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Morphometric Study of the Palm Weevils, *Rhynchophorus vulneratus* and *R. ferruginous* (Coleoptera: Curculionidae) in View of Insular and Mainland Populations of Malaysia

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ABSTRACT

A morphometric analysis was conducted on *Rhynchophorus vulneratus* and *R. ferruginous* (Coleoptera: Curculionidae) insular and mainland populations. Twenty-three morphological characters were measured and the data were analysed using independent *t*-test, principal component analysis (PCA) and discrimination function analysis (DFA). Using independent *t*-test, all characters were found to be significant at $p < 0.05$, except distance between eyes (ED), mesotarsus length (F2Ta) and metatarsus length (F3Ta). In PCA, cumulative variations of 80.7% were recorded from the first two principal components, resulting from high loadings in elytra length (EL), elytra width (EW) and pronotum length (PL). For DFA, a single function explained a canonical correlation of 0.952 with 100.0% of variation and the Wilk's Lambda statistics (0.094) was strongly supported with $p < 0.0001$. The highest character loadings were the total length (TL), elytra width (EW) and pronotum length (PL), which were useful as diagnostic characters for separating both *Rhynchophorus* species. Therefore, this study suggests that *R. vulneratus* and *R. ferruginous* are morphologically distinct species. This finding proves that the insular population of *R. vulneratus* fits the generality of the 'island rule' as being larger compared to the mainland counterparts of *R. ferruginous*.

Keywords: Morphometrics, mainland, insular population, *Rhynchophorus*, weevils

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INTRODUCTION

The palm weevils of the genus *Rhynchophorus* (Herbst, 1795) are large insect pests that cause massive destruction to a broad range of palm species (family Aracaceae) including sago, coconut, oil palm and dates worldwide (Hoddle & Hoddle, 2011; Murphy & Briscoe, 1999; Wattanapongsiri, 1966). The red palm weevils, *R. ferruginous* (Olivier, 1790) was reported to be indigenously distributed throughout the Southeast Asia and Melanesia; including Sri Lanka, Thailand, Cambodia, Vietnam, Peninsular Malaysia, Singapore, Sumatra, Java, Boneo, Philippines and Taiwan (Rugman-Jones, Hoddle, Hoddle, & Stouthamer, 2013; Wattanapongsiri, 1966).

A comprehensive taxonomic revision on the genus *Rhynchophorus* by Wattanapongsiri (1966) had separated the two species of the red stripe weevils, *R. vulneratus* and the red palm weevils, *R. ferruginous*, primarily by the shape of pronotum and body coloration. However, the extreme colour polymorphism shared between the two species caught the attention of many taxonomists especially since 1990s (Rugman-Jones et al., 2013). Many taxonomical studies had been conducted including by Hallett, Crespi and Borden (2004), Hoddle and Hoddle (2011), Rugman-Jones et al. (2013), Abad et al. (2014), and Lannino, Sineo, Bianco, Arizza and Manachini (2016), but none could validate the specific status of these pests due to high polymorphism and phenotypic plasticity expressed by different populations.

Apart from that, the occurrence of the red stripe weevils, *R. vulneratus* singularly in Sarawak (Malaysian Borneo) had provided a good natural study site for examining the effect of island rule (van Valen, 1973) towards the body size of *Rhynchophorus* species. Due to the unique features of island environments, distinctive selection pressures led to less predators, relaxed competition and limited food supplies that can yield complex evolutionary trajectories in body size (McClain, Boyer, & Rosenberg, 2006; Palkovacs, 2003; van Valen, 1973).

Animal species inhabiting islands have a smaller body size (dwarfism) among large-sized species, while the opposite, a larger body size (being gigantism) among small-sized species are seen compared with mainland organisms (Lomolino, 2005; van Valen, 1973). However, it is not a conclusive evidence for every single organism, since the discrepancies of findings were reported from other vertebrates, such as in carnivores (Meiri, Dayan, & Simberloff, 2004, 2006) and elephant (Smith et al., 2003).

The objectives of this study were to identify unique characteristics of two *Rhynchophorus* species in Malaysia and to examine whether the size variation follows the generality of the 'island rule'. *R. vulneratus* represented the insular population, meanwhile *R. ferruginous* represented the mainland population. In addition, this study is important to complement the lack of research on Malaysian specimens, especially in Borneo.

