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Author(s): G. Manjeri R. Muhamad Q. Z. Faridah and S. G. Tan
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Morphometric Analysis of *Oryctes rhinoceros* (L.) (Coleoptera: Scarabaeidae) from Oil Palm Plantations

G. Manjeri and R. Muhamad
Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia
43400 UPM Serdang, Selangor, MALAYSIA
manjeri_g@yahoo.com; ritamuhamad@yahoo.com

Q. Z. Faridah
Department of Biology, Faculty of Science, University Putra Malaysia
43400, Serdang, Selangor, MALAYSIA
faridahqz@gmail.com

and

S. G. Tan
Department of Cell and Molecular Biology, Faculty of Biotechnology and Molecular Sciences, Universiti Putra Malaysia
43400 UPM Serdang, Selangor, MALAYSIA
sgtan_98@yahoo.com

Abstract

Morphometric analysis was performed on the oil palm pest *Oryctes rhinoceros* (L.) as part of a study to determine the possibility of a cryptic species complex occurring in this organism. Six beetle populations, each with a sample of 30 individuals, were examined. The morphometric variables measured were total body length, elytral length, pronotal length, pronotal width, and length of the cephalic horn. Principal component analysis and canonical discriminant analysis indicated no distinct discontinuity or clustering of populations. The morphology of individuals from different populations was observed to overlap on one another. These results indicate that *O. rhinoceros* show stability in morphometric character variance across a wide geographical range.

Key Words: rhinoceros beetles, morphology, cryptic species complex, Asia

Oryctes rhinoceros (L.), commonly known as the rhinoceros beetle, is a pest of interest in oil palm plantations. The adults feed on the unopened fronds of young palms in replanting sites, inflicting serious damage which could lead to crop loss. Presence of the pest in replanting sites has been recorded as early as six months after replanting (Kamarudin and Wahid 1997). Being a nocturnal organism with a gregarious feeding habit, it is difficult to eliminate this pest (Young 1986). Attacks by this beetle have resulted in major crop losses in many oil palm plantations and studies have revealed high rates of damage (Liau and Ahmad 1991; Samsuddin *et al*. 1993; Kamarudin and Wahid 1997; Chung *et al*. 1999).

Pheromone trapping is currently the most common procedure to reduce infestations of the beetle. A species–specific aggregation pheromone is placed in young oil palm replanting sites to trap and monitor the beetles (Hallet *et al*. 1995). However, not all populations of *O. rhinoceros* in the field were observed to be attracted by it. Therefore, based on the discrepancy in attraction toward the species-specific pheromone, there is a possibility for the occurrence of a cryptic species complex in this insect. Morphometric analysis was the first step taken to investigate this notion and to further understand the population structure of *O. rhinoceros* beetles. Morphometrics, the quantitative analysis of shapes, is based on measurements of an organism's morphological structures (Mehrparvar *et al*. 2012). To date, morphometric techniques have been successfully applied to differentiate closely related species, populations, and biotypes in various insects (Pungerl 1986; Barari *et al*. 2005; Barahoei *et al*. 2011). The aim of this study was to ascertain if characteristic morphometric variations could be observed within and among *O. rhinoceros* populations.

Material and Methods

Study Location and Sample Collection. Samples of *O. rhinoceros* beetles were collected from young oil palm replanting sites. Four locations were chosen

for this study, with three situated in Peninsular Malaysia and one in Sumatra, Indonesia. The specific locations were Felcra Berhad in Perak, Tennamaram Estate in Selangor, Kuantan Trading Plantation in Pahang, and Paya Pinang Plantation in Medan, Sumatra. Sample collections at all sites were carried out using two procedures: Rothamstead light trapping and pheromone trapping using Sime RB pheromone lures. The distance between both traps was less than 15 m at all the four study sites. Beetles trapped via the different procedures were categorized as different populations. Therefore, at each study location, two populations were collected. Here after, the beetle populations are coded based on the location and trapping procedure; thus, Selangor pheromone (SP), Selangor light (SL), Perak pheromone (PP), Perak light (PL), Pahang pheromone (PaP), and Medan pheromone (MP). No beetles were captured in the Pahang and Medan light traps.

Morphometric Measurement. Morphometric measurements were taken on 30 beetles from each population. Care was taken to measure only intact individuals to ensure accurate values. Measurements were taken using a digital Mitutoyo caliper at 0.01 mm precision. Five variables were measured. Total body length was measured along the midline from the clypeal apex to the apex of the longest elytron. Elytral length was measured along the midline from the anterior margin to the apex of the longest elytron. Pronotal length was measured along the midline from the anterior margin to the posterior margin. Pronotal width was the greatest transverse width measured between the anterior pronotal angles. Length of cephalic horn was measured from base to apex (Cook *et al*. 2006).

