JOURNAL OF NATURAL HISTORY, 2004, **38,** 2863–2882

Synonymy of *Rhynchophorus ferrugineus* (Olivier), 1790 and *R. vulneratus* (Panzer), 1798 (Coleoptera, Curculionidae, Rhynchophorinae)

R. H. HALLETT†, B. J. CRESPI‡ and J. H. BORDEN§

†*Department of Environmental Biology, University of Guelph, Guelph, ON, Canada N1G 2W1; e-mail: rhallett@evb.uoguelph.ca*
‡*Department of Biological Sciences, Simon Fraser University, Burnaby, BC, Canada V5A 1S6*
§*Centre for Pest Management, Department of Biological Sciences, Simon Fraser University, Burnaby, BC, Canada V5A 1S6*

(*Accepted 10 October 2003*)

Morphological, molecular-genetic and breeding data were collected to investigate the species status of the Asian palm weevils, *Rhynchophorus ferrugineus* (Olivier) and *R. vulneratus* (Panzer) (Coleoptera: Curculionidae). These weevils are distinguished by characteristic colouring of the pronota and elytra, but naturally occurring colour intermorphs were observed. Contrary to the literature, quantitative measurements of the concavity of subgenal sutures and of pronotal shape indicated no differences between the two species. Larvae did not differ significantly in labral characteristics. Random amplified polymorphic DNA (RAPD) banding patterns were identical for nine of 14 primers, indicating that these weevils are very closely related. Sequences of the cytochrome oxidase gene for 201 base pairs read were identical for *R. ferrugineus* and *R. vulneratus*, but the congener *R. bilineatus* differed from them by 10%, suggesting divergence of these lineages about 5 million years ago. Hybrid F1s were obtained from all heterospecific crosses, and one surviving hybrid F1 female produced viable eggs. Previous studies have revealed no pheromonal differences. On the basis of this evidence, *R. ferrugineus* and *R. vulneratus* should be considered colour morphs of the same species and be synonymized under the name *Rhynchophorus ferrugineus* (Olivier), with the common name Asian palm weevil.

KEYWORDS: Asian palm weevil, red palm weevil, cross-breeding, mitochondrial DNA sequencing, morphological comparisons, RAPD analyses, Rhynchophorinae, *Rhynchophorus ferrugineus*, *Rhynchophorus vulneratus*, synonymization.

Introduction

The Asian palm weevils, *Rhynchophorus ferrugineus* (Olivier) and *R. vulneratus* (Panzer), are serious pests of palms throughout South and South-East Asia (Sivapragasam *et al.*, 1990; Sadakathulla, 1991), and are sympatric throughout

Journal of Natural History
ISSN 0022-2933 print/ISSN 1464-5262 online © 2004 Taylor & Francis Ltd
http://www.tandf.co.uk/journals
DOI: 10.1080/00222930310001657874

virtually the whole of the range of *R. vulneratus* (figure 1). Introductions of *R. ferrugineus* to the Arab Gulf States (Bokhari and Abuzuhari, 1992) and their spread westward into Egypt are causing the devastation of date palms throughout that region.

Several observations made while investigating production of and response to aggregation pheromone by *R. ferrugineus* and *R. vulneratus* (Hallett *et al.*, 1993; Hallett, 1996; Perez *et al.*, 1996) led us to question the validity of recognizing these weevils as separate species. In three experiments, 27 captured weevils (10.6% of total captured) had colour markings intermediate between those of *R. ferrugineus* and *R. vulneratus* (Hallett *et al.*, 1993). These individuals had the black pronotum with a red stripe characteristic of *R. vulneratus*, but their elytra and abdomens were reddish brown, like *R. ferrugineus*. A single field-collected individual (Hallett, 1996) had the characteristic pronotum of *R. ferrugineus*, but the black elytra and abdomen typical of *R. vulneratus*.

A cross-attraction experiment indicated no differences in the natural pheromone blends produced by males of either species (Hallett *et al.*, 1993; Hallett, 1996). In the scolytid genus *Ips* closely related species are cross-attractive, but unlike *R. ferrugineus* and *R. vulneratus* they maintain reproductive isolation through allopatric or parapatric distributions (Lanier and Wood, 1975). In two sympatric *Gnathotrichus* spp., specificity in aggregation pheromones is maintained by chirality of a single compound, one species requiring both enantiomers and the other only one enantiomer, the antipode of which is repellent (Borden *et al.*, 1976, 1980). Despite extensive investigation, no evidence for species specificity imparted by

FIG. 1. Geographic distributions of *Rhynchophorus ferrugineus* (shaded) and *R. vulneratus* (hatched), based on country (mainland and New Guinea) or island records (Indonesia and Philippines) in Wattanapongsiri (1966) and additional reports. Unreliable records (Wattanapongsiri, 1966) of both species from South and Central America have been excluded.

blends of compounds or chirality was disclosed for sympatric *R. ferrugineus* and *R. vulneratus* (Hallett, 1996; Perez *et al*., 1996).

These observations alone do not constitute a basis for the synonymization of the species. Colour variation could occur within a species. Compounds not detected in our experiments, e.g. epicuticular contact pheromones, could impart species specificity to communication mechanisms. Other pre-mating mechanisms that could ensure reproductive isolation include: ecological stratification of habitat, differences in flight periods or diel activity, differences in host compounds used as synergists, food preferences, specificity of courtship rituals and genital incompatibility (Lanier and Burkholder, 1974). Potential post-mating mechanisms of isolation include infertility of eggs and sterility of hybrids (Lanier and Burkholder, 1974).

Although there are reports that *R. ferrugineus* more often attacks the trunk and *R. vulneratus* the terminal bud of the tree (Sivapragasam *et al*., 1990), both attack the same variety of palm types (Wattanapongsiri, 1966), and co-attacked trees have been observed (Banks, 1906). Antennal responses of *R. ferrugineus* and *R. vulneratus* to host volatiles were almost identical with both species responding to five coconut compounds; the only difference was in a minor antennal response by *R. vulneratus* to a sixth coconut compound (Hallett, 1996). Therefore, it is unlikely that reproductive isolation is occurring through different food preferences or the use of different host compounds as pheromone synergists.

In terms of differences in flight periods or diel activity, no formal study was made, but *R. ferrugineus* and *R. vulneratus* were repeatedly observed flying at the same times of day. Where the two species are sympatric in Indonesia, individuals of both species were always captured throughout the year (Hallett, personal observation).

Rochat (1991) reported evidence for a female-produced cuticular pheromone for *R. palmarum*, which may be important in sex recognition and mating behaviour. However, interspecific mating pairs of *R. ferrugineus* and *R. vulneratus* are often observed in caged populations, suggesting that incompatibilities in courtship rituals or genitalia do not exist or are insufficient on their own to maintain isolation.

Our objective was to challenge the null hypothesis that despite the existence of two distinct colour morphs, *R. ferrugineus* and *R. vulneratus* do not constitute valid species. Investigations included a re-examination of morphological characters used in part to justify separation of these two species (Wattanapongsiri, 1966), comparative analysis of DNA variability and a cross-breeding study.

Methods

Adult specimens were obtained from four sources for this study: (1) Indonesian specimens of *R. ferrugineus* and *R. vulneratus* were collected from four locations (Bogor 6°30′S, 106°15′E; Bojong Kalong 6°48′S, 106°58′E; Cikancana 6°50′S, 107°02′E; and Pakuwon 6°45′S, 106°45′E) in West Java; (2) *R. ferrugineus* was also collected in Ras Al Kaimeh, United Arab Emirates (UAE); (3) specimens of *R. ferrugineus* and *R. vulneratus* included in Wattanapongsiri's 1966 study were borrowed from the American Museum of Natural History; and (4) *R. bilineatus* (Montr.) adults were collected in Rabaul, Papua New Guinea. The type specimens could not be located for comparison with other specimens.

Voucher specimens of weevils used in morphological analyses and F1s from the cross-breeding study have been deposited in the collection of the Canadian Museum of Nature, Ottawa. Larval specimens were obtained from cross-breeding studies conducted herein.

Morphological comparisons

Submentum and subgenal sutures. Forty-three specimens of *R. ferrugineus* (22 female, 16 male, five unknown), 50 *R. vulneratus* (17 female, 23 male, 10 unknown) and 12 *R. vulneratus* colour intermorphs (five female, seven male) (Wattanapongsiri, 1966) were mounted on Styrofoam blocks with the ventral surface of the rostrum exposed. Source collection, country of origin and number of weevils examined are given in table 1. The basal area of the rostrum (figure 2) was drawn under the microscope (40–50 ×) using a camera lucida (Wild M5, Heerbrug, Switzerland). Because the weevils were inverted and both species were mixed in a single group, the species of the weevil being drawn was concealed from the researcher. Three measurements were made from the drawings: length of concave section of submentum (L), central width of submentum (CW) and maximum distance between suture lines (W). Two estimates of concavity were developed: (1) (central width of submentum)/(maximum distance between subgenal sutures) or CW/W, and (2) (length of concave section)/(maximum distance between subgenal sutures) or L/W (figure 1). Both estimates are inverse measures of the concavity of the subgenal sutures. By the first estimate (CW/W), a score of one indicates a submentum with straight subgenal sutures, while scores close to zero indicate highly concave subgenal sutures.

