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# A Morphometric Analysis Of Malaysian *Rhinolophus* Species

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## ABSTRACT

A preliminary investigation on the morphometric variations among four *Rhinolophus* species was carried out using voucher specimens from the Universiti Malaysia Sarawak (UNIMAS) Zoological Museum and the Department of Wildlife and National Park (DWNP), Kuala Lumpur. A total of 19 individuals from *R. acuminatus*, *R. affinis*, *R. creaghi* and *R. stheno* were morphologically analysed where 27 linear measurements of body, skull and dental were appropriately recorded. The data were subjected to Discriminant Function Analysis (DFA) and Canonical Variate Analysis (CVA) using SPSS Version 15.0 and Cluster Analysis of Euclidean distance using Minitab Version 14.4. The highest character loadings observed in Function 1, Function 2 and Function 3 were the fifth digit metacarpal length (D5MCL), the fourth digit metacarpal length (D4MCL) and the palatal length (PL) with the standardized canonical discriminant function coefficient value of 22.384, 14.235 and 8.122, respectively. These three characters are identified as the best morphological predictor in for differentiating the four species of *Rhinolophus* in this study. Thus, the morphometric approach which as being more cost-effective could be useful in addition to DNA sequencing for aiding in species identification.

**Keywords**: Morphometric, Discriminant Function Analysis, *Rhinolophus*, species identification.

## INTRODUCTION

The horseshoe bats of genus *Rhinolophus* Lacepède, 1799 (Rhinolophidae) are known to be well distributed throughout the tropics, subtropics and temperate zones (Corbet and Hill, 1992; Findley, 1993; Francis, 2001). In Malaysia, there are currently 15 *Rhinolophus* species recorded; 10 species in Borneo and 12 species in Peninsular Malaysia

(Payne *et al*., 1985; Corbet and Hill, 1992; Khan, 1992; Hutson *et al.,* 2001; Yoshiyuki and Lim, 2005; Simmons, 2005). Generally, the rhinolophids are small to medium in size with forearm length range of 33-50 milimeter (mm), having an elaborate complex noseleaf and a raised portion called sella that is very useful for identification among the species of genus *Rhinolophus* (Payne *et al*., 1985; Corbet and Hill, 1992; Francis, 2008). Besides that, the ears are sorted from moderate to large sized (15-42 mm) with a moderate long tail (13-58 mm) that is completely enclosed within their interfemoral membrane (Payne *et al*., 1985; Vaughan, 1986; Corbet and Hill, 1992; Francis, 2008). *Rhinolophus* species are usually found roosting in caves, buildings, hollow trees and foliage including rock crevices and were recorded mostly from the forest understorey (Payne *et al*., 1985; Corbet and Hill, 1992). However, there is still lack of knowledge and studies regarding to the taxonomic, systematics and phylogenetic relationships among Malaysian *Rhinolophus* (Maree and Grant, 1997; Wang *et al.,* 2003). In this study, we focused on the morphometric relationships among four species within this genus, namely, *R. acuminatus*, *R. affinis*, *R. creaghi* and *R. stheno* due to their overlapping body sizes and close similarities of their facial structure. This study was designed to evaluate the morphometric variations using the 27 morphological characters and to show the main characters for discriminating these examined species.

## METHODOLOGY

A total of 23 adult individuals from four species, namely, *R. acuminatus* (assigned as Group 1), *R. affinis* (Group 2), *R. creaghi* (Group 3) and *R. stheno* (Group 4) were examined from voucher specimens from the Universiti Malaysia Sarawak (UNIMAS) Zoological Museum and the Department of Wildlife and National Park (DWNP), Kuala Lumpur. All specimens were collected from secondary forest, national park or nature reserve around Sarawak and Peninsular Malaysia. The adult specimens were determined following Kunz (1988) by observing the epiphyseal-diaphyseal fusion on the third, fourth and fifth metacarpals.

Twenty-seven morphological characters including body, skull and dental (Figure 1) were measured using digital caliper (Mitutoyo™; calibrated to 0.01mm) and steel ruler with the aid of microscope following the methods by Kitchener *et al*. (1993) and Jayaraj *et al*. (2005, 2006). These data were analysed for Discriminant Function Analysis (DFA) and Canonical Variate Analysis (CVA) using Statistical Package for Social

Science (SPSS) program version 15.0 and Cluster Analysis (Euclidean distance) with Unweighted Pair-Group Method Average (UPGMA) method using Minitab program version 14.40. A probability of $P < 0.05$ was considered significant in all analysis.