Statistical Analysis. Pearson's correlation coefficient, principal component analysis, and discriminant analysis in SAS software version 9.0 (SAS Institute, Inc. 2002) were used to determine whether there was sexual dimorphism in measured variables for each population, and whether or not each beetle population formed a distinct unit.

Results

Correlation between Morphometric Variables and Sex of *O. rhinoceros*. Prior to multivariate analysis, the interaction between variables and sexes due to sexual dimorphism among species was assessed. No significant correlation existed between male and female beetles for each of the measured variables (Table 1). Therefore separation of beetles according to sex was not necessary for the subsequent principal component analysis and discriminant analysis.

Table 1. Pearson correlation parameters for each of five morphometric variables comparing sexes of *Oryctes rhinoceros.*

Variables	*r*	*P*-value
Elytral length	−0.10287	0.3346
Pronotal length	−0.03981	0.7095
Length of cephalic horn	0.10798	0.3111
Pronotal width	0.04382	0.6817
Total body length	−0.03828	0.7202

Principal Component Analysis. Based on principal component analysis, five principal components were extracted, however, only the first two components accounted for the majority of the total variance (Table 2). In addition, all five variables were well represented in the first two principal components based on their loading values. Principal Component 1 (PC1) had an eigenvalue of 3.0940 which explained 61.9% of total variation in the *O. rhinoceros* populations. In this component, all variables indicated a reasonably high loading value; however, three variables, total body length, pronotal width, and pronotal length, showed the highest loading values. Principal Component 2 (PC2) accounted for only 15.0% of the variance, with an eigenvalue of 0.7511. Although a low variance was indicated, this second component was considered as the loading value for length of cephalic horn and elytron length. In addition, based on these two extracted principal components,

Table 2. Contribution of five morphometric variables to the extracted principal components (PC).

Variables	PC 1	PC 2	PC 3	PC 4	PC 5
Total body length	0.4689	0.0774	0.0231	−0.8583	0.1919
Pronotal width	0.5013	0.0541	−0.4172	0.0998	−0.7494
Pronotal length	0.4843	−0.1586	−0.4626	0.3764	0.6201
Length of cephalic horn	0.3860	−0.6729	0.6052	0.1422	−0.1083
Elytral length	0.3812	0.7164	0.4950	0.3021	0.0714
Eigenvalue	3.0949	0.7511	0.5125	0.4160	0.2253
Variance (%)	61.9	15.0	10.3	8.3	4.5
Cumulative variance (%)	61.9	76.9	87.2	95.5	100.0

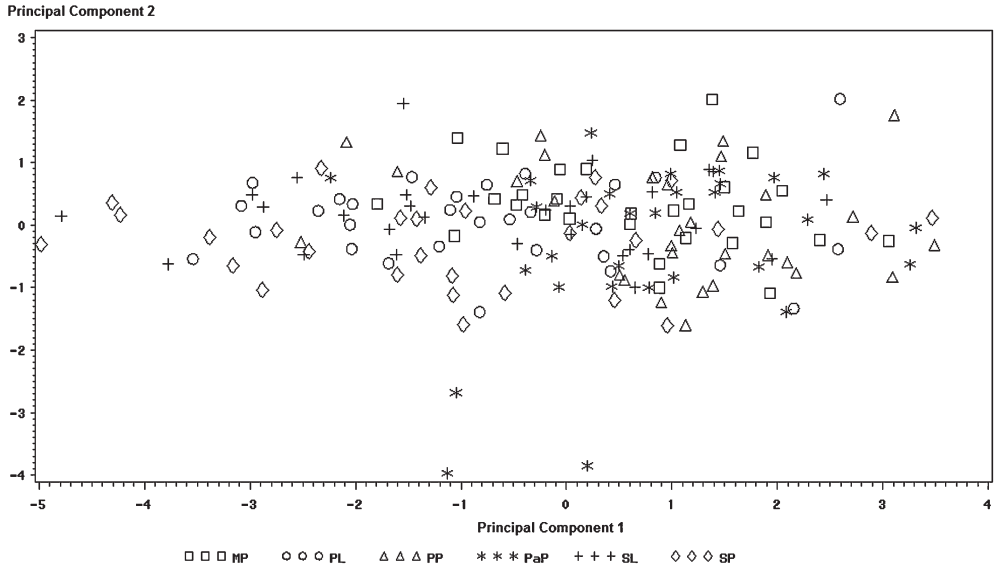

Fig. 1. Scatter plot based on the component scores from principal component analysis of Principal Component 2 against Principal Component 1. SP = Selangor pheromone; SL = Selangor light, PP = Perak pheromone; PL = Perak light; PaP = Pahang pheromone; MP = Medan pheromone.

a cumulative variance of 76.9% was achieved. The scatter plot based on the component scores of PC1 and PC2 indicated no distinct discontinuity or clustering of populations based on the five variables (Fig. 1).