Pronotal shape. Seventy-five specimens of *R. ferrugineus* (35 females, 31 males and nine unknown), 64 *R. vulneratus* pure colour types (23 females, 31 males and 10 unknown) and 14 intermediate *R. vulneratus* colour types (five females, nine males) were mounted dorsal side up, and drawings were made of the shape of the pronotum using a microscope (6–6.4 ×) and camera lucida. Four measurements were taken from the drawings: maximum and minimum width of the

Table 1. Source collection, country of origin and number of adult specimens of *R. ferrugineus*, *R. vulneratus* and *R. vulneratus* intermediate colour morphs examined in morphological studies of the submentum and pronotum.

Species	Collection†	Country	Submentum (no. of specimens)	Pronotum (no. of specimens)
R. ferrugineus	AMNH	Unknown	–	1
		India	5	5
		Indonesia	1	1
		Philippines	4	9
		Taiwan	5	7
		Thailand	1	1
		Vietnam	1	1
	RHH	Indonesia	26	24
		UAE	–	26
R. vulneratus	AMNH	Unknown	1	2
		Borneo	6	8
		Indonesia	5	15
		Philippines	4	4
	RHH	Indonesia	34	35
R. vulneratus intermediate colour morphs	AMNH	Borneo	2	3
		Philippines	–	1
	RHH	Indonesia	10	10

†AMNH, American Museum of Natural History; RHH, Rebecca H. Hallett.

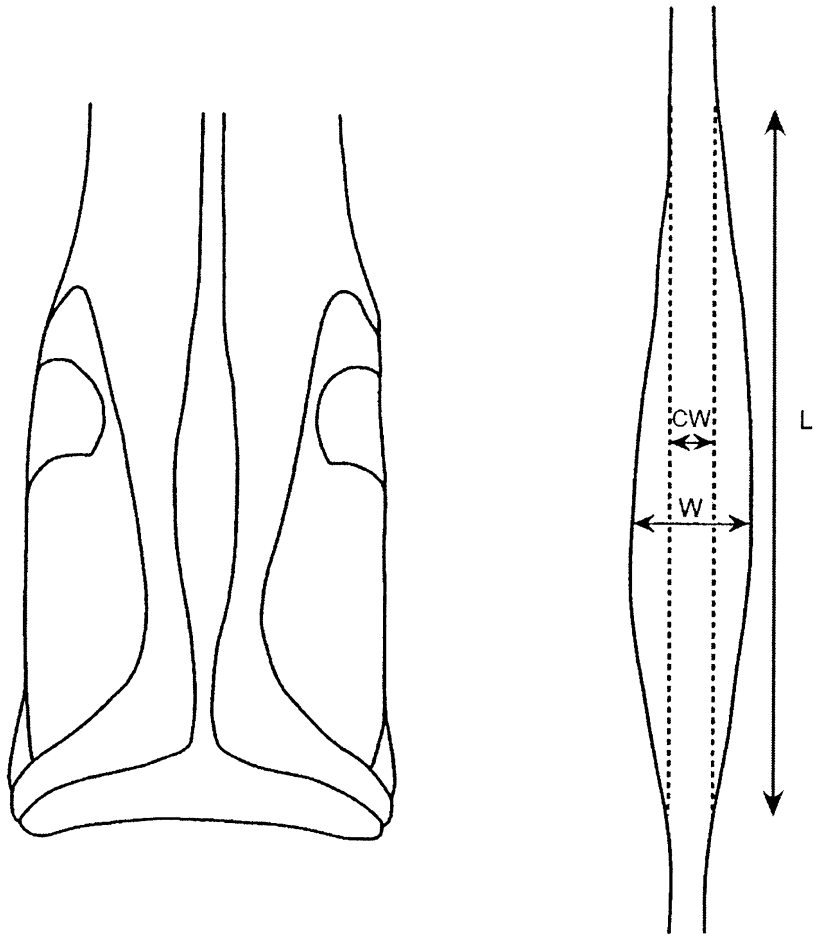

FIG. 2. Representative diagram of ventral view of basal area of rostrum of *R. ferrugineus* or *R. vulneratus* as drawn using a camera lucida. Measurements depicted in enlarged drawing are the central width of the submentum (CW), maximum width of submentum (W) and length of concave section of submentum (L).

pronotum, pronotal length and the length of a transect from the midline at rear to the point at which the maximum width line met the antero-lateral margin (figure 3). Measurements taken from pronotal figures by Wattanapongsiri (1966) were designated as 'taxonomic standards'. Three estimates of pronotal shape were then devised: ratio of minimum to maximum pronotal width (MinW/MaxW), ratio of minimum pronotal width to length (MinW/PL) and ratio of pronotal length to transect length (PL/TL). A MinW/MaxW ratio close to 1.0 would represent a specimen with a square pronotum, while a lower value would indicate a more vertically oval or circular pronotum. A low MinW/PL indicates a vertically oval pronotum, while a large value indicates a horizontally oval specimen. A high PL/TL ratio indicates an oval pronotum, while a lower ratio indicates a circular or square pronotum.

Larval characters. Larvae of *R. ferrugineus* ($N=27$) and *R. vulneratus* ($N=40$), as well as hybrid F1 offspring of both ♀ *ferrugineus* × ♂ *vulneratus*

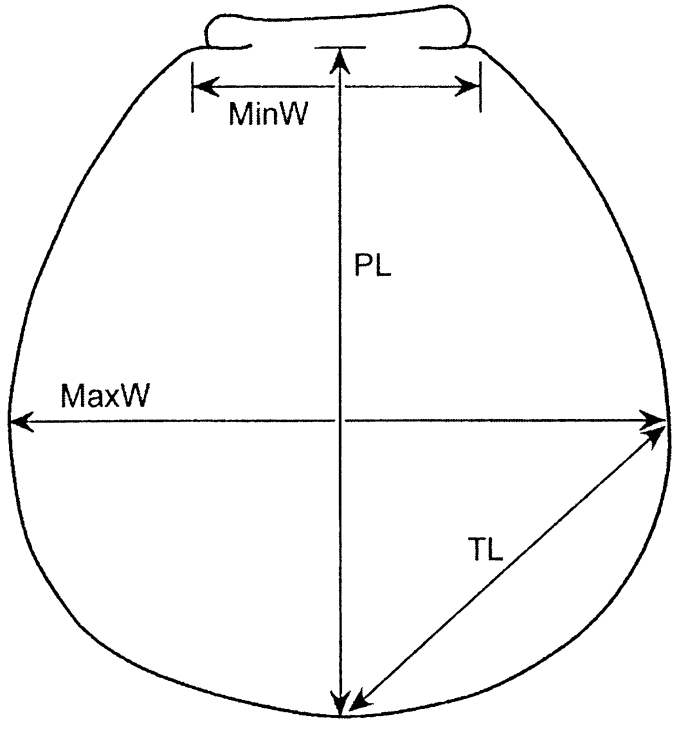

FIG. 3. Representative pronotum of *R. ferrugineus* or *R. vulneratus* as drawn using a camera lucida, and showing four measurements used in calculating estimates of pronotal shape: maximum (MaxW) and minimum (MinW) width of pronotum, pronotal length (PL), and length of transect (TL) from distal mid-point to the anterolateral margin at point of maximum width.

($N=19$) and ♀ *vulneratus* × ♂ *ferrugineus* ($N=16$) crosses were obtained from cross-breeding studies described below. The width of the head capsule was measured and the labrum removed from each specimen. The labrum was cleared using Peacocks' technique (Barbosa, 1974), except that glacial acetic acid was substituted for xylene in the final neutralization step. Each labrum was then permanently mounted on a microscope slide using Permount mounting agent (Fisher Scientific, Fair Lawn, NJ, USA). The number of lateral labral setae (Wattanapongsiri, 1966) (figure 4) was counted on each specimen. Distances between the epipharyngeal sensory pore and seta 1 (D1) and seta 2 (D2) (figure 5) were measured using an optical micrometer. The ratio of the distance from the epipharyngeal pore to seta 1 and the distance between the epipharyngeal pore and seta 2 was determined (D1/D2).

Statistical analyses. Comparisons between morphological measurements were made with ANOVA and Bonferroni *t*-tests (PROC GLM, SAS Institute, 1985).

Genetic comparisons

Live adults of both *R. ferrugineus* and *R. vulneratus* were collected at four locations in West Java, Indonesia and shipped to Simon Fraser University (SFU). Weevils were frozen at −80°C until needed for analysis.

