1) absent.
- 35. Basal theca in lateral view: (0) saddle-like; (1) not saddle-like.
- 36. Basal thecae seen dorsally: (0) swollen laterally; (1) not swollen laterally; (2) slightly swollen laterally.
- 37. Length of basal theca (BTH) vs. anterior theca (ATH): (0) BTH > ATH; (1) BTH = ATH; (2) BTH < ATH.
- 38. Shape of tip of anterior theca: (0) pointed; (1) truncate or blunt; (2) rounded.

- 39. Tip of membranous conjunctiva: (0) narrowly to deeply concave; (1) widely or shallowly concave; (2) narrowly pointed; (3) widely rounded; (4) truncate to sub-truncate; (5) U to W shaped.
- 40. Tip of penis: (0) rounded tip near each other; (1) rounded tip separated; (2) tapered tip separated; (3) truncated only (4) subtruncated
- 41. Ejaculatory duct: (0) exserted; (1) not exserted; (2) slightly exserted.
- 42. Basal plates of phallobase: (0) short; (1) long and porrect; (2) long, oblique and forward.
- 43. Shape of spermathecae: (0) globular to sub-globular; (1) oblongate/elongate without stripes; (2) oblongate with stripes; (3) thinly or slenderly elongate; (4) broadly elongate.
- 44. Distal spermathecal duct (DSD): (0) short and straight; (1) long and coiled; (2) shortly coiled.
- 45. Spermathecal pump vs. distal spermathecal duct: (0) subequal; (1) 2x to 2.75x; (2) 3x to 3.8x; (3) 4 to 4.5x.
- 46. Spermathecal bulb processes: (0) 0; (1) 2; (3) 3.
- 47. Loops in distal spermathecal duct: (0) absent; (1) 6 to 8 coils; (2) 9 to 10 coils.
- 48. Proctiger: (0) square; (1) subquadrate; (2) L > W; (3) W > L.
- 49. Paratergite IX: (0) not exserted; (1) exserted.
- 50. 7th abdominal mid sternum in male: (0) narrowly convex; (1) widely convex; (2) moderately convex; (3) triangular.
- 51. R+M Cu1 triangle: (0) absent; (1) large; (2) moderate.
- 52. Junction of vein R+M: (0) absent; (1) square; (2) rectangular.
- 53. 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.
- 54. Longitudinal veins below discal cell: (0) 3; (1) 4; (2) 5; (3) 6; (4) 7.
- 55. Proboscis reach: (0) abdominal segment I; (1) coxae III; (2) before coxae III; (3)_between coax I and II; (4) midcoxa III.
- 56. Proboscis segment IV vs. III in males: (0) subequal; (1) IV > III; (2) IV < III.
- 57. Proboscis segment IV vs. III in females: (0) subequal; (1) IV > III; (2) IV < III.
- 58. Pronotal lobes: (0) distinct; (1) indistinct.
- 59. Feeding status: (0) non-pest; (1) pest.

CONCLUSION AND RECOMMENDATION

The genus *Scotinophara* spp. is probably among the most economically important taxa in the rice agroecosystem. Because of this, it has been the object of several morphological studies which have revealed it to be a complex of species in the Philippines. Thus, there are probably more than one species causing damage to rice in the islands of Mindanao, Siquijor, Negros, Panay, Bohol, and Luzon in the Philippines. The 14 new species of rice black bug belonging to the *coarctata* group recently described where 4 new species belong to the *lurida* group and a single taxon to the *serrata* group are considered new to science. Practical consequences of proper species identification at the species level may affect the design and implementation of quarantine restrictions, management and control programs.

Therefore, this study therefore investigated the systematic relationships among the RBB species in the Philippines using the tools of phenetics and cladistics and determined patterns of variation among the species groups. Results of the phenetic analysis using MDSCALE showed that the species groups were distinct and that the members of each group were similar to one another. Few morphological differences distinguished species among the groups. Overall, the plot of the minimum spanning tree showed a stable classification of the RBB into four species groups. The species within each group were connected, respectively.

The ten equally parsimonious trees resulted in a consensus tree of 12 steps and CI of 58. Of the 60 characters used in the analysis, nine proved uninformative. Inspection of the per-character *ci* should have a high level of homoplasy in the current data matrix. The results of the parsimony analysis did not support the contention that the species belong to four groups previously identified based on the MDSCALE plot. A number of monophyletic groups are apparent in the strict consensus tree. Six of the

species scored are autapomorphic species supported by few unique character states. Noteworthy, the character states diagnostic for *S. tarsalis*, *S. arkwata*, *S. molavica*, *S. mlanga*, *S. latiuscula* (Breddin), *S. ilonga*, *S. pirurotonga* and *S. alegria*.

This study is far from being complete. While the true nature of the relationships of these rice black bugs are beginning to unfold, there are other traits that need to be explored to shed light to our basic understanding of the various evolutionary processes involved in the differentiation of these species of black bugs. Molecular data, behavior, feeding ecology, embryology and more informative morphological characters might generate new information that will be very useful in establishing the relationships of the species.

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