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Population variation of the red stripe weevils, *Rhynchophorus vulneratus* (Coleoptera: Curculionidae) isolated by geographical limit

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Abstract. Morphological variation of the red stripe weevils, *Rhynchophorus vulneratus* (Coleoptera: Curculionidae) was investigated using three different populations from Kota Samarahan (Sarawak), Mukah (Sarawak), and central of Peninsular Malaysia. A total of 145 individuals were morphologically examined in which 23 morphological characters of head, body and legs were linearly measured. All data were analysed using Principal Component Analysis in Minitab Version 17.1. The first three principal components explained 82.5% of variance with eigenvalues greater than 1.0 and the principal component plot of PC1 against PC2 showed the clustering of the three populations, in which Mukah population forms its own cluster whereas Kota Samarahan and Peninsular Malaysia populations show overlapping clusters. The morphometric findings indicated that the pronotum and elytra were the most diagnostic characters for discriminating the three examined populations. This suggests that the populations of *R. vulneratus* might have undergone adaptive changes on its morphological characters possibly due to different environmental factors including the availability of food resources, habitat types,and human activities and this requires further testing.

Key words. variation, morphometrics, *Rhynchophorus vulneratus*, Principal Component Analysis (PCA)

INTRODUCTION

Variations can be observed through differences among individuals within a population and also between members of different species that involved alteration and displacement of some morphological key characters. According to Ferreira & McKinlay (1999), variation is a necessity in biology and is the most important feature of evolutionary theory. Divergences occurring within a population is considered as a vital point for speciation (Balon, 1993; Margurran, 1998), crucial for understanding the basis of biodiversity processes (Alibert et al., 2001) as well as microevolution (Garnier et al., 2005). In some studies of the ground beetles (Carabidae), morphology seems to be more informative than genetics, where significant morphological divergences may be detected despite little differences in molecular markers (Palestrini et al., 2012).

Variations described from morphological characters are associated with many factors including geographical areas, resource availability, nutrition quality, competition and temperature (Angilletta & Dunham, 2003; Sota et al., 2007; Chown & Gaston, 2010; Laparie et al., 2010). These factors however may vary across different habitats due to different surroundings and environmental conditions for particular insects (Laparie et al., 2010). As a consequence, polymorphism and phenotypic plasticity are evident in insects, in response to environmental change and surroundings. These alterations and variations subsequently increase survivability, fecundity, fitness, population density and species range (West-Eberhard, 2003; DeWitt & Scheiner, 2004; Whitman & Ananthakrishnan, 2009).

Many population studies had been conducted in insects to show morphological variations based on different geographical areas, habitat types and food availability (see Laparie et al., 2010; El-Mergawy et al. 2011a, 2011b; Tambe et al., 2013; Rugman-Jones et al., 2013; Hassan et al., 2017). Studying a particular group within a small geographical area may also indicate the ability of this population to utilise different resources at the same time (Selander, 1966; Daly & Wilson, 1983; Kalaisekar et al., 2012). The outcomes are beneficial to understand the adaptation shown by this local population, which promotes diversity in insect morphology and thus possibly lead to speciation (Sota et al., 2007). As a result, recognition of diagnostic morphological character(s) at the population level is essential to give better insights into the study population.

In this study, the morphological variation among different populations of the red stripe weevil, *Rhynchophorus vulneratus* is investigated. The adults are large-sized insects ranging from about 30 to 45 mm in body length. The overall body coloration is black with a visible red stripe in the middle of its pronotum. The sexual dimorphism in *R. vulneratus* is