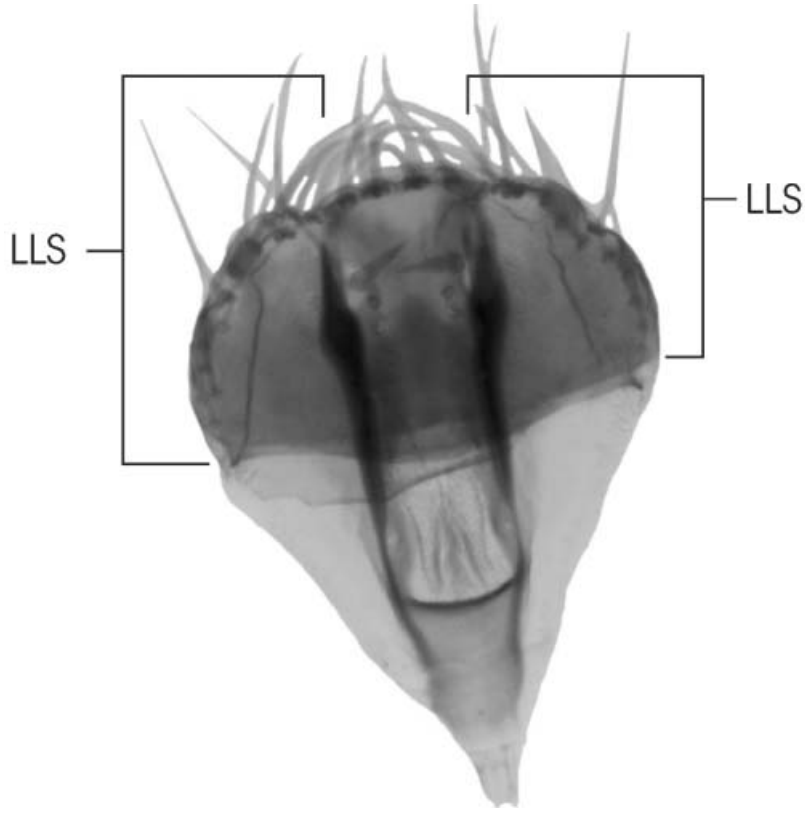

FIG. 4. Larval labrum of *R. ferrugineus* showing the location of the lateral labral setae (LLS). Magnification 40×. Photograph: J. Gibson.

One middle leg of each beetle was ground with a glass pipette in a 1.5-ml microtube containing 0.9 ml of Lifton buffer (0.2 M sucrose, 0.05 M EDTA, 0.1 M Tris, 0.5% sodium dodecyl sulphate, pH 9.0). After 15 min on ice, 100 μl of 8 M potassium hydroxide was added. The suspension was vortexed briefly, returned to ice for another 15 min and then centrifuged at high speed (14 000 rpm) for 15 min. The supernatant was transferred to a new 1.5-ml microtube, 100 μl of 1:24 isoamyl alcohol:chloroform and 1.3 ml of equilibrated phenol was added, and the mixture was shaken and then centrifuged at 14 000 rpm for 10 min. The aqueous phase was transferred to a new tube, 100 μl of 1:24 isoamyl alcohol:chloroform was added and the mixture was centrifuged again as above. The aqueous phase was then removed to a new tube and 30 μl of ammonium acetate and one-half volume of isopropanol was added. After 1 h at −20°C, solutions were centrifuged for 30 min to pellet the DNA. The pellet was washed twice in 1 ml of cold 70% ethanol, air-dried for 1 h, resuspended in 100 μl of distilled deionized water at room temperature, and stored at −20°C. Prior to polymerase chain reaction (PCR) amplifications, DNA was quantified in a spectrophotometer and amounts were adjusted so that equal amounts of DNA were used in each amplification.

Random amplified polymorphic DNA–polymerase chain reaction (RAPD-PCR) analyses. RAPD amplification reactions each contained 22 μl of water, 0.5 μl of 10 μM dNTPs, 2.5 μl of 10× buffer (100 mM Tris-HCl, 15 mM $MgCl_2$, 500 mM

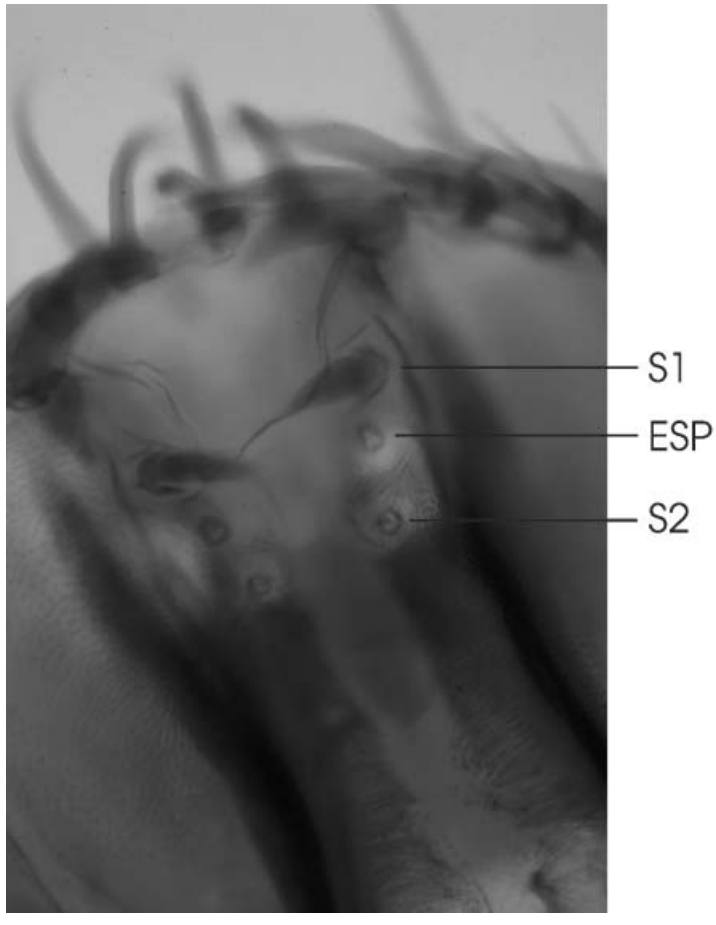

FIG. 5. Larval labrum of *R. vulneratus* showing the location of the epipharyngeal sensory pore (ESP), seta 1 (S1) and seta 2 (S2). Magnification 100×. Photograph: J. Gibson.

KCl, pH 8.3), 0.33 μl of 10 μM primer (University of British Columbia oligonucleotide facility RAPD primer set), 0.125 μl of *Taq* DNA polymerase and 0.83 μl of weevil genomic DNA. All 24 primers used were random 10-base oligomers, the sequences of which are given in the captions of figures 6–8. Amplifications were performed in a Perkin-Elmer-Cetus 480 thermocycler programmed for 35 cycles with denaturation at 94°C for 1 min, annealing at 35°C for 1 min and extension at 72°C for 2 min, for specimens of both *R. ferrugineus* and *R. vulneratus* from Bojong Kalong. Amplified DNA was analysed by electrophoresis on 1.5% agarose gels, stained with ethidium bromide and photographed.

Mitochondrial DNA sequencing. PCR amplification of the mitochondrial cytochrome oxidase subunit 1 (CO1) gene was conducted with specimens of both *R. ferrugineus* and *R. vulneratus* from three locations (Bogor, Cikancana and Pakuwon) and with *R. bilineatus* from Rabaul, Papua New Guinea. Double- and single-stranded mitochondrial DNA (mtDNA) amplifications used C1-J-1718 and TL2-N-3014, and sequencing was conducted by annealing with TL2-N-3014 and C1-J-2441 (Simon *et al.*, 1994). Double-stranded mtDNA amplifications each contained 39 μl of water, 1 μl of dNTPs, 5 μl of 10× buffer, 2.5 μl each

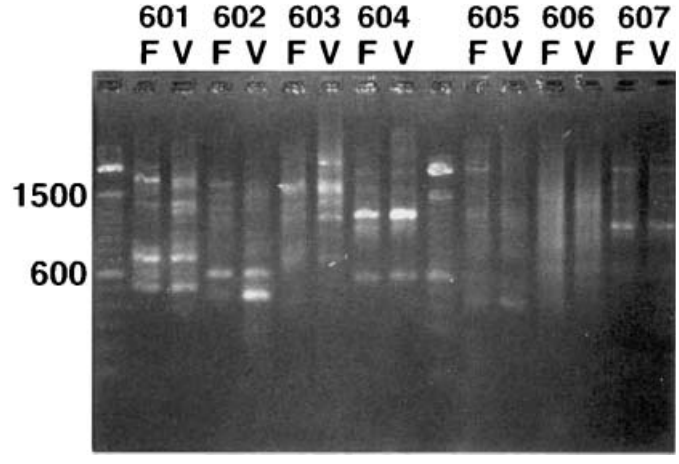

FIG. 6. RAPD fragments amplified using primers 601–608 on DNA from *R. ferrugineus* (F) and *R. vulneratus* (V) specimens from Bojong Kalong, Java. Lanes 1 and 10 contain a 100 bp ladder for reference. Primer nucleotide sequences (5′–3′): 601, CCG-CCC-ACT-G; 602, GCG-AAG-ACT-A; 603, ACC-CAC-CGC-G; 604, GGC-CCA-TTG-C; 605, CCG-ATC-ATT-C; 606, CGG-TCG-GCC-A; 607, AGT-GTC-GTC-G; 608, GAG-CCC-GAA-A.