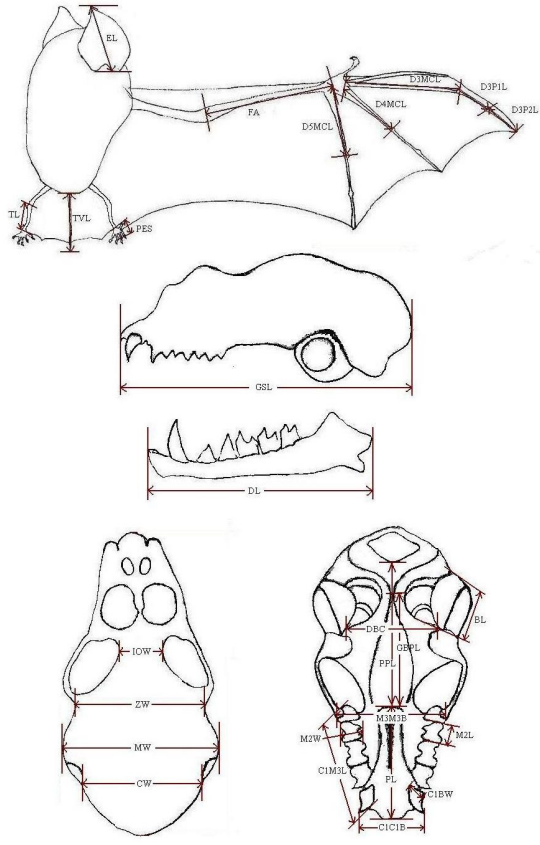


Figure 1: Twenty-seven morphological characters measured in *Rhinolophus* (Drawing is not to scale).

## RESULTS AND DISCUSSION

Descriptive statistics for the study species are listed as in Table 1. Discriminant function analysis successfully extracted three significant functions in which the Functions 1, 2 and 3 explained 59.7%, 39.0% and 1.3% of the variance, respectively (Table 2). This showed that the Function 1 with higher character loadings has higher variability of characters in the analysis.

The Wilk's lambda statistic (Table 3) for the tests of Function 1 through 3 functions and Function 2 through 3 functions (Wilk's lambda = 0.000) have a probability of $p = 0.000$ respectively, whereas the Function 3 (Wilk's lambda = 0.060) has the probability of $p = 0.030$. Highest character loadings observed in both Function 1 and Function 2 were the fifth digit metacarpal length (D5MCL) and the fourth digit metacarpal length (D4MCL) respectively. Highest character loading observed in Function 3 was the palatal length (PL) (Table 4). Therefore, these diagnostic characters were useful to differentiate among the species of *R. acuminatus*, *R. affinis*, *R. creaghi* and *R. stheno*.

TABLE 1: Descriptive statistics for the studied species.