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

Locality	Kota Samarahan (n=81)				Mukah (n=49)				Peninsular Malaysia (n=15)			
Character	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**
Snout length	10.5035	1.1781	7.71	13.28	10.7304	0.5550	9.61	12.21	10.7167	1.4198	7.81	12.69
Snout width	1.6672	0.1349	1.34	2.06	1.7598	0.0870	1.53	1.96	1.6833	0.1412	1.43	1.96
Scape of antenna length	3.9235	0.4414	2.75	5.04	4.1253	0.2287	3.69	4.64	4.0747	0.5035	3.25	4.91
Antenna length	1.3665	0.1014	1.03	1.59	1.7038	0.3223	1.24	2.21	1.3753	0.1147	1.21	1.58
Antenna width	1.8904	0.1793	1.47	2.33	1.5719	0.2966	1.19	2.11	2.0380	0.1981	1.65	2.29
Distance between eyes	0.9623	0.1269	0.72	1.30	1.0604	0.1308	0.82	1.36	1.0420	0.1705	0.76	1.37
Pronotum length	12.6104	1.4375	8.49	16.53	13.3676	0.6260	11.89	14.95	12.7667	1.6883	9.18	14.92
Pronotum width	10.6098	1.2604	7.05	13.94	11.2092	0.4894	10.18	12.48	10.8340	1.4228	8.12	12.66
Elytra length	16.2225	1.7235	10.93	20.05	16.9000	0.7919	15.39	19.01	16.5933	1.9687	12.56	19.30
Elytra width	13.2958	1.4782	8.74	16.95	13.9096	0.6106	12.42	15.36	13.5347	1.7527	9.92	15.93
Distance between mesocoxae	2.5490	0.3717	1.34	3.44	2.6327	0.2193	2.03	3.05	2.6707	0.3858	1.88	3.39
Distance between metacoxae	3.1874	0.4224	2.16	4.42	3.3261	0.2596	2.93	3.96	3.4393	0.5104	2.47	4.42
Distance between meso-metacoxae	7.6596	0.8720	5.24	9.68	7.9147	0.4604	6.66	8.98	7.9267	1.0276	5.77	9.03
Profemur length	6.5789	0.9523	4.67	9.09	6.9718	0.4904	6.02	8.25	6.9480	0.9662	5.17	8.26
Protibia length	6.9999	0.8408	4.83	9.26	7.6016	0.5712	5.52	8.84	6.9573	0.8211	5.27	8.76
Protarsus length	3.2284	0.3885	2.11	4.36	3.5209	0.2558	2.75	4.17	3.1907	0.3161	2.57	3.70
Mesofemur length	7.2257	0.8169	4.88	9.30	7.7643	0.3725	6.82	8.79	7.4267	0.7988	5.93	8.99
Mesotibia length	5.7344	0.6764	3.97	7.56	6.1416	0.3579	5.41	7.11	5.6886	0.6984	4.27	7.08
Mesotarsus length	3.0268	0.4181	2.02	4.34	3.4100	0.2550	2.70	3.84	2.9479	0.3329	2.22	3.57
Metafemur length	8.1305	0.8722	5.58	10.61	8.6098	0.3946	7.71	9.44	8.2340	0.9566	6.22	9.60
Metatibia length	6.8678	0.7762	4.84	8.93	7.3188	0.4017	6.40	8.45	6.8740	0.7311	5.62	8.27
Metatarsus length	2.9173	0.3763	2.20	4.18	3.2115	0.2315	2.50	3.63	2.9146	0.3814	2.06	3.69
Total length	30.7429	3.2664	20.72	38.75	31.8741	1.4630	28.04	35.63	31.2228	2.8920	24.06	36.11

expressed through males having a shorter snout compared to females (Hill & Abang, 2005). In females, the longer snout aids the drilling of deeper holes in seeds and fruits for oviposition (Tung, 1983).

R. vulneratus is reported as a native species throughout Southeast Asia including Thailand, Peninsular Malaysia, Singapore, Borneo, Indonesia, Philippines and additionally, Japan (Murphy & Briscoe, 1999; Hill & Abang, 2005; Rugman-Jones et al., 2013). As it is widely distributed, this pest exhibits high polymorphism and is extensively studied due to its taxonomic ambiguity with *R. ferrugineus* (Hallett et al., 2004; Rugman-Jones et al., 2013). It is known to be one of the most damaging pests to many palms such as coconut, oil palm, and sago palm (Murphy & Briscoe, 1999; Hill & Abang, 2005). However, the presence of this pest species locally is not of a major concern, since the damage caused by its larvae to sago and palm trees is not at a severe level. In Sarawak (Malaysian Borneo), the larvae of *R. vulneratus* serves as one of the protein sources consumed by the natives. It can be easily found where the sago plantations are traditionally or commercially cultivated. In the present study, three localities were selected comprising of Kota Samarahan and Mukah divisions from Sarawak, and another population representing the central region of Peninsular Malaysia. The objective was to examine the population variation using external morphological characters as well as to document the phenotypic plasticity from different localities in Malaysia. Additionally, some potential factors of environment and surroundings including food availability, habitat types, human activities and disturbance effect are also discussed.