of 10 μM primers (C1-J-1718 and TL2-N-3014), 0.25 μl of *Taq* DNA polymerase and 0.7 μl of weevil genomic DNA. Amplifications were performed in a Perkin-Elmer-Cetus 480 thermocycler programmed for 30 cycles with denaturation at 94°C for 1 min, annealing at 47°C for 1 min and extension at 72°C for

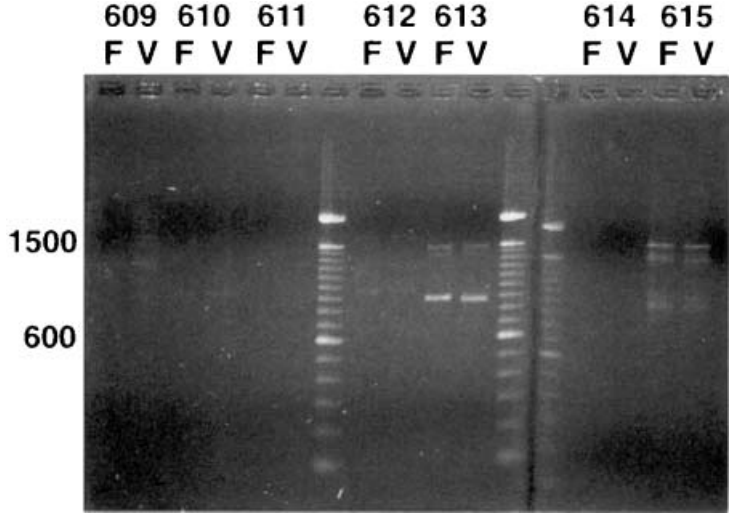

FIG. 7. RAPD fragments amplified using primers 609–616 on DNA from *R. ferrugineus* (F) and *R. vulneratus* (V) specimens from Bojong Kalong, Java. Lanes 7, 12 and 13 contain a 100 bp ladder for reference. Primer nucleotide sequences (5′–3′): 609, ACA-GCA-CCA-T; 610, TTT-GCC-GCC-C; 611, CCA-TCG-TAC-C; 612, CCG-TGA-GTA-T; 613, TGC-ACC-CAC-G; 614, GTA-GTC-TCG-C; 615, CGT-CGA-GCG-G; 616, CGG-AAG-AAA-C.

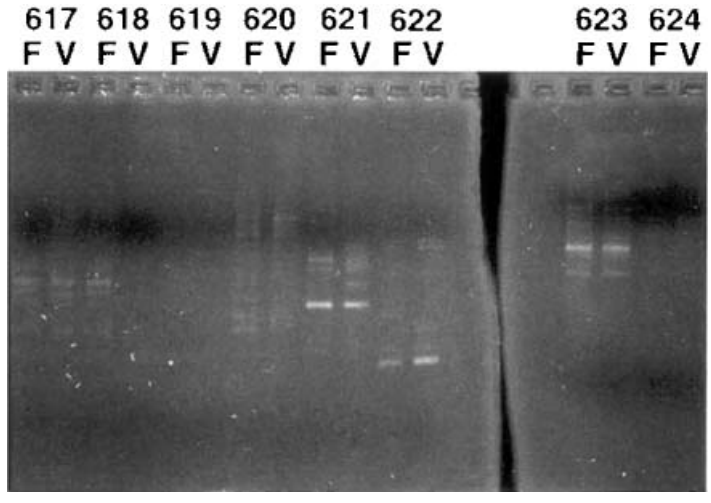

FIG. 8. RAPD fragments amplified using primers 617–624 on DNA from *R. ferrugineus* (F) and *R. vulneratus* (V) specimens from Bojong Kalong, Java. Primer nucleotide sequences (5′–3′): 617, CGG-ACT-ATG-T; 618, CGG-ACT-ATG-T; 619, TTC-CCT-AGC-G; 620, TTG-CGC-CCG-G; 621, GTC-TGC-GCT-A; 622, ACA-GGT-GGT-T; 623, TGC-GGG-ACT-G; 624, GTG-ATA-AGC-C.

1 min. Single-stranded amplifications were then conducted using 3 μl of amplified double-stranded DNA, 39 μl of water, 1 μl of dNTPs, 5 μl of 10 × buffer, 0.6 μl of 10 μM primer (C1-J-1718 or TL2-N-3014) and 0.25 μl of *Taq* DNA polymerase. There were 30 amplification cycles with denaturation at 94°C for 1 min, annealing at 52°C for 1 min and extension at 72°C for 1 min. In order to remove dNTPs, primers and enzymes, amplified DNA was centrifuged in Millipore Ultrafree MC 30,000 NMWL polysulfone membrane tubes (Millipore, Bedford, MA) and resuspended in 20 μl of water prior to sequencing by annealing with TL2-N-3014 or C1-J-2441.

Double-stranded mtDNA amplification of the CO1 gene was conducted with the primers C1-J-1718 and TL2-N-3014 and sequenced by annealing with C1-J-2441 and TL2-N-3014. Amplifications each contained 17 μl of water, 2.5 μl of 10 × buffer, 0.6 μl of bovine serum albumin (100 ng μl^{-1}), 0.1 μl of *Taq* DNA polymerase and 0.7 μl of weevil genomic DNA. The thermocycler was programmed for 30 cycles with denaturation at 94°C for 1 min, annealing at 47°C for 1 min and extension at 72°C for 45 s in the first six cycles with duration increased by 10 s in each subsequent cycle. Prior to sequencing, 3 μl of double-stranded DNA was treated with exonuclease I and shrimp alkaline phosphatase to digest primers and deactivate dNTPs.

Cross-breeding studies

Pupal chambers containing live pupae were collected in Bojong Kalong, Indonesia and brought to SFU. All newly eclosed adults used in this study had the distinctive colour markings of either *R. ferrugineus* or *R. vulneratus*. They were kept separately for at least 3 days before pairing with another adult. All possible con- and heterospecific pairings were performed (*ferrugineus* × *ferrugineus*, female *ferrugineus* × male *vulneratus*, female *vulneratus* × male *ferrugineus* and *vulneratus* ×

vulneratus). The conspecific pairing for *R. ferrugineus* was made only once. All other pairings were performed with two pairs of weevils.

Adult pairs were kept in 150-mm tissue culture dishes (Corning Inc., Corning, NY) and given slices of apple on which to feed and oviposit. Two pieces of paper towelling (one under and one over the apple slices) were provided to help any overturned weevil to right itself. Three times a week, paper towelling and apple slices were replaced and inspected for eggs and early instar larvae. Eggs were removed, soaked for 2 min in a 1:840 solution of benzalkonium chloride (Sigma Chemical Co., St Louis, MO) and dried on filter paper. Both eggs and larvae were placed individually in 29.6 ml plastic containers (Solo Cup Co., Urbana, IL) containing an artificial rearing medium adapted from Rahalkar *et al.* (1985): sugarcane bagasse, 53 g; medium desiccated coconut, 60 g; Brewer's yeast, 20 g; granulated sugar, 76 g; agar, 20 g; Wesson salt, 2 g; vitamin diet fortification, 12 g; distilled water, 760 ml; 4 M potassium hydroxide, 3 ml; methyl *para*-hydroxybenzoate 14% solution in 95% ethanol, 10 ml; sorbic acid 12.5% solution in 95% ethanol, 15 ml. Containers were inspected weekly for signs of feeding and larvae were transferred into new containers of diet as required. Later instars were transferred to 100-ml glass containers, and as larvae approached pupation they were given diet that contained jute fibres for construction of the pupal chamber. The rearing chamber was maintained at 27–30°C and 90–95% relative humidity.

Upon emergence, F1 adults were held individually with apples until they could be mated. Due to the long time span over which F1 adults emerged and high mortality, only four F1 weevils were subsequently paired for mating (female *vulneratus–ferrugineus* × male *vulneratus–vulneratus*, and female × male *vulneratus–vulneratus*). Paper towelling and apple slices were examined for eggs and early instar larvae as above. Containers of artificial diet were examined for early instar larvae 5 and 8 weeks after the last egg was laid.

Results

Morphological comparisons

Submentum and subgenal sutures. No significant differences were found between *R. ferrugineus* and *R. vulneratus* for either estimate of concavity (table 2). However, the subgenal sutures of males are more concave than those of females according to both estimates (table 2). In addition, significant differences were found in the concavity of sutures according to country of origin, with individuals from Indonesia having more concave subgenal sutures than those from Taiwan, India and Borneo.