| **Species** | ***R. acuminatus* (n=7)** | | | ***R. affinis* (n=8)** | | | ***R. stheno* (n=4)** | | | ***R. creaghi* (n=4)** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Character** | **Mean ± SD** | **Min** | **Max** | **Mean ± SD** | **Min** | **Max** | **Mean ± SD** | **Min** | **Max** | **Mean ± SD** | **Min** | **Max** |
| **FA** | 48.98 ± 1.10 | 47.64 | 50.30 | 48.26 ± 1.14 | 46.77 | 50.03 | 46.69 ± 1.11 | 45.12 | 47.74 | 51.17 ± 0.45 | 50.72 | 51.78 |
| **E** | 15.91 ± 1.61 | 13.83 | 17.85 | 19.43 ± 1.35 | 17.77 | 22.06 | 14.15 ± 0.44 | 13.63 | 14.71 | 20.82 ± 0.20 | 20.58 | 21.06 |
| **TB** | 22.51 ± 0.59 | 21.47 | 23.40 | 22.07 ± 0.53 | 21.02 | 22.88 | 21.99 ± 0.56 | 21.20 | 22.48 | 24.73 ± 0.29 | 24.56 | 25.16 |
| **PES** | 9.30 ± 0.42 | 8.88 | 10.02 | 8.78 ± 0.72 | 7.11 | 9.62 | 7.83 ± 0.39 | 7.28 | 8.15 | 9.50 ± 0.60 | 8.63 | 9.92 |
| **TVL** | 23.86 ± 1.00 | 22.00 | 24.94 | 22.16 ± 1.53 | 18.82 | 24.06 | 16.62 ± 1.28 | 15.75 | 18.51 | 18.74 ± 1.49 | 17.98 | 20.97 |
| **D3MCL** | 37.37 ± 0.68 | 36.35 | 38.01 | 37.20 ± 0.93 | 36.20 | 38.75 | 34.70 ± 0.89 | 33.90 | 35.68 | 39.23 ± 0.71 | 38.53 | 40.14 |
| **D3P1L** | 15.93 ± 0.40 | 15.25 | 16.33 | 14.73 ± 0.36 | 14.15 | 15.31 | 14.03 ± 0.73 | 13.30 | 14.83 | 14.85 ± 0.43 | 14.41 | 15.36 |
| **D3P2L** | 20.97 ± 1.02 | 19.57 | 22.52 | 23.57 ± 0.88 | 22.07 | 24.82 | 21.86 ± 1.55 | 19.54 | 22.88 | 23.66 ± 0.57 | 23.09 | 24.35 |
| **D4MCL** | 38.79 ± 0.70 | 37.56 | 39.71 | 38.01 ± 0.90 | 36.52 | 38.79 | 35.99 ± 1.22 | 34.64 | 37.10 | 38.87 ± 2.83 | 34.66 | 40.62 |
| **D5MCL** | 39.57 ± 0.86 | 38.18 | 40.43 | 38.70 ± 0.83 | 37.28 | 39.76 | 36.62 ± 0.77 | 35.93 | 37.38 | 40.01 ± 0.92 | 38.71 | 40.65 |
| **GSL** | 22.99 ± 0.42 | 22.20 | 23.40 | 22.54 ± 0.53 | 21.45 | 23.34 | 20.39 ± 0.62 | 19.82 | 21.22 | 24.19 ± 0.20 | 24.00 | 24.42 |
| **IOW** | 2.59 ± 0.26 | 2.34 | 3.12 | 2.33 ± 0.18 | 1.97 | 2.55 | 1.92 ± 0.14 | 1.74 | 2.08 | 2.55 ± 0.78 | 2.45 | 2.62 |
| **CW** | 8.29 ± 0.37 | 7.82 | 8.92 | 8.58 ± 0.37 | 8.09 | 9.16 | 8.34 ± 0.21 | 8.15 | 8.55 | 8.81 ± 0.34 | 8.44 | 9.26 |
| **MW** | 11.40 ± 0.27 | 10.91 | 11.71 | 10.81 ± 0.29 | 10.19 | 11.16 | 10.05 ± 0.21 | 9.85 | 10.32 | 11.17 ± 0.09 | 11.12 | 11.30 |
| **ZW** | 10.11 ± 0.21 | 9.84 | 10.45 | 10.15 ± 0.41 | 9.79 | 11.12 | 9.21 ± 0.16 | 9.04 | 9.37 | 10.39 ± 0.17 | 10.28 | 10.64 |
| **PPL** | 11.42 ± 1.48 | 8.31 | 12.52 | 11.67 ± 0.44 | 11.06 | 12.63 | 11.04 ± 0.52 | 10.40 | 11.56 | 13.00 ± 0.19 | 12.76 | 13.08 |
| **PL** | 8.41 ± 1.40 | 7.42 | 11.52 | 7.37 ± 0.36 | 6.55 | 7.82 | 6.29 ± 0.41 | 5.75 | 6.72 | 7.51 ± 0.20 | 7.28 | 7.72 |
| **DBC** | 5.84 ± 0.36 | 5.43 | 6.32 | 5.39 ± 0.37 | 4.92 | 5.98 | 5.32 ± 0.22 | 5.00 | 5.50 | 5.67 ± 0.16 | 5.52 | 5.88 |
| **BL** | 3.02 ± 0.17 | 2.81 | 3.31 | 3.34 ± 0.36 | 2.63 | 3.92 | 2.78 ± 0.10 | 2.65 | 2.88 | 3.40 ± 0.10 | 3.28 | 3.48 |
| **GBPL** | 9.93 ± 0.58 | 9.16 | 10.68 | 9.99 ± 0.63 | 9.09 | 11.27 | 8.43 ± 1.93 | 5.58 | 9.89 | 11.09 ± 0.25 | 10.72 | 11.28 |
| **DL** | 15.51 ± 0.23 | 15.08 | 15.74 | 15.21 ± 0.51 | 14.48 | 15.63 | 13.19 ± 0.80 | 12.08 | 14.00 | 16.00 ± 0.37 | 15.59 | 16.39 |
| **C1BW** | 1.75 ± 0.08 | 1.62 | 1.87 | 1.73 ± 0.13 | 1.50 | 1.93 | 1.48 ± 0.15 | 1.27 | 1.63 | 1.88 ± 0.08 | 1.76 | 1.93 |
| **C1C1B** | 5.63 ± 0.20 | 5.33 | 5.85 | 5.67 ± 0.23 | 5.27 | 5.93 | 4.93 ± 0.20 | 4.72 | 5.12 | 6.04 ± 0.18 | 5.80 | 6.20 |
| **M3M3B** | 7.88 ± 0.24 | 7.48 | 8.17 | 7.76 ± 0.12 | 7.58 | 7.94 | 6.93 ± 0.16 | 6.78 | 7.16 | 8.31 ± 0.17 | 8.11 | 8.45 |
| **C1M3L** | 7.12 ± 0.17 | 6.89 | 7.34 | 6.95 ± 0.29 | 6.43 | 7.27 | 6.24 ± 0.09 | 6.15 | 6.35 | 7.52 ± 0.16 | 7.33 | 7.71 |
| **M2L** | 1.75 ± 0.16 | 1.51 | 1.99 | 1.73 ± 0.15 | 1.49 | 2.06 | 1.46 ± 0.13 | 1.34 | 1.61 | 1.67 ± 0.07 | 1.56 | 1.74 |
| **M2W** | 2.10 ± 0.19 | 1.83 | 2.36 | 2.19 ± 0.24 | 1.95 | 2.63 | 1.65 ± 0.08 | 1.53 | 1.70 | 2.18 ± 0.16 | 2.03 | 2.37 |