MATERIAL AND METHODS

A total of 145 adult specimens comprising of 81 individuals from Kota Samarahan (Sarawak), 49 individuals from Mukah (Sarawak) and 15 individuals from Pulau Gadong (Melaka) and Chembong (Negeri Sembilan) were morphologically examined. The sampling site for Kota Samarahan was located in the UNIMAS east campus, surrounded by peat swamp and secondary forests, whereas the sampling site for Mukah population was conducted at Kampung Ladang Teh, which is known to be the center of sago production within the state. Specimens from Pulau Gadong and Chembong were obtained from the insect collection at Department of Agriculture (Kuala Lumpur) where no further details about the specific sampling sites were provided. Hence in this present study, it is assumed that both localities were situated nearby the urban area.

For the locality coding, Kota Samarahan was denoted as locality (1), Mukah as locality (2) and specimens from Pulau Gadong and Chembong were combined to represent the central region of Peninsular Malaysia as locality (3). The specimens representing Sarawak's populations were baited using freshly cut sago trunk left for four to seven days, the adult specimens were collected by hand and later deposited at UNIMAS Zoological Museum. Identification of field specimens, except samples from Pulau Gadong and Chembong followed the taxonomic key provided by Wattanapongsiri (1966) and Arnett et al. (2002). The date of collection, habitat or vegetation (surrounding) at time of collection, host plants the specimens were collected from (if any), and detailed locations were recorded as reference. In this study, both males and females for each population were combined as the aim was to differentiate the populations from different localities.

Twenty-three characters were linearly measured using a digital caliper (Mitutoyo TM) calibrated to 0.01 mm and recorded to two decimal points, following descriptions provided by Chown (1989) and Sazali et al. (2018). The characters involved were the snout length, snout width, scape of antenna length, antenna length, antenna width, distance between eyes, pronotum length, pronotum width, elytra length, elytra width, total length (without snout), profemur length, protibia length, protarsus length, mesofemur length, mesotibia length, mesotarsus length, metafemur length, metatibia length, metatarsus length, distance between mesocoxae, distance between metacoxae and distance between meso-metacoxae.

All data were assumed to have a normal distribution as the number of samples for each population exceeds 30, except for the population of Peninsular Malaysia with only 15 samples. In order to avoid bias due to possible outliers presented prior to the analysis, normality tests were conducted exclusively on this under-represented population where all characters were subjected to Q-Q distribution plots test and validated using Shapiro-Wilks methods.

The data were then analysed using principal component analysis (PCA). The analysis was conducted using Minitab version 17.1 (Minitab Inc., 2013), 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). The analysis was based on the correlation matrix and the first two components were visualised to facilitate the separation among the three populations.

RESULTS

The data from the 23 morphological characters were summarised as mean length ± standard deviation (in mm) following respective localities as shown in Table 1. Normality of the data were satisfied for each character across different populations as Q-Q distribution plots and Shapiro-Wilks methods confirmed the test and hence the samples were subjected to further statistical analysis (data not shown). In the PCA, the analysis showed the first three principal components that showed the most variations among the three populations, resulted in cumulative variations of 82.5%.

The component load for each principal component is shown together with respective eigenvalue and percentage of variation in Table 2. In the first principal component

Table 2. The first three principal components.

Character	**PC1**	**PC2**	**PC3**
Snout length	0.207	0.201	*–0.338
Snout width	0.220	–0.075	0.185
Scape of antenna length	0.231	0.098	0.026
Antenna length	0.066	*–0.548	*–0.339
Antenna width	0.064	*0.582	0.248
Distance between eyes	0.120	–0.162	–0.258
Pronotum length	*0.246	0.037	–0.015
Pronotum width	0.235	0.094	–0.146
Elytra length	*0.243	0.082	–0.094
Elytra width	*0.246	0.085	–0.103
Distance between mesocoxae	0.176	0.117	*–0.340
Distance between metacoxae	0.210	0.143	–0.186
Distance between meso-metacoxae	0.234	0.150	–0.111
Profemur length	0.205	–0.020	0.052
Protibia length	0.218	–0.142	*0.372
Protarsus length	0.190	–0.194	0.148
Mesofemur length	0.233	–0.055	0.188
Mesotibia length	0.222	–0.132	0.325
Mesotarsus length	0.188	–0.226	0.021
Metafemur length	*0.242	–0.005	0.126
Metatibia length	0.232	–0.081	0.216
Metatarsus length	0.185	–0.217	–0.016
Total length	0.237	0.123	-0.193
Eigenvalue	15.540	2.159	1.274
% variation	67.6	9.4	5.5
% cumulative	67.6	77.0	82.5

* Diagnostic characters with high loadings in each principal component.