Pronotal shape. According to Wattanapongsiri's (1966) figures, *R. ferrugineus* would be expected to have a score of 0.6 for both MinW/MaxW and MinW/PL, while *R. vulneratus* should have scores of around 0.4 for both. No significant differences were found between *R. ferrugineus* and *R. vulneratus* specimens for MinW/MaxW; however, a significant difference was found between species for the estimate MinW/PL (table 3). Significant differences were also found between countries of origin for both MinW/MaxW and MinW/PL, with UAE specimens differing from Indonesian and Filipino specimens for MinW/MaxW, and UAE specimens differing from all other countries for the estimate MinW/PL. No differences were found between sexes for any of the three estimates. In addition,

Table 2. Estimates of the concavity of the subgenal sutures of specimens of *R. ferrugineus* and *R. vulneratus*, using three sources of variation: species, sex and country of specimens.

Source of variation	*N*	CW/W[†] (mean ± SE)	L/W[‡] (mean ± SE)
Species		$P=0.699$	$P=0.153$
R. ferrugineus	43	0.40 ± 0.02 a	6.74 ± 0.28 a
R. vulneratus	50	0.38 ± 0.02 a	6.67 ± 0.21 a
R. vulneratus intermediate colour morphs	12	0.37 ± 0.02 a	7.58 ± 0.61 a
Sex		$P=0.015$	$P=0.018$
Female	44	0.42 ± 0.02 a	7.27 ± 0.30 a
Male	46	0.37 ± 0.02 b	6.39 ± 0.20 b
Country		$P=0.0001$	$P=0.171$
Taiwan	5	0.57 ± 0.06 a	6.58 ± 0.54 a
Vietnam	1	0.57	8.86
India	5	0.53 ± 0.04 a	8.09 ± 1.21 a
Borneo	8	0.52 ± 0.05 a	7.95 ± 0.43 a
Thailand	1	0.43	7.20
Philippines	8	0.41 ± 0.03 ab	6.08 ± 0.42 a
Indonesia	76	0.34 ± 0.01 b	6.68 ± 0.20 a

CW, central width of submentum; W, maximum width of submentum; L, length of concave section of submentum.

Values in the same category and column followed by the same letter are not significantly different, Bonferroni *t*-test, $\alpha=0.05$. Probabilities above each column for species, sex and country refer to significance of these factors in the ANOVA model.

[†]ANOVA, df = 9, 79, $F=5.67$, $P=0.0001$.

[‡]ANOVA, df = 9, 79, $F=2.12$, $P=0.0375$.

no differences were found between species or countries for the estimate PL/TL (table 3).

Larval characters. There were no significant differences in the mean number of lateral labral setae between *R. ferrugineus*, *R. vulneratus* and hybrid F1s (table 4). The number of larval lateral setae was not dependent on head capsule width (means not shown, but term included in ANOVA model).

There was no significant difference between the four crosses in the mean ratio of the distances between the epipharyngeal pore and seta 1 and seta 2 (table 4). This ratio was approximately 1.0 for all four larval types, indicating that the epipharyngeal sensory pore is equidistant from setae 1 and setae 2. Again, this character was not dependent upon head capsule width (means not shown, but term included in ANOVA model).

Genetic comparisons

RAPD-PCR analysis. RAPD banding patterns were visualized for 14 primers (601, 602, 603, 604, 607, 610, 612, 613, 615, 617, 620, 621, 622, 623) (figures 6–8). In nine of these (604, 607, 610, 612, 613, 615, 617, 622, 623), no differences in banding patterns were observed between *R. ferrugineus* and *R. vulneratus* from Bojong Kalong, Java (figures 6–8). For all five of the remaining primers (601, 602, 603, 620, 621), some amplification fragments were common to both *R. ferrugineus* and *R. vulneratus* (figures 6, 8). The few differences in

Table 3. Estimates of pronotal shape of specimens of *R. ferrugineus* and *R. vulneratus* compared to taxonomic standard measures taken from Wattanapongsiri's (1966) drawings.

Source of variation	*N*	MinW/MaxW[†] (mean ± SE)	MinW/PL[‡] (mean ± SE)	PL/TL[§] (mean ± SE)
Species		$P = 0.230$	$P = 0.001$	$P = 0.443$
R. ferrugineus	75	0.48 ± 0.01 a	0.44 ± 0.01 a	1.72 ± 0.01 a
R. vulneratus intermediate colour morphs	14	0.46 ± 0.01 a	0.41 ± 0.01 ab	1.74 ± 0.01 a
R. vulneratus	64	0.46 ± 0.01 a	0.40 ± 0.01 b	1.71 ± 0.02 a
R. ferrugineus taxonomic standard		0.61	0.63	1.63
R. vulneratus taxonomic standard		0.42	0.43	1.14
Sex		$P = 0.083$	$P = 0.198$	$P = 0.549$
Female	63	0.45 ± 0.01 a	0.41 ± 0.01 a	1.73 ± 0.01 a
Male	71	0.47 ± 0.01 a	0.43 ± 0.01 a	1.73 ± 0.01 a
Country		$P = 0.035$	$P = 0.003$	$P = 0.279$
UAE	26	0.53 ± 0.01 a	0.49 ± 0.01 a	1.74 ± 0.01 a
Indonesia	86	0.46 ± 0.01 b	0.42 ± 0.01 b	1.73 ± 0.01 a
Borneo	11	0.45 ± 0.01	0.40 ± 0.01 b	1.76 ± 0.03 a
Vietnam	1	0.45	0.39	1.63
India	5	0.44 ± 0.02	0.38 ± 0.01 b	1.67 ± 0.04 a
Philippines	14	0.44 ± 0.02 b	0.40 ± 0.01 b	1.71 ± 0.03 a
Taiwan	7	0.44 ± 0.01	0.38 ± 0.01 b	1.68 ± 0.03 a
Thailand	1	0.43	0.38	1.61

MinW, minimum width of pronotum; MaxW, maximum width of pronotum; PL, pronotum length; TL, length of transect from mid-point to anterolateral margin at maximum width.

Excluding taxonomic standards, values in the same category and column followed by the same letter are not significantly different, Bonferroni *t*-test, $\alpha = 0.05$. Probabilities above each column for species, sex and country refer to significance of these factors in the ANOVA model.

[†]ANOVA, df = 10, 121, $F = 2.18$, $P = 0.0235$.

[‡]ANOVA, df = 10, 121, $F = 3.97$, $P = 0.0001$.

[§]ANOVA, df = 10, 121, $F = 1.08$, $P = 0.385$.

Table 4. Comparison of morphological characteristics of larval F1 specimens obtained from inter- and intraspecific crosses between *R. ferrugineus* (F) and *R. vulneratus* (V).

Cross (female–male)	*N*	No. of lateral labral setae		D1/D2 (mean ± SE)[‡]
		Range	Mean ± SE[†]	
FF	27	20–24	21.67 ± 0.31	0.94 ± 0.05
FV	25	20–26	21.36 ± 0.98	0.94 ± 0.04
VF	18	18–24	21.89 ± 0.46	0.98 ± 0.07
VV	42	18–26	20.51 ± 1.07	1.02 ± 0.03

D1, distance between epipharyngeal sensory pore and seta 1; D2, distance between epipharyngeal sensory pore and seta 2 on larval labrum.

[†]No significant differences between means, Bonferroni *t*-test, $\alpha = 0.05$. ANOVA, df = 4, 107, $F = 0.78$, $P = 0.538$.

[‡]No significant differences between means, Bonferroni *t*-test, $\alpha = 0.05$. ANOVA, df = 4, 102, $F = 0.72$, $P = 0.579$.

banding between *R. ferrugineus* and *R. vulneratus* for these primers may be due to stochastic variation in the efficacy of the amplification reactions.

Mitochondrial DNA sequencing. Sequences were obtained in two segments of the CO1 gene, corresponding to nucleotides 2563–2661 on the sense (or major) strand and 2765–2877 on the antisense (or minor) strand of the mitochondrial DNA molecule of *Drosophila yakuba* (Clary and Wolstenholme, 1985). *Rhynchophorus ferrugineus* and *R. vulneratus* from Bogor, Cikancana and Pakuwon were identical in the sequence of these segments (201 base pairs read) of the CO1 gene (figure 9). Over the same segments, *R. bilineatus* differed from *R. ferrugineus* and *R. vulneratus* at 21 nucleotides (figure 9). All but one of these differences were point substitutions at third codon positions.

Cross-breeding studies

Numbers of eggs laid, hatching success and survival to pupal and adult stages were comparable regardless of whether weevils were paired with con- or heterospecific mates (table 5).