TABLE 2: Eigenvalues for DFA of four selected *Rhinolophus*.

| Function | **Eigenvalue** | **% of Variance** | **Cumulative %** | **Canonical Correlation** |
|---|---|---|---|---|
| 1 | 725.613* | 59.7 | 59.7 | 0.999 |
| 2 | 474.113* | 39.0 | 98.7 | 0.999 |
| 3 | 15.552* | 1.3 | 100.0 | 0.969 |

* First 3 canonical discriminant functions were used in the analysis

TABLE 3: Wilks' Lambda for DFA of four selected *Rhinolophus*.

| **Test of Function(s)** | **Wilks' Lambda** | **Chi-square** | **df** | **Sig.** |
|---|---|---|---|---|
| **1 through 3** | 0.000 | 163.364 | 57 | 0.000 |
| **2 through 3** | 0.000 | 94.185 | 36 | 0.000 |
| **3** | 0.060 | 29.468 | 17 | 0.030 |

TABLE 4: Standardised Canonical Discriminant Function coefficients of four selected *Rhinolophus*. Highest character loadings for each function were indicated with an arrow.

| **Character** | **Function** | | |
|---|---|---|---|
| | **1** | **2** | **3** |
| **FA** | -9.901 | 5.632 | 3.440 |
| **E** | 1.218 | 3.923 | 1.075 |
| **TB** | -2.798 | 4.894 | 1.692 |
| **PES** | -1.738 | 5.704 | -0.498 |
| **TVL** | -1.947 | -2.619 | -0.884 |

| | | | | |
|---|---|---|---|---|
| | **D3MCL** | 6.910 | -0.061 | -2.501 |
| | **D3P1L** | -2.474 | -2.438 | 1.490 |
| | **D3P2L** | 3.500 | -1.255 | 0.054 |
| → | **D4MCL** | -19.585 | 14.235* | 3.412 |
| → | **D5MCL** | 22.384* | -11.953 | -0.600 |
| | **GSL** | 2.474 | -4.336 | -4.818 |
| | **IOW** | 5.134 | 0.847 | 2.072 |
| | **CW** | -4.818 | 8.007 | 1.180 |
| | **MW** | -1.857 | -3.928 | -0.778 |
| | **ZW** | 5.485 | -3.877 | -2.041 |
| | **PPL** | 2.911 | 5.094 | 5.172 |
| → | **PL** | -7.415 | 9.588 | 8.122* |
| | **DBC** | 10.290 | -8.204 | -0.156 |
| | **BL** | -0.955 | -4.196 | -0.650 |

* Diagnostic character in each function.