MATERIALS AND METHODS

A total of 98 adult specimens consisting of 53 individuals of the red stripe weevils, *R. vulneratus* from Samarahan, Sarawak and 45 individuals of the red palm weevils, *R. ferruginues*, collected from Kuala Nerus, Terengganu was linearly measured. The specimens of *R. vulneratus* from Samarahan were collected from several field samplings while *R. ferruginous* from Terengganu were obtained from the insects collection at Department of Agriculture, Semenggoh (Sarawak) and the Centre for Insect Systematics (CIS), Universiti Kebangsaan Malaysia (UKM).

Identification of field specimens follows the taxonomy suggested by Arnett, Thomas, Skelley and Frank (2002) and Wattanapongsiri (1966). Twenty-three characters (Figure 1) were linearly measured using a digital caliper (Mitutoyo TM) calibrated to 0.01 mm and recorded to two decimal points. Snout length (SL), snout width (SW), scape of antenna length (SAL), antenna length (AL), antenna width (AW), distance between eyes (ED), pronotum length (PL), pronotum width (PW), elytra length (EL), elytra width (EW), total length (without snout) (TL), profemur length (F1L), protibial length (F1Tb), protarsus length (F1Ta), mesofemur length (F2L), mesotibia length (F2Tb), mesotarsus length (F2Ta), metafemur length (F3L), metatibia length (F3Tb), metatarsus length (F3Ta), mesocoxal distance (MSD), metacoxal distance (MTD) and meso-metacoxal distance (MMD) were measured.

All 23 variables were subjected to independent *t*-test to define the significant characteristics among the examined species

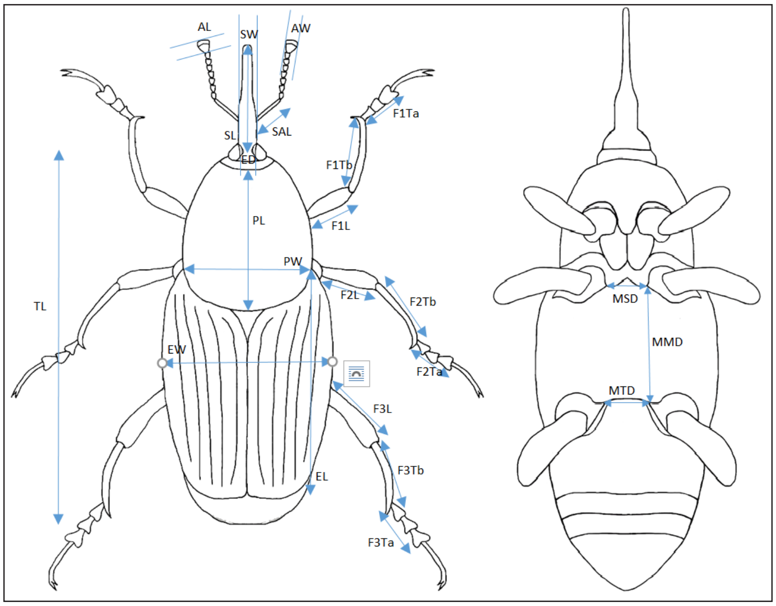

Figure 1. Twenty three morphological characters measured in this study

at *p*-value less than 0.05 (Hair, Rolph, Roland, & William, 1995). The variables were then subjected to Principal Component Analysis (PCA) to enable condensation of data on a multivariate phenomenon into its main, representative features by projection of the data into a two-dimensional presentation (Janžekovič & Novak, 2012) and analysed using Minitab program version 17.1 (Minitab Inc, 2013). The analysis was based on the correlation matrix and the first two components were visualised to facilitate the separation among the two species.

The discriminant function analysis (DFA) was also conducted to define the significant characters for distinguishing *R. vulneratus* and *R. ferruginous*. The analysis was conducted following procedures described by Sazali and Aziz (2012), and Sazali and Juary (2012) using SPSS program version 22.0 for Windows (SPSS Inc., 2017). All character loadings were performed at once to investigate the integrity of pre-defined groups using the measure of distance of Wilk's Lambda, based on generalised squared Euclidean distance that adjusts for unequal variances (Hair et al., 1995). A cross-validation testing procedure was also performed to assess the ability of the predictive model built. Statistical differences are considered significant where *p*-value is less or equals 0.05 (Hair et al., 1995).

RESULTS

Descriptive data from the 23 morphological characters for *R. vulneratus* and *R. ferruginous* is summarised in Table 1. Data was assumed to follow normal distribution as the sample size for each species is greater than 30, which satisfy the normality requirement for further statistical analysis. Using independent *t*-test, all tested variables were significant at $p < 0.05$ which were found useful for differentiating both *R. vulneratus* and *R. ferruginous*. This was followed by principal component analysis (PCA) using all characters before the discriminant function analysis (DFA), except for three characters, namely, distance between eyes (ED), mesotarsus length (F2Ta) and metatarsus length (F3Ta).