Seventeen adult F1s were obtained from the crosses, of which nine arose from paired conspecific *R. vulneratus*. The four heterospecific pairings produced six hybrid adults. In all cases, coloration of the F1 adults was identical to that of the

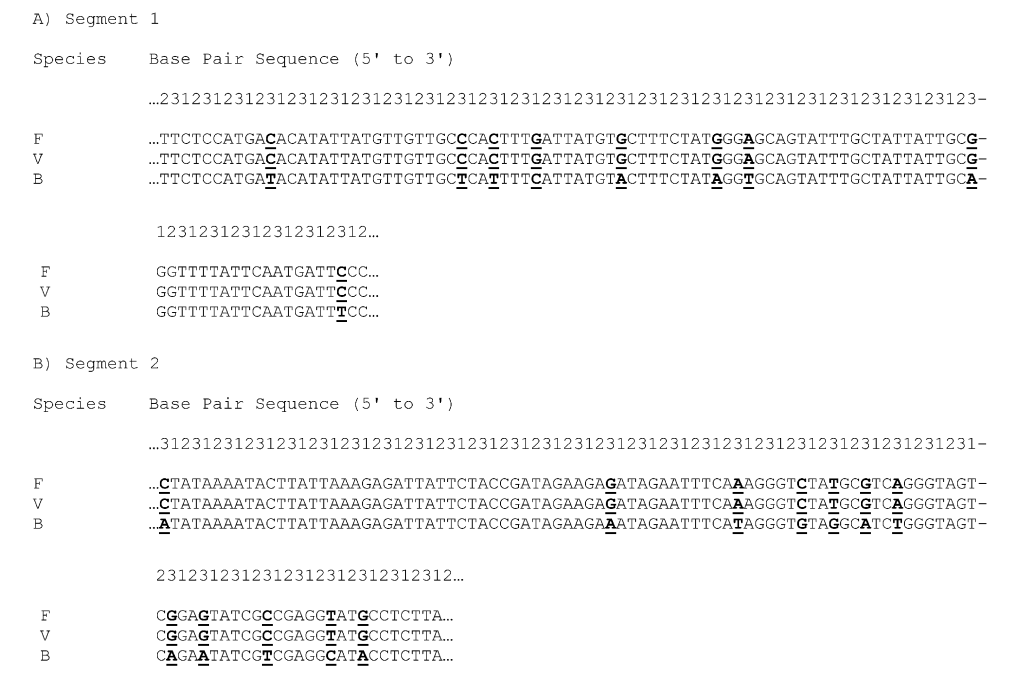

FIG. 9. Two segments of the cytochrome oxidase subunit 1 gene as sequenced in *R. ferrugineus* (F), *R. vulneratus* (V) and *R. bilineatus* (B). (A) Segment 1: 97 nucleotide segment of the sense strand sequenced by annealing with C1-J-2441 (Simon *et al.*, 1994); (B) segment 2: 104 nucleotide segment of the anti-sense strand sequenced by annealing with TL2-N-3014 (Simon *et al.*, 1994). These segments correspond to nucleotides 2563–2661 and 2765–2877, respectively, of the mtDNA molecule of *Drosophila yakuba* (Clary and Wolstenholme, 1985). Bold font and underlining indicates nucleotide at which some difference exists between species.

Table 5. Summary of eggs produced, number of emerged larvae, number of pupal chambers produced and number of adult F1s obtained from inter- and intraspecific crosses between *R. ferrugineus* (F) and *R. vulneratus* (V).

Pair (female–male)	Total eggs laid	Total larvae emerged	Total pupal chambers produced	Total F1 adults emerged
FF-1	414	208 (50.2%)	3 (1.4%)	2 (67%)
VV-1	355	148 (41.7%)	4 (2.7%)	3 (75%)
VV-2	401	159 (39.7%)	9 (5.7%)	6 (67%)
FV-1	341	159 (46.6%)	1 (0.6%)	1 (100%)
FV-2	359	135 (37.6%)	2 (1.5%)	1 (50%)
VF-1	260	105 (40.4%)	4 (4.8%)	2 (40%)
VF-2	296	169 (50.1%)	4 (2.4%)	2 (50%)

Numbers in parentheses indicate percentage of individuals successfully reaching that stage from previous stage.

male parent. The F1 *vulneratus–vulneratus* pair both died within 5 days of being paired and no eggs were laid. The F1 female *vulneratus–ferrugineus* × male *vulneratus–vulneratus* pair produced 66 eggs, from which 24 larvae emerged, demonstrating that a hybrid female could produce viable eggs.

Discussion

None of our investigations produced evidence that could be used to invalidate the hypothesis that *R. ferrugineus* and *R. vulneratus* are the same species.

Major morphological differences between *R. ferrugineus* and *R. vulneratus* were described by Wattanapongsiri (1966) in his revision of the genus *Rhynchophorus*. Foremost amongst these were differences in the shapes of the submentum and the pronotum. Wattanapongsiri (1966) erroneously referred to the subgenal sutures (Lyal, 1995) delineating the submentum and running down the ventral surface of the rostrum as the 'gular sutures'. These sutures were described in *R. vulneratus* as 'concave at both sides medially before reaching base of rostrum', and in *R. ferrugineus* as 'oval at base, but less concave than in *vulneratus*' (Wattanapongsiri, 1966). According to our estimate of concavity, *R. ferrugineus* would be expected to have a higher score than *R. vulneratus*, but no such differences were found (table 2).

Rhynchophorus ferrugineus was described by Wattanapongsiri (1966) as having the sides of the pronotum gradually curved to the apex and then abruptly constricted anteriolaterally, whereas the pronotum of *R. vulneratus* was described as broadly rounded at the base and then strongly narrowed to the apex. In our study, significant differences were found between the species for only one of three estimates of pronotal shape (table 3). The values for this estimate are much more similar for *R. ferrugineus* and *R. vulneratus* than is expected from the taxonomic standards calculated from Wattanapongsiri's (1966) diagrams. In addition, all specimens from UAE are *R. ferrugineus* by colour determination, and differed significantly in pronotal shape from those of other countries. This likely accounts for the statistically significant difference found between the species (table 3). *Rhynchophorus ferrugineus* and *R. vulneratus* cannot be differentiated on the basis of adult morphological characters.

Wattanapongsiri (1966) described larval *R. ferrugineus* as having 22 long lateral labral setae, and *R. vulneratus* as having 28 lateral labral setae. However, this study

indicates that both *R. ferrugineus* and *R. vulneratus* are best described as having 20–22 lateral labral setae.

Wattanapongsiri (1966) also described larval labra of *R. ferrugineus* as having the epipharyngeal pore placed closer to seta 2 than to seta 1, but with the distance from the epipharyngeal pore to seta 1 less than twice the distance to seta 2. He described *R. vulneratus* as having the epipharyngeal pore equidistant from setae 1 and 2. Head capsule width was not a significant source of variation for the ratio D1/D2, indicating that this morphological characteristic is consistent in all larval instars. In this study, the epipharyngeal pore is best described as being equidistant from both seta 1 and seta 2 for both *R. ferrugineus* and *R. vulneratus*.

Analysis of RAPDs (Williams *et al.*, 1990) is very useful in determining the existence of different species and populations (Ballinger-Crabtree *et al.*, 1992; Chalmers *et al.*, 1992; Kambhampati *et al.*, 1992; Perring *et al.*, 1993; Puterka *et al.*, 1993). In some insect taxa, RAPD analyses can detect higher levels of genetic variation than detected by allozyme analysis (Kambhampati *et al.*, 1992; Black, 1993; Puterka *et al.*, 1993). Allozyme analyses were used by deGroot (1992) to support synonymization of two sympatric scolytid cone beetles, *Conophthorus resinosae* Hopkins and *C. banksianae* McPherson. Differences in RAPD banding patterns observed in amplification products of primers 601–603 and 620–621 (figures 6, 8) may be due to stochastic variation in amplification reactions or, perhaps, genetic differences between individuals. In order to determine the source of this variation, a more comprehensive study is required, examining RAPD banding patterns of many individuals from each population. However, the clearly identical banding patterns seen for both *R. ferrugineus* and *R. vulneratus* from nine other primers (604, 607, 610, 612, 613, 615, 617, 622, 623) (figures 6–8) suggests that these weevils are very closely related and possibly a single species.

The complete absence of differences in mtDNA sequencing between *R. ferrugineus* and *R. vulneratus* from three different locations (figure 9) provides strong evidence that they are a single species. The parapatric species, *R. bilineatus*, differed from *R. ferrugineus* and *R. vulneratus* at 10% of nucleotides examined. Sequence divergence estimates of 1.7–2.3% per million years have been determined for a number of arthropod species (Martin and Simon, 1990; Boyce *et al.*, 1994; Brower, 1994; Funk *et al.*, 1995). The degree of sequence divergence suggests that *R. bilineatus* diverged from *R. ferrugineus* and *R. vulneratus* about 5 million years ago. *Rhynchophorus bilineatus* is morphologically distinct from *R. ferrugineus* and *R. vulneratus* (Wattanapongsiri, 1966), but all three utilize ferrugineol as a major aggregation pheromone (Hallett *et al.*, 1993; Oehlschlager *et al.*, 1995). Thus geographical separation apparently precluded any selection pressure for evolution of pheromone specificity.