Both canonical discriminant function (Figure 2) and Cluster Analysis (Figure 3) show clear separation and grouping by each species. *R. acuminatus*, *R. affinis*, *R. creaghi* and *R. stheno* are morphologically similar in terms of overlapping forearm length as each are recorded to have a forearm range of 44-50 mm, 48-54 mm, 46-51 mm and 42-48 mm, respectively (Payne *et al*., 1985; Francis, 2008). Their body coloration is also resembles each other, where they can only be distinguished through observation of the sella shapes and the connecting process (Payne *et al*., 1985; Corbet and Hill, 1992; Kingston *et al*., 2006). In addition, close

similarities shared between *R. acuminatus* and *R. affinis* often result to species misidentification.

In our study, the morphometric analyses subsequently revealed the misidentified species where four specimens of *R. affinis* were wrongly assigned as *R. acuminatus*. This finding also supported by the molecular data obtained from DNA sequencing of partial mitochondrial DNA (mtDNA) cytochrome *b* gene, in which they were found to be aligned together within the *affinis* group (Sazali *et al*., 2006).

Besides that, normal identification procedures even though practiced by experienced zoologists, may still result in some identification error. It is thus shown that morphometric analyses can help in morphologically similar species identification or misidentification. Moreover, the morphometric approach, which is affordable and cheaper in term of cost, may be used in addition to the DNA sequencing for aiding in species identification.

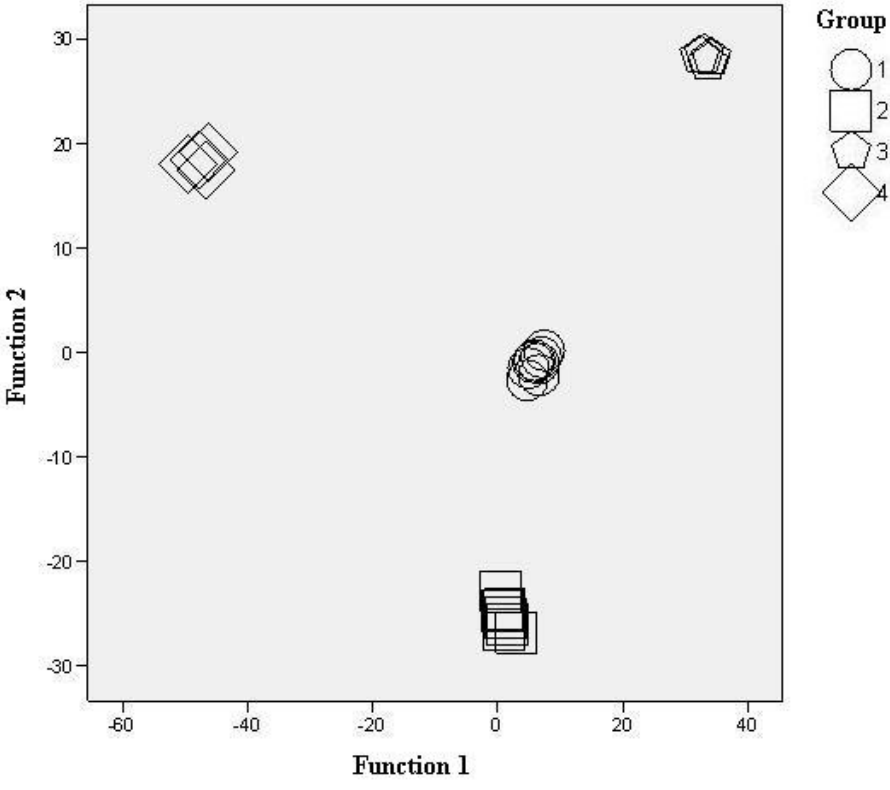


Figure 2: CVA plot of Functions 1 and 2 of four selected *Rhinolophus*.
1 = *R. acuminatus*, 2 = *R. affinis*, 3 = *R. creaghi*, 4 = *R. stheno*.