In the PCA, the first two principal components showed the most variations among the two species with cumulative variations of 80.7%. Only those with eigenvalues greater than 1.0 were considered for data interpretation. In the first principal component (PC1), the characteristics that showed higher loadings were the elytra length (0.236), elytra width (0.236) and pronotum length (0.234), supported with eigenvalue of 17.815 and 74.7% variation. Meanwhile, for the second principal component (PC2), e higher loadings were due to metafemur length (F3L, -0.498) and antenna length (0.418) with eigenvalue of 1.368 and 5.9% variation. Although the species-grouping is not really obvious as shown in Figure 2 since few samples overlapped, the cumulative variations of more than 80% is proven and supported in the analysis.

Further test of discriminant function analysis (DFA) revealed a single significant function that explains a canonical correlation

Table 1
Summary of the 23 morphological characters measured (mm) in R. vulneratus *and* R. ferruginous *for this study*

Species	*R. vulneratus* (n=53)				*R. ferruginous* (n=45)				*p*
Character	Mean	SD	Min	Max	Mean	SD	Min	Max	
SL	10.7408	1.0917	8.88	13.28	9.1444	0.8513	7.8	11.05	<0.001
SW	1.6891	0.1263	1.41	2.06	1.6127	0.0769	1.40	1.78	0.001
SAL	4.0396	0.4065	3.24	5.04	3.4282	0.2720	2.38	3.98	< 0.001
AL	1.3779	0.0905	1.20	1.58	1.0831	0.0774	0.94	1.25	< 0.001
AW	1.9213	0.1729	1.57	2.33	1.7622	0.1088	1.50	1.97	<0.001
ED	0.9723	0.1255	0.80	1.30	0.9302	0.1017	0.73	1.20	0.075*
PL	12.8749	1.3656	10.10	16.53	11.5109	0.7034	9.11	13.00	< 0.001
PW	10.8866	1.2505	8.45	13.94	9.4089	0.6161	7.58	10.50	< 0.001
EL	16.6117	1.6107	13.15	20.05	14.1091	0.9092	11.50	15.86	< 0.001
EW	13.6032	1.4049	10.71	16.95	11.4389	0.7105	9.28	12.78	< 0.001
TL	31.3221	3.0401	25.84	38.75	28.1244	1.7203	22.77	32.12	< 0.001
F1L	6.6419	0.8911	4.91	9.07	6.2816	0.4960	4.53	7.25	0.018
F1Tb	7.1898	0.8183	5.22	9.26	5.9822	0.4372	4.70	6.93	< 0.001
F1Ta	3.2851	0.3513	2.42	4.08	3.0284	0.2667	2.31	3.62	< 0.001
F2L	7.3857	0.8059	5.66	9.30	6.1387	0.3763	4.93	7.00	< 0.001
F2Tb	5.8389	0.6402	4.44	7.47	4.9862	0.3760	4.14	5.77	< 0.001
F2Ta	3.0738	0.3795	2.25	4.05	2.9498	0.2601	2.27	3.70	0.067*
F3L	8.2860	0.7744	6.51	10.21	6.9000	0.3988	5.40	7.73	< 0.001
F3Tb	6.9877	0.7496	5.17	8.71	5.8822	0.3638	4.82	6.71	< 0.001
F3Ta	2.9155	0.3720	2.20	3.74	2.8600	0.2556	2.25	3.36	0.400*
MSD	2.6457	0.3314	2.02	3.44	2.3031	0.1920	1.78	2.86	< 0.001
MTD	3.2515	0.4146	2.53	4.42	2.9782	0.2555	2.46	3.76	< 0.001
MMD	7.8362	0.8222	5.86	9.68	6.8249	0.4485	5.57	7.76	< 0.001

Note: Snout length (SL), snout width (SW), scape of antenna length (SAL), antenna length (AL), antenna width (AW), distance between eyes (ED), pronotum length (PL), pronotum width (PW), elythra length (EL), elythra width (EW), total length (without snout) (TL), profemur length (F1L), protibia length (F1Tb), protarsus length (F1Ta), mesofemur length (F2L), mesotibia length (F2Tb), mesotarsus length (F2Ta), metafemur length (F3L), metatibia length (F3Tb), metatarsus length (F3Ta), mesocoxal distance (MSD), metacoxal distance (MTD) and meso-metacoxal distance (MMD). * Not significant

of 0.952 which accounted for 100.0% of the variation (Table 2). The Wilk's Lambda statistics of 0.094 was supported with a highly significant function ($p < 0.0001$) (Table 3). Meanwhile, the standardised canonical discriminant function coefficient (Table 4) shows the highest character loadings observed for the model, namely total length (TL), followed by elytra width (EW) and pronotum length (PL).