Adult F1s were obtained from all crosses performed, indicating that genital mismatch and infertility of eggs are not operating as isolating mechanisms between *R. ferrugineus* and *R. vulneratus*. The influence of the male parent on coloration suggests that it is sex-linked, although this result may be an artefact of the small number of adult F1s obtained. *Rhynchophorus ferrugineus* has a typical curculionid chromosome formula of 10 $A + Xy_p$ (Bartlett and Rananavare, 1983). Due to the presence of intermediate colour morphs in the wild, it is likely that coloration is controlled by more than one gene locus. High mortality and loss of limbs among adult F1s may have been the result of deficiencies in the artificial diet. The production of viable eggs from the female *vulneratus–ferrugineus* × male *vulneratus–*

vulneratus pair demonstrates that hybrid F1s can be fertile and that reproductive isolation maintained by hybrid sterility is unlikely.

deGroot (1992) supported synonymization of the sympatric scolytid species *C. resinosae* and *C. banksianae* on the basis of similarities in morphology (Wood, 1982), cuticular hydrocarbons (Page *et al*., 1990), karyology (deGroot and Ennis, 1990), life history (deGroot and Borden, 1991), host selection behaviour (deGroot and Borden, 1992), allozymes (deGroot *et al*., 1992), and pheromone production and response (Pierce *et al*., 1995). Synonymization of the bark beetles, *Dendroctonus ponderosae* Hopkins and *D. monticolae* Hopkins (Wood 1963) was confirmed by mating experiments, developmental rates, karyology and morphological similarities (Lanier and Wood, 1968). Synonymization of sympatric *Ips cribricollis* (Eich.) and *I. grandicollis* (Eich.) was disputed on the basis of morphological and chromosomal differences, and their inability to interbreed (Lanier, 1987). The bark weevils, *Pissodes approximatus* Hopkins and *P. nemorensis* Germar were synonymized as *P. nemorensis* on the basis of identical pheromones (Phillips *et al*., 1984), cross-attraction (Phillips and Lanier, 1986), and similarities in allozymes, morphology and behaviour (Phillips *et al*., 1987). The sibling species, *P. strobi* (Peck), has been upheld as a distinct species due to evidence of reproductive isolation (Phillips and Lanier, 1983) and mtDNA sequence divergence (Boyce *et al*., 1994).

In comparison, *R. ferrugineus* and *R. vulneratus* are alike in morphological characters (tables 2–4), RAPD banding patterns (figures 6–8), mitochondrial DNA sequencing (figure 9), host plant preference (Hallett, 1996), and pheromone production and response (Hallett *et al*., 1993; Hallett, 1996; Perez *et al*., 1996). Given these similarities, the lack of reproductive isolating mechanisms and the existence of colour intermorphs it is unlikely that *R. ferrugineus* and *R. vulneratus* constitute two valid species. On the basis of accumulated evidence, we propose that *R. ferrugineus* and *R. vulneratus* be considered as colour morphs of the same species and that by the law of priority (International Commission on Zoological Nomenclature, 1999) they be synonymized under the name *Rhynchophorus ferrugineus* (Olivier), with *R. vulneratus* becoming a junior synonym. We further propose that the common name Asian palm weevil be universally adopted for *R. ferrugineus*.

Synonymies for *Rhynchophorus ferrugineus*

[*Curculio*] *ferrugineus* Olivier, 1790: 473; [*Curculio*] *vulneratus* Panzer, 1798: x, syn. n.; *Rhynchophorus vulneratus* (Panzer) Gyllenhal, 1838; *pascha* Boheman, 1845; *indostanus* Chevrolat, 1882; *ferrugineus* var. *tenuirostris* Chevrolat, 1882; *signaticollis* Chevrolat, 1882; *pascha* var. *cinctus* Faust, 1892; *ferrugineus* var. *seminger* Faust, 1894; *signaticollis* var. *dimidiatus* Faust, 1894.

Acknowledgements

We thank the following people and organizations: W. Yuandi, R. Edmonds, W. Rumini, W. Baringbing, S. Laup and Drs N. P. D. Angerilli, R. N. B. Prior and A. Rauf, for collecting and shipping weevils; Dr L. H. Herman for the loan of specimens from AMNH; J. Gibson and M. Ferguson-Gratton for assistance in examining specimens; Roger's Sugar for provision of sugarcane bagasse; the Eastern Indonesia University Development Project, Bogor Agricultural Institute and Coconut Research Station (Pakuwon) for logistical support; Dr C. Lyal for providing key references; Drs M. Cox, C. Lyal and S. Marshall for their comments

on an earlier draft of this paper; and M. Castledine for graphical assistance. This work was supported by an International Development Research Centre Young Canadian Researchers' Award and a Natural Science and Engineering Research Council (NSERC) Postgraduate Scholarship to R.H.H. and an NSERC research grant to B.J.C.

References

BALLINGER-CRABTREE, M. E., BLACK, W. C., IV. and MILLER, B. R., 1992, Use of genetic polymorphisms detected by the random-amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) for differentiation and identification of *Aedes aegypti* subspecies and populations, *American Journal of Tropical Medicine and Hygiene*, **47**, 893–901.

BANKS, C., 1906, *The Principal Insects Attacking the Coconut Palm. Parts I and II, New Philippine Insects* (Manila: Bureau of Printing).

BARBOSA, P., 1974, *Manual of Basic Techniques in Insect Histology* (Amherst, MA: Autumn Publishers).

BARTLETT, A. C. and RANANAVARE, H. D., 1983, Karyotype and sperm of the red palm weevil (Coleoptera: Curculionidae), *Annals of the Entomological Society of America*, **76**, 1011–1013.

BLACK, W. C. IV., 1993, PCR with arbitrary primers: approach with care, *Insect Molecular Biology*, **2**, 1–6.

BOKHARI, U. G. and ABUZUHARI, R., 1992, Diagnostic tests for redpalm weevil, *Rhynchophorus ferrugineus* infested datepalm trees, *Arab Gulf Journal of Scientific Research*, **10**, 93–104.

BORDEN, J. H., CHONG, L. J., MCLEAN, J. A., SLESSOR, K. N. and MORI, K., 1976, *Gnathotrichus sulcatus*: synergistic response to enantiomers of the aggregation pheromone sulcatol, *Science*, **192**, 894–896.

BORDEN, J. H., HANDLEY, J. R., MCLEAN, J. A., SILVERSTEIN, R. M., CHONG, L. J., SLESSOR, K. N., JOHNSTON, B. D. and SCHULER, H. R., 1980, Enantiomer-based specificity in pheromone communication by two sympatric *Gnathotrichus* species (Coleoptera: Scolytidae), *Journal of Chemical Ecology*, **6**, 445–456.

BOYCE, T. M., ZWICK, M. E. and AQUADRO, C. F., 1994, Mitochondrial DNA in the bark weevils: phylogeny and evolution in the *Pissodes strobi* species group (Coleoptera: Curculionidae), *Molecular Biology and Evolution*, **11**, 183–194.

BROWER, A. V. Z., 1994, Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution, *Proceedings of the National Academy of Science of the USA*, **91**, 6491–6495.

CHALMERS, K. J., WAUGH, R., SPRENT, J. I., SIMONS, A. J. and POWELL, W., 1992, Detection of genetic variation between and within populations of *Gliricidia sepium* and *G. maculata* using RAPD markers, *Heredity*, **69**, 465–472.

CLARY, D. O. and WOLSTENHOLME, D. R., 1985, The mitochondrial DNA molecule of *Drosophila yakuba*: nucleotide sequence, gene organization, and genetic code, *Journal of Molecular Evolution*, **22**, 252–271.

DEGROOT, P., 1992, Biosystematics of *Conophthorus* Hopkins (Coleoptera: Scolytidae) in eastern North America. PhD thesis, Simon Fraser University, Burnaby, BC, Canada, 210 pp.

DEGROOT, P. and BORDEN, J. H., 1991, Life history of the jack pine tip beetle, *Conophthorus banksianae* McPherson (Coleoptera: Scolytidae): implications for species status, *Canadian Entomologist*, **123**, 211–217.

DEGROOT, P. and BORDEN, J. H., 1992, Host acceptance behaviour of the jack pine tip beetle, *Conophthorus banksianae* and the red pine cone beetle, *C. resinosae*, *Entomologica Experimentalis et Applicata*, **65**, 149–155.

DEGROOT, P. and ENNIS, T. J., 1990, Cytotaxonomy of *Conophthorus* (Coleoptera: Scolytidae) in eastern North America, *Canadian Entomologist*, **122**, 1131–1135.

DEGROOT, P., HARVEY, G. T. and RODEN, P. M., 1992, Genetic divergence among eastern

North American cone beetles, *Conophthorus* (Coleoptera: Scolytidae), *Canadian Entomologist*, **124**, 189–199.