Discriminant Analysis. A stepwise discriminant analysis was performed on all five morphometric measurements. The stepwise discriminant analysis indicated three morphometric characters, elytral length, pronotal width, and total body length, as suitable linear measures that permitted discrimination among *O. rhinoceros* populations (Table 3). The remaining two morphometric characters did not enter the discriminant functions due to lack of discriminatory power. A discriminant analysis was performed to classify the individual beetles into their respective populations based on the three selected morphometric variables. However, 100% re-classification of individuals into their respective populations was not achieved. Nevertheless, 67 individuals (37.2%) were correctly re-classified to their respective populations. The correct re-classification of individuals in each population occurred at a level of 26.7% to 56.7% (Table 4).

Based on canonical discriminant analysis, three canonical functions were extracted (Table 5). Canonical Function 1 (CF1) accounted for 70.4% of the variance in the data set , Canonical Function 2 (CF2) explained 20.8% of the variance, and Canonical Function 3 (CF3) accounted for the remaining 8.9% of the variance. The first three canonical functions explained 100% of the total variance. The highest coefficient loading for CF1 was the pronotal

Table 3. Stepwise discriminant analysis parameters for three morphological characters measured from six populations of *Oryctes rhinoceros*.

Parameters	Pronotal width	Total body length	Elytral length
Partial R^2	0.2773	0.1023	0.0642
F value	13.3500	3.9400	2.3600
$P > F$ value	<0.0001	0.0021	0.0422
Wilk's Lambda	0.7227	0.6487	0.6071
$P <$ Wilk's Lambda	<0.0001	<0.0001	<0.0001
Average squared canonical correlation	0.0554	0.0757	0.0881
$P >$ Average squared canonical correlation	<0.0001	<0.0001	<0.0001

Table 4. Classification results for the discriminant analysis of six populations of *Oryctes rhinoceros*. SP = Selangor pheromone; SL = Selangor light, PP = Perak pheromone; PL = Perak light; PaP = Pahang pheromone; MP = Medan pheromone.

Actual population	*n*	Predicted population membership					
		SP	SL	PP	PL	PaP	MP
SP	30	17	3	4	2	4	0
		56.7%	10.0%	13.3%	6.7%	13.3%	0.0%
SL	30	5	10	9	4	1	1
		16.7%	33.3%	30.0%	13.3%	3.3%	3.3%
PP	30	1	6	10	3	4	6
		3.3%	20.0%	33.3%	10.0%	13.3%	20.0%
PL	30	5	5	2	9	5	4
		16.7%	16.7%	6.7%	30.0%	16.7%	13.3%
PaP	30	1	1	6	4	13	5
		3.3%	3.3%	20.0%	13.3%	43.3%	16.7%
MP	30	0	3	10	4	5	8
		0.0%	10.0%	33.3%	13.3%	16.7	26.7%

Table 5. Summary of parameters of three canonical functions from canonical discriminant analysis of six *Oryctes rhinoceros* populations.

Canonical function	Eigenvalue	Variance (%)	Cumulative variance (%)	Canonical correlation
1	0.4016	70.6	70.6	0.5352
2	0.1187	20.8	91.2	0.3257
3	0.0505	8.9	100.0	0.2192

Table 6. Standardized discriminant function coefficients of the first three canonical functions (CF) for three morphological characters measured from six populations of *Oryctes rhinoceros*. * = major loading in each canonical function.

Variables	CF 1	CF 2	CF 3
Pronotal width	1.384780466*	−0.644913074	0.368058132
Elytral length	−0.163661394	0.983252784*	0.741438042
Total body length	−0.246742919	0.538374971	−1.262761463*

Table 7. Total canonical structure of the first three canonical functions (CF) for three morphological characters measured from six populations of *Oryctes rhinoceros*.

Variables	CF 1	CF 2	CF 3
Pronotal width	0.9751	0.2068	−0.0794
Elytral length	0.3662	0.8802	0.3017
Total body length	0.4992	0.5771	−0.6462

width, CF2 was elytral length, and CF3 was total body length (Table 6). Pronotal width in CF1, elytral length in CF2, and total body length in CF3 are important variables in their respective canonical functions (Table 7). Scatter plots were produced between CF1 and CF2 (Fig. 2) and CF1 and CF3 (Fig. 3) based on the extracted canonical variable scores. Population ordinations were observed to overlap in both graphs, indicating much similarity among individual members of the six populations of *O. rhinoceros*.