(PC1), the characteristics that showed higher loads were the pronotum length (0.246), elytra width (0.246), elytra length (0.243) and metafemur length (0.242), supported with eigenvalue of 15.540 and 67.6% variation. For the second principal component (PC2), the higher loads were resulted from the antenna width (0.582) and antenna length (–0.548) with eigenvalue of 2.519 and 9.4% variation. Furthermore, in the third principal component (PC3), the higher loadings were observed in the protibia length (0.372), mesocoxal distance (–0.340), antenna length (–0.339) and snout length (–0.338) with eigenvalue of 1.274 and 5.5% variation. Based on the principal component plot (Fig. 1), the Mukah population formed its own cluster whereas populations of Kota Samarahan and Peninsular Malaysia showed overlapping clusters.

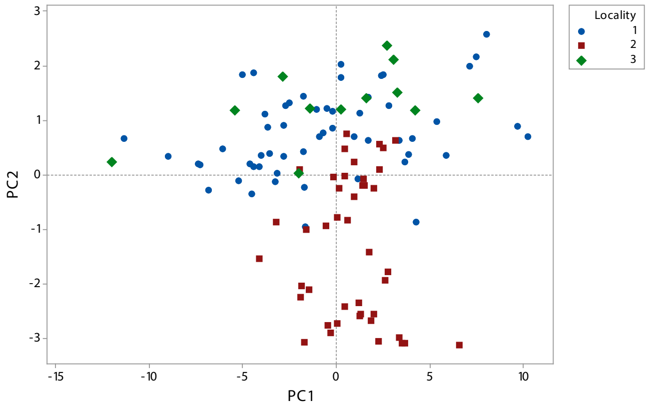

Fig. 1. Principal component plot (PC1 vs PC2) of *R. vulneratus* populations from Kota Samarahan (1), Mukah (2) and central region of Peninsular Malaysia (3).

Overall, the Mukah population showed the largest sizes (in mm; Table 1) for most of the characters, especially in pronotum length (Mukah = 13.37 ± 0.63; Samarahan = 12.61 ± 1.44; Peninsular Malaysia = 12.77 ± 1.69), elytra width (Mukah = 13.91 ± 0.61; Samarahan = 13.30 ± 1.48; Peninsular Malaysia = 13.53 ± 1.75), elytra length (Mukah = 16.90 ± 0.79; Samarahan = 16.22 ± 1.72; Peninsular Malaysia = 16.59 ± 1.97) and metafemur length (Mukah = 8.61 ± 0.39; Samarahan = 8.13 ± 0.87; Peninsular Malaysia = 8.23 ± 0.96).

DISCUSSION

Due to the condition of the voucher specimens, the morphometrics study was restricted to only external morphological characters where the measurement readings can be taken. Thus, the genitalia was deliberately omitted and not measured in this study. A study by Wahizatul et al. (2012) on the red palm weevils *R. ferrugineus*, found evidence that the body and pronotum sizes between males and females of the red palm weevils were generally similar. Besides that, Abad et al. (2014) also suggested that sexual dimorphism is not a factor in size variation of the red palm weevils, *R. ferrugineus* from central and southern populations in the Philippines. A multivariate study by van Rensburg et al. (2003) on the weevil species of the genus *Ectemnorhinus* suggested that both sexes could be combined in their statistical analysis since sexual dimorphism in this species was not significant. We observed the same case in our study and did not pick up size dimorphism between the sexes.

The variation of body sizes among different localities might indicate that different environmental conditions during their juvenile development, such as variation with respect to food availability or quality, larval density, abiotic factors, predations or other related factors are present (Tambe et al., 2013). These factors were not quantitatively measured here and requires further testing. The relative abundance of the species in each of the localities does suggest that they are adapting well to each of the respective modified environments.