FUNK, D. J., FUTUYAMA, D. J., ORTI, G. and MEYER, A., 1995, A history of host associations and evolutionary diversification for *Ophraella* (Coleoptera: Chrysomelidae): new evidence from mitochondrial DNA, *Evolution*, **49**, 1008–1017.

HALLETT, R. H., 1996, Aggregation pheromones of coleopteran pests of palms. PhD thesis, Simon Fraser University, Burnaby, BC, Canada, 220 pp.

HALLETT, R. H., GRIES, G., GRIES, R., BORDEN, J. H., CZYZEWSKA, E., OEHLSCHLAGER, A. C., PIERCE, H. D., JR., ANGERILLI, N. P. D. and RAUF, A., 1993, Aggregation pheromones of two Asian palm weevils, *Rhynchophorus ferrugineus* and *R. vulneratus*, *Naturwissenschaften*, **80**, 328–331.

INTERNATIONAL COMMISSION ON ZOOLOGICAL NOMENCLATURE, 1999, *International Code of Zoological Nomenclature Adopted by the International Union of Biological Sciences, with Effect from 1 January 2000*, 4th edn (London: International Trust for Zoological Nomenclature).

KAMBHAMPATI, S., BLACK, W. C., IV. and RAI, K. S., 1992, Random amplified polymorphic DNA of mosquito species and populations (Diptera: Culicidae): techniques, statistical analysis, and applications, *Journal of Medical Entomology*, **29**, 939–945.

LANIER, G. N., 1987, The validity of *Ips cribricollis* (Eich.) (Coleoptera: Scolytidae) as distinct from *I. grandicollis* (Eich.) and the occurrence of both species in Central America, *Canadian Entomologist*, **119**, 179–187.

LANIER, G. N. and BURKHOLDER, E., 1974, Pheromones in speciation of Coleoptera, in M. C. Birch (ed.) *Pheromones* (Amsterdam: North-Holland Publishing Co.), pp. 161–189.

LANIER, G. N. and WOOD, D. L., 1968, Controlled mating, karyology, morphology, and sex-ratio in the *Dendroctonus ponderosae* complex, *Annals of the Entomological Society of America*, **61**, 517–526.

LANIER, G. N. and WOOD, D. L., 1975, Specificity of response to pheromones in the genus *Ips* (Coleoptera: Scolytidae), *Journal of Chemical Ecology*, **1**, 9–23.

LYAL, C. H. C., 1995, The ventral structures of the weevil head (Coleoptera: Curculionidae), *Memoirs of the Entomological Society of Washington*, **14**, 35–51.

MARTIN, A. and SIMON, C., 1990, Differing levels of among-population divergence in the mitochondrial DNA of periodical cicadas related to historical biogeography, *Evolution*, **44**, 1066–1080.

OEHLSCHLAGER, A. C., PRIOR, R. N. B., PEREZ, A. L., GRIES, R., GRIES, G., PIERCE, H. D., JR and LAUP, S., 1995, Structure, chirality, and field testing of a male-produced aggregation pheromone of Asian palm weevil, *Rhynchophorus bilineatus* (Montr.) (Coleoptera: Curculionidae), *Journal of Chemical Ecology*, **21**, 1619–1629.

OLIVIER, G., 1790, *Encyclopédie méthodique. Histoire naturelle. Insectes*, Vol. 5 (Paris: Panckoucke), 793 pp.

PAGE, M., NELSON, L. J., HAVERTY, M. I. and BLOMQUIST, G. J., 1990, Cuticular hydrocarbons of eight species of North American cone beetles, *Conophthorus* Hopkins, *Journal of Chemical Ecology*, **16**, 1173–1198.

PANZER, G. F., 1798, *Johann Euseb Voets Beschreibungen und Abbildungen hartschaaligter Insekten, Coleoptera Linn: aus dem Original getreu übersetz, mit der in selbigem fehlenden Synonymie und beständingen Commentar versehen. Vierter Theil* (Erlangen: J. J. Palm), xii + 120 pp.

PEREZ, A. L., HALLETT, R. H., GRIES, R., GRIES, G., OEHLSCHLAGER, A. C. and BORDEN, J. H., 1996, Pheromone chirality of Asian palm weevils, *Rhynchophorus ferrugineus* (Oliv.) and *R. vulneratus* (Panz.) (Coleoptera: Curculionidae), *Journal of Chemical Ecology*, **22**, 357–368.

PERRING, T. M., COOPER, A. D., RODRIGUEZ, R. J., FARRAR, C. A. and BELLOWS, T. S., JR, 1993, Identification of a whitefly species by genomic and behavioral studies, *Science*, **259**, 74–77.

PHILLIPS, T. W. and LANIER, G. N., 1983, Biosystematics of *Pissodes* Germar (Coleoptera: Curculionidae): feeding preferences and breeding site specificity of *P. strobi* and *P. approximatus*, *Canadian Entomologist*, **115**, 1627–1636.

PHILLIPS, T. W. and LANIER, G. N., 1986, Interspecific activity of semiochemicals among

sibling species of *Pissodes* (Coleoptera: Curculionidae), *Journal of Chemical Ecology*, **12**, 1587–1601.

PHILLIPS, T. W., WEST, J. R., FOLTZ, J. L., SILVERSTEIN, R. M. and LANIER, G. N., 1984, Aggregation pheromone of the deodar weevil, *Pissodes nemorensis* (Coleoptera: Curculionidae): isolation and activity of grandisol and grandisal, *Journal of Chemical Ecology*, **10**, 1417–1423.

PHILLIPS, T. W., TEALE, S. A. and LANIER, G. N., 1987, Biosystematics of *Pissodes* Germar (Coleoptera: Curculionidae): seasonality, morphology, and synonymy of *P. approximatus* Hopkins and *P. nemorensis* Germar, *Canadian Entomologist*, **119**, 465–480.

PIERCE, H. D., JR, GROOT, P. DE, BORDEN, J. H., RAMASWAMY, S. and OEHLSCHLAGER, A. C., 1995, Pheromones in red pine cone beetle, *Conophthorus resinosae* Hopkins, and its synonym, *C. banksianae* McPherson (Coleoptera: Scolytidae), *Journal of Chemical Ecology*, **21**, 169–185.

PUTERKA, G. J., BLACK, W. C., IV., STEINER, W. M. and BURTON, R. L., 1993, Genetic variation and phylogenetic relationships among worldwide collections of the Russian wheat aphid, *Diuraphis noxia* (Mordvilko), inferred from allozyme and RAPD-PCR markers, *Heredity*, **70**, 604–618.

RAHALKAR, G. W., HARWALKAR, M. R., RANANAVARE, H. D., TAMHANKAR, A. J. and SHANTHRAM, K., 1985, *Rhynchophorus ferrugineus*, in P. Singh and R. F. Moore (eds) *Handbook of Insect Rearing*, Vol. I (Amsterdam: Elsevier Science Publishers), pp. 279–286.

ROCHAT, D., 1991, Ecologie chimique du charançon des palmiers, *Rhynchophorus palmarum* (L.) (Coleoptera: Curculionidae). PhD thesis, Université Paris IV, Paris, 135 pp.

SADAKATHULLA, S., 1991, Management of red palm weevil, *Rhynchophorus ferrugineus* F., in coconut plantations, *Planter*, **67**(786), 415–419.

SAS INSTITUTE, 1985, *SAS User's Guide: Statistics*, Version 5 (Cary, NC: SAS Institute).

SIMON, C., FRATI, F., BECKENBACH, A., CRESPI, B., LUI, H. and FLOOK, P., 1994, Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers, *Annals of the Entomological Society of America*, **87**, 651–701.

SIVAPRAGASAM, A., ARIKIAH, A. and RANJIT, C. A., 1990, The red stripe weevil, *Rhynchophorus schach* Olivier (Coleoptera: Curculionidae): an increasing menace to coconut palms in Hilir Perak, *Planter*, **66**, 113–123.

WATTANAPONGSIRI, A., 1966, *A Revision of the Genera Rhynchophorus and Dynamis (Coleoptera: Curculionidae)*. Department of Agriculture Science Bulletin 1 (Bangkok: Department of Agriculture).

WILLIAMS, J. G. K., KUBELIK, A. R., LIVAK, K. J., RAFALSKI, J. A. and TINGEY, S. V., 1990, DNA polymorphisms amplified by arbitrary primers are useful as genetic markers, *Nucleic Acids Research*, **18**, 6531–6535.

WOOD, S. L., 1963, A revision of bark beetle genus *Dendroctonus* Erichson (Coleoptera: Scolytidae), *Great Basin Naturalist*, **23**, 1–117.

WOOD, S. L., 1982, The bark and ambrosia beetles of North and Central America (Coleoptera: Scolytidae). A taxonomic monograph, *Great Basin Naturalist Memoirs*, **6**, 1–1359.