Discussion

In this study, multivariate techniques were applied to study the variability in measured characters of six *O. rhinoceros* groups. Variation in beetle body form and size are known to be related to specific habitat demands and physiological and behavioral characteristics (Den Boer 1986). Therefore, species-specific morphological characters are capable of reflecting closely related species. In our study, the selected morphometric variables, according to Cook *et al*. (2006), are reflective of body shape relevant to habitat, locomotion, burrowing, and flying (Talarico *et al*. 2011). Morphometric studies using the selected variables are also important in assessing ecomorphological diversity of any

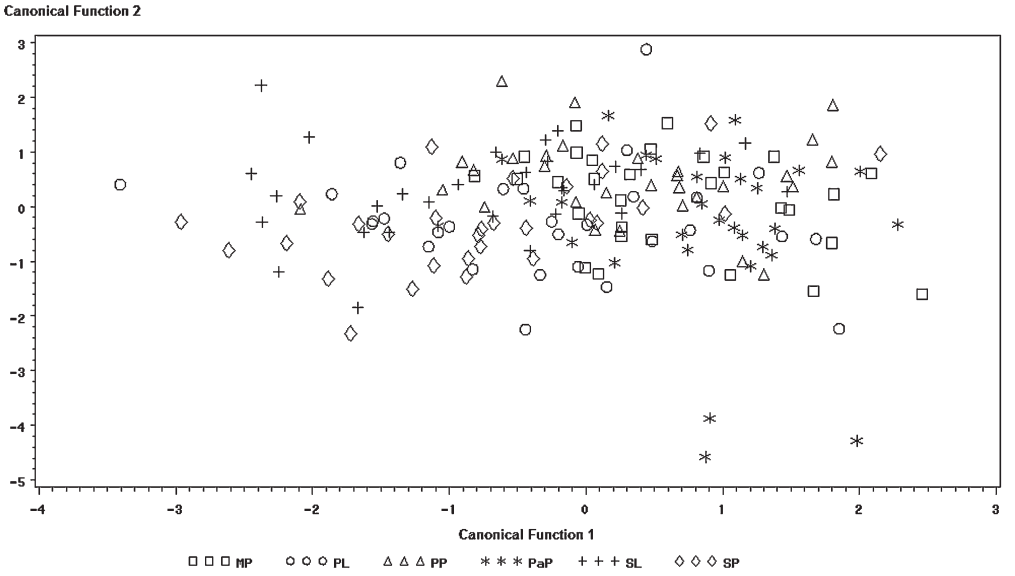

Fig. 2. Scatter plot of the canonical scores from the canonical discriminant analysis of Canonical Function 2 against Canonical Function1. SP = Selangor pheromone; SL = Selangor light, PP = Perak pheromone; PL = Perak light; PaP = Pahang pheromone; MP = Medan pheromone.

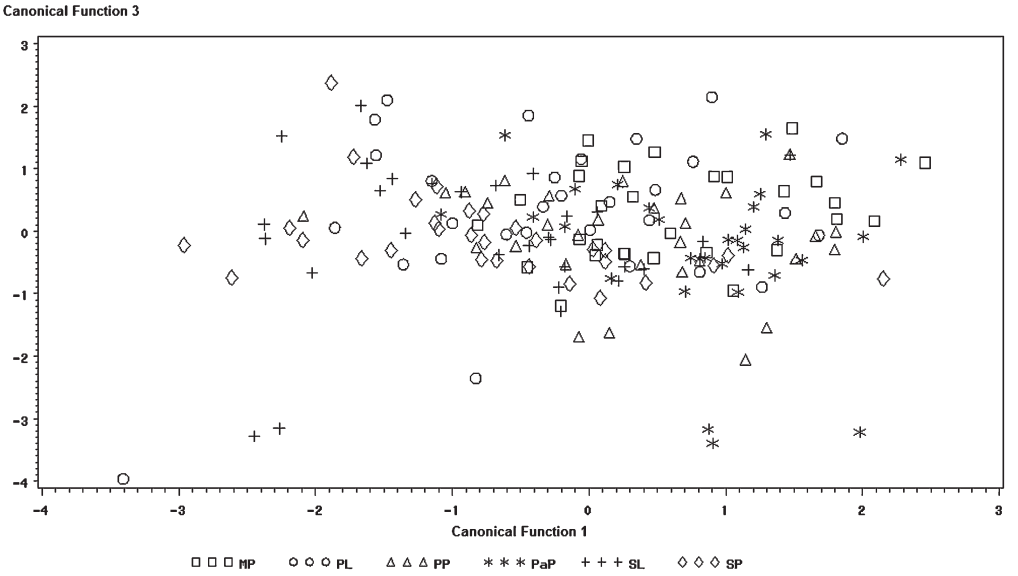

Fig. 3. Scatter plot of the canonical scores from the canonical discriminant analysis of Canonical Function 3 against Canonical Function1. SP = Selangor pheromone; SL = Selangor light, PP = Perak pheromone; PL = Perak light; PaP = Pahang pheromone; MP = Medan pheromone.