In the present study, the Mukah population (2) was generally larger in size for most characters except smallest antenna width and having moderate distance between mesocoxae, distance between metacoxae and distance between meso-metacoxae. These changes were not directly proportionate to their overall body dimensions but this finding is in accordance with Macagno et al. (2011) who mentioned that the interspecific divergences of body size and relative size in dung beetles do not necessarily evolve in concert. Palestrini et al. (2012) also proved that the smallest species of *Nebriola fontinallis* did not possess the smallest external characters, whereas the largest subspecies of *N. cordicollis winkleri* did not have the largest elytron. The allometric size variation observed here suggests that there may be differential constraints present during development as a response to the local environment respectively. At this point it is unclear if the observed size differences is purely an expression of the phenotypic variation of the species, or an adaptative response to the local environment based on novel mutations, or a combination of both.

There is no significant differences in between the two populations of *R. vulneratus* from Kota Samarahan and central region of Peninsular Malaysia. It is of interest to acknowledge the similarity of their body sizes, which might have resulted from similar environmental conditions and surroundings or mixture of genetic stocks due to possible transplantations. Future genetic studies may help determine whether the morphological similarities observed here are due to shared ancestry or convergent evolution. On the other hand, it is postulated here that the abundance of food resources in Mukah's sago plantations has led to the size increment of the local red stripe weevils. Mukah is a suburban area in the state, known as the main area for sago plantation in Sarawak, contributing to the economic growth in the local and domestic industry. The population of this pest is under control and never reached the economic threshold that affect sago production. Despite being one of the agricultural pests, the larvae of *R. vulneratus* is of local people's interest as it serves as a protein source and it is marketable. This in fact helps to control the population of *R. vulneratus* and thus, its presence does not significantly affect the production of sago starch in that particular area. This unintentional control measure may likely be reducing the population density such that food resources are not limiting, allowing for maximum growth potential for the local red stripe weevils. If true, our hypothesis predicts that under similar circumstances, we can expect comparable increments in body size in other types of plantations whereby population densities are not strained. Further investigations in the population density thresholds present in this species can provide insights on the key factors regulating their growth rates. The natural inference of whether larger sizes leads to greater fecundity in this species is unknown and deserves further study as well.

In Kota Samarahan, the UNIMAS east campus is situated in a rapidly developing area for many housing settlements and urbanisation. Many areas of the peatland were also converted for agricultural purposes for oil palm and other plantations such as rubber, sago, coconut, cocoa and other short term crops (Ipor, 2006). Similar to Mukah, to date, there is no report of infestations caused by *R. vulneratus* in any of these plantations in Sarawak. Due to the smaller scale of cultivation present in Kota Samarahan, mixed with increasing urban development, limitation of food resources might explain the lack of corresponding results with that of Mukah. A more quantitative approach will need to be conducted to verify the differences recorded here.

From this study, the pronotum and elytra were found to be significant diagnostic characters that are effective for discriminating Mukah population from Kota Samarahan and central of Peninsular Malaysia. As found in other insects, the elytra was useful to indicate size variation for other coleopteran species such as the ground beetles, *Carabus nemoralis* (Carabidae; Blanckenhorn & Demont, 2004), the stripe flea beetles, *Phyllotreta striolata* (Chrysomelidae; Blanckenhorn & Demont, 2004), the seed-feeding beetles, *Stator limbatus* (Chrysomelidae; Stillwell et al., 2007) and the seven-spotted ladybird beetles, *Coccinella septempunctata* (Coccinellidae; Kalaisekar et al., 2012). However, further studies should be conducted to validate the effect of this variations in regards to the availability of food resources, habitat types and human disturbance.

CONCLUSIONS

Using linear measurement of twenty three morphological characters, the morphological variation of the red stripe weevils, *R. vulneratus* was evident from different populations of Kota Samarahan (1), Mukah (2) and central region of Peninsular Malaysia (3). Phenotypic plasticity is most evident in the red stripe weevils, *R. vulneratus* in the form of elytral, pronotal, metafemoral and antennal length, in response to different habitats or surroundings.

The population differences of this pest species might result from different environmental factors such as the availability of food resources during larval growth stage and development, habitat types, the intensity of human activities and disturbance effect. Morphometry is a reliable method to test for the phenotypical variations between populations of *R. vulneratus*. However, other possible factors including genetic alterations of the populations and additional testing such as designated experiment on feeding and diets should be also examined, in order to further support the morphometrics findings.

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