The discriminant function scores for each group are clearly separated for both *R. vulneratus* and *R. ferruginous* in which all the examined parameters were useful in

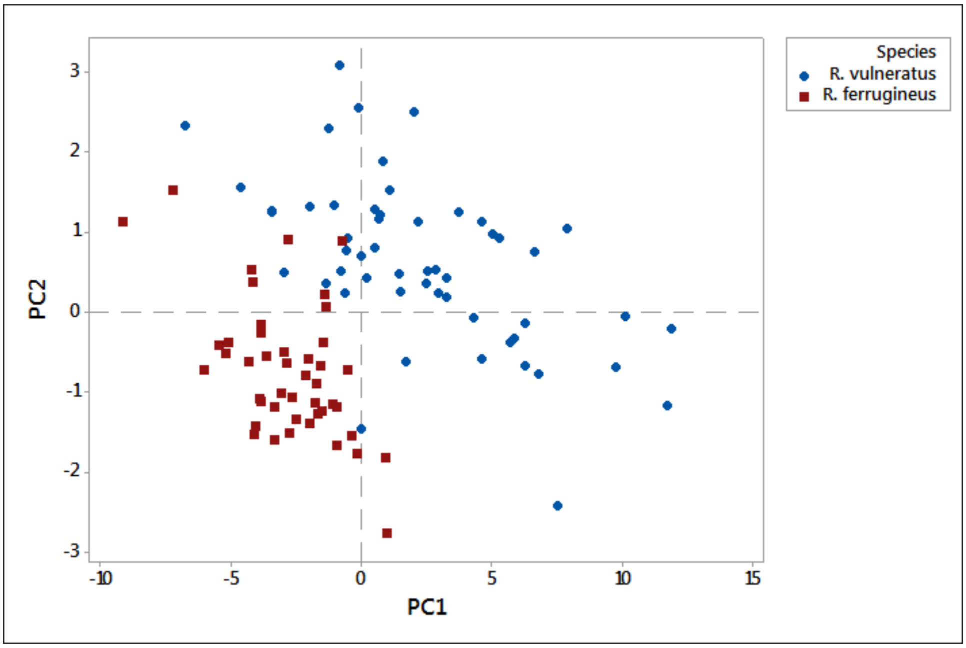

Figure 2. Principal component plot (PC1 vs PC2) of *R. vulneratus* and *R.ferrugineus*

Table 2
Eigenvalues for the DFA

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	9.588[a]	100.0	100.0	0.952

Note. [a] First 1 canonical discriminant functions were used in the analysis

Table 3
Wilks' Lambda values for the DFA

Test of Function(s)	Wilk's Lambda	Chi-Square	df	Sig.
1	0.094	202.939	20	0.000

separating both species as shown in Figure 3. Through a predictive group membership procedure, a perfect score of 100.0% of original group cases correctly classified into each respective species group, whereas in the cross-validating test, 98.0% of cases were correctly classified (data not shown). Hence, this outcome strongly suggests a valid species status of *R. vulneratus* and *R. ferruginous*.