possible co-existing species. Slight differences identified among biogeographical regions may indicate selection favoring local environment and climatic and evolutionary history which may be indicative of variation in gene pool (Inward *et al*. 2011).

Although five principal components were extracted in the principal component analysis, only

PC1 and PC2 were chosen for analysis because the subsequent components captured a small amount of the variance and thus were inefficient (Hatcher and Stepanski 1994). Based on the visual interpretation of PC1 and PC2, no clear clustering of *O. rhinoceros* individuals was observed. Individuals that were trapped via both light and pheromone trap from all six sampled localities were observed to be grouped in one morphocluster. The morphology of individuals from both light and pheromone traps from different locations was observed to overlap with one another. This situation indicated that *O. rhinoceros* showed stability in morphometric character variance across a wide geographical range.

The discriminant analysis was performed based on a stepwise discrimination procedure which permitted the selection of the three most discriminating variables (pronotal width, total body length, and elytral length) that had the possibility to visualize separations (Yakubu *et al*. 2010) among the *O. rhinoceros* populations. According to Herrera *et al*. (1996), suitable morphometric characters allow easy monitoring of variables, assisting the applications of ethnological characterizations and permitting reliable racial discrimination. The three selected morphological variables for *O. rhinoceros* had highly significant Wilk's Lambda values and average squared canonical correlations. According to Anderson (2003), small values of Wilk's Lambda indicate strong group differences, while values close to one indicate no group differences. When looking at the Wilk's Lambda values for the three best chosen variables, it was possible to make an early observation that very low group differences occurred among *O. rhinoceros* populations as the values were close to one.

The output of the discriminant analysis based on the chosen variables further indicated that misclassifications of individuals were recorded at a very high rate (62.8%). This highlights the fact that, although the populations were of different geographical origins, high similarities in morphometric features were exhibited. The lack of pattern in morphometric distinctiveness among population members suggested no direct relationship between the extent of morphometric divergence and geographical separation. Three canonical functions were extracted based on the three selected variables. In addition, the degree of relationship between the predictors and groups (known as canonical correlation) was higher in CF 1 (0.5352) compared to CF 2 (0.3257) and CF 3 (0.2192). Each independent variable contributes differently to the derived canonical functions (Tabachnick and Fidell 2001). The scatterplots of the first three canonical functions based on the canonical discriminant analysis did not produce any clear separation among the six populations examined in this study, due to morphological overlaps among individuals within and between populations. Discrimination was difficult due to overlaps in the range of interpopulation variabilities (Falniowski *et al*. 1996; Chiu *et al*. 2002). Looking at the whole picture, the studied morphometric variables exhibited much similarity between populations, resulting in overlaps between populations.

Accurate detection and monitoring of individuals is extremely important when dealing with pest organisms (Pollock *et al*. 2002). Generally, the detection of a cryptic complex is difficult as it often occurs in small population sizes and individuals are morphologically indistinguishable (Vine *et al*. 2009). However, Bickford *et al*. (2007) highlighted that the pheromones of sibling species are often distinct. Differences in the ratios of compounds or chirality of compounds often aids in distinguishing the pheromone of one species from that of another species (Byers 1989; Kozlov *et al*. 1996; Bengtsson 2008). The observed similarity in morphological characters among different populations of *O. rhinoceros* coupled with the selective attraction exhibited towards the pheromone trapping system does suggest, however, the possibility of a cryptic species complex within this species.

In our study, it was clearly seen that the morphometric variables studied showed no isolated cluster of *O. rhinoceros* beetles. Although three major characters were highlighted as suitable variables for discrimination purposes, it was found that all the populations shared similarities in morphological characters, thus resulting in overlap of morphological variation in individuals collected from different populations as observed in the clustering pattern. It can be concluded that the samples tested were not morphologically distinguishable, but the possibility of a cryptic complex in *O. rhinoceros* remains in question. A comprehensive study of the population genetics of this species from throughout Southeast Asia should be done for further confirmation.

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