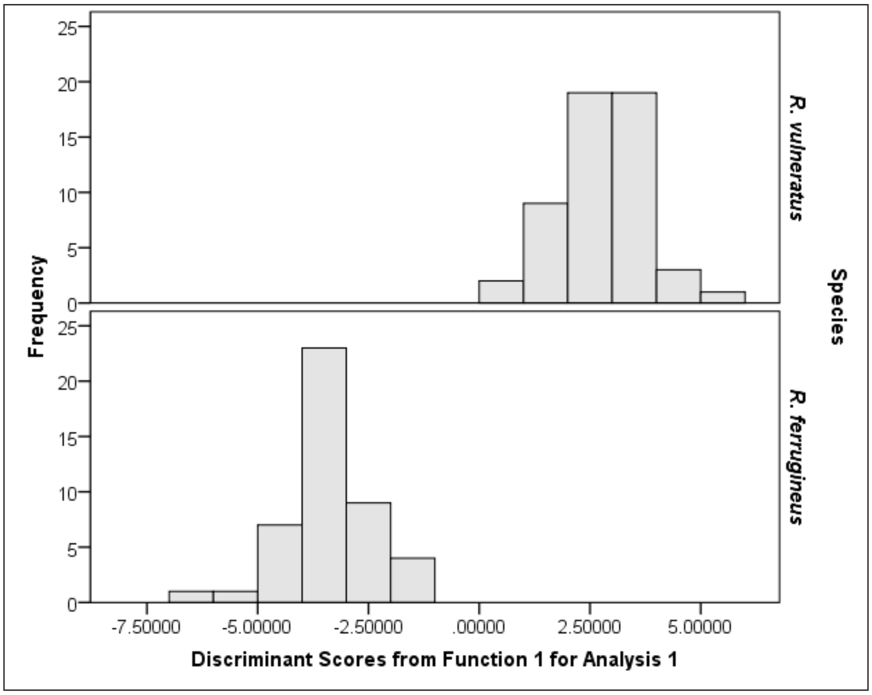

Figure 3. Separate-group histograms of discriminant function scores for *R. vulneratus* and *R. ferruginous*

Table 4
Standardised canonical discriminant function coefficients for the 20 characters

	Function 1
SL	1.347
SW	-0.493
SAL	-0.070
AL	0.337
AW	0.021
PL	-1.583*
PW	0.163
EL	1.276
EW	1.769*
TL	-2.286*

Table 4 *(continue)*

	Function 1
F1L	-0.809
F1Tb	1.198
F1Ta	-0.107
F2L	0.473
F2Tb	-0.281
F3L	0.134
F3Tb	0.176
MSD	0.137
MTD	-0.856
MMD	-0.102

Note: *Diagnostic character(s) with high coefficient in Function 1

DISCUSSION

To date, the red palm weevils are taxonomically still considered as a single species of *R. ferruginous* comprising other colour-morph type of R. *vulneratus* (Hallett et al., 2004; Rugman-Jones et al., 2013). It is important to understand the specific status of both colour morphs species because the red palm weevils have become a severe threat to date plantation and production, ornamental landscape plants and native palm trees worldwide (Rugman-Jones et al., 2013).

In both principal component analysis (PCA) and discriminant function analysis (DFA), the differences between *R. vulneratus* and *R. ferruginous* were found in the elytra width (EW) and pronotum length (PL). According to Wattanapongsiri (1966), the two species can be distinguished based on the shape of their pronotum and gular suture. In *R. vulneratus*, the pronotum is strongly and narrowly curved anteriorly, whereas in *R. ferruginous*, its pronotum is curved at the sides and it has a more uniform anterior (Wattanapongsiri, 1966). Meanwhile for the gular suture, the shape is more concave at both sides before reaching the base of rostrum in *R. vulneratus*, whereas in *R. ferruginous*, the shape is oval at base but less concaved than the former species (Wattanapongsiri, 1966).

However, the description by Wattanapongsiri was not informative enough as *Rhynchophorus* species expressed high polymorphism and phenotypic plasticity across a wide geographical distribution (Abad et al., 2014; Lannino et al., 2016; Rugman-Jones et al., 2013) leading to its taxonomic ambiguity. Phenotypic plasticity refers to changes in phenotypes and morphology induced by different surroundings and environment (DeWitt & Scheiner, 2004; West-Eberhard, 2003; Whitman & Ananthrakrishnan, 2009). Additionally, these changes are beneficial to insects for increasing survival, fecundity, fitness, population density and species range.

Moreover, the origin of specimens examined here has provided a platform to generally review the 'island rule' as proposed by van Valen (1973), who reported the body size variation in insular population of mammals. Many studies had been conducted to review the generality of the 'island rule' on vertebrates (Lomolino et al., 2013) but only one known study had been reviewed for invertebrate, particularly in tenebrionid beetle by Palmer (2002).

The size divergent of body and shape of wings in natural populations may represent adaptive modification due to island environments or habitat heterogeneity (Lee & Lin, 2012). Even when the geographical effect is considered, the evolutionary divergence of insects does not exactly follow the pattern of neutral evolution indicated by the genetic marker (Lee & Lin, 2012). This was the case study by Lee and Lin (2012) who proposed that morphological variations resulted from the morphology and wing shape of damselflies, *Euphaea formosa* and *E. yayeyamana* (Odonata: Euphaeidae) are most likely corresponded to natural and sexual selection for fitness optimization. Palmer (2002) in his study on tenebrionid beetle, *Asida planipennis* (Coleoptera: Tenebrionidae) also stated that size has evolved by selection related to site-specific environmental factors linked with island area.

The overall body size of *R. vulneratus* is relatively larger than *R. ferruginous* from the two localities (Table 1). Wattanapongsiri (1966) noted the considerable variation in size for the two species was due to food availability and limitation during the larval stage, which later affected the adult form. Being larger in size will give benefits to *R. vulneratus* as it provides greater fecundity in females, access to mates among males and advantages of resource sequestration (Blanckenhorn, 2000; Honik, 1993; Lighton, Quinlan, & Feener, 1994; Parker & Simmons, 1994; Rivero & West, 2002). In contrast, being smaller in size to *R. ferruginous* may reduce viability costs of growth and development, enhance agility and reduced detectability, minimise maintenance costs of energy, lower heat stress and costs of reproduction and increase scramble competitive ability (Blanckenhorn, 2000, 2005; Moya-Lara*ño, El-Sayyid, & Fox, 2007).*

As a matter of geographical concern, the chosen localities for *R. vulneratus* and *R. ferruginous* in the present study was believed to provide no beneficial impact towards the development of the weevils' larva, as the sampling sites for both species were not situated or located near any large plantation area that could give plenty of food supply. In fact, the sites were surrounded by human settlements and can be categorised as disturbed areas. Therefore, the effect of size variation in regards to possible consequence of food availability from the sites can be ignored or at least minimised.

The insular population of *R. vulneratus* was collected from the UNIMAS East Campus, Samarahan Division of Sarawak. The campus and its surroundings are developing areas, constructed from peat lands and surrounded by a secondary forest and are in close proximity to many human settlements. As a developing sub-urban, parts of Samarahan are transformed into agricultural lands for plantation of oil palm, coconut, pineapple, banana, paddy and vegetables farms, be it commercially cultivated or as a small-scale business managed by local people. However, the occurrence of *R. vulneratus* in these plantation areas are hardly detected and there is no established documentation that particularly report the damages caused by this red stripe weevil in the state, unless found abundantly in the sago plantations which is not commercially cultivated in this district.

Meanwhile, the mainland population of *R. ferruginous* was collected from Seberang Takir, located in Kuala Nerus District of Terengganu. The sampling site is a non-commercial coconut plantation situated nearby fisherman villages. Being one of the most destructive insect pests of palms (Aracaceae) in the world (Rugman-Jones et al., 2013), this red palm weevil was first reported by the Department of Agriculture (Terengganu) when many of their coconut trees lose their leaf crowns at Rhu Tapai, Setiu (Wahizatul, Zazali, Abdul, & Nurul, 2013). Indeed, this weevil was never being reported as a lethal pest of coconut in the country (Murphy & Briscoe, 1999), hence the invasion of *R. ferruginous* in Terengganu had urged the authority to take immediate actions to control the weevils before it could become aggressive enough to affect the coconut and oil palm industry in Malaysia, which greatly contributes to the country's economic growth (Wahizatul et al., 2013).

As initially suggested by van Valen (1973), the insular population of the red stripe weevils, *R. vulneratus* fits the generality of the 'island rule', as being larger compared with the mainland counterparts of the red palm weevils, *R. ferruginous*. The larger size of *R. vulneratus* may be due to decreased competition or predators (Lomolino, 1985) in the Bornean island, since *R. ferruginous* is a quarantine species in Sarawak (Department of Agriculture, personal communication, 2013). It survives due to abundance of food resources, especially sago trees. Meanwhile, the reduction of body size in *R. ferruginous* might be related to resource limitation (Lomolino, 1985) in Peninsular Malaysia, as the competition with its sibling species, *R. vulneratus* and other insect pests, is also expected.

CONCLUSION

The findings of this study suggest that the red stripe weevils, *R. vulneratus* and the red palm weevils, *R. ferruginous* are morphologically distinct species. The locality's selection of Kota Samarahan, Sarawak and Seberang Takir, Terengganu was believed to satisfactorily represent the nature of island and mainland populations. As an insect, the insular population of *R. vulneratus* in Sarawak is relatively larger in size compared with the mainland population of *R. ferruginous* in Peninsular Malaysia, which adhered to the generality of 'island rule' proposed by van Valen (1973).

However, future studies should look other study sites to examine the morphometric variation across the latitudinal or altitudinal gradients. Other possible factors including climatic condition, annual mean temperature, sexual size dimorphisms and island or mainland size should be included for detailed assessment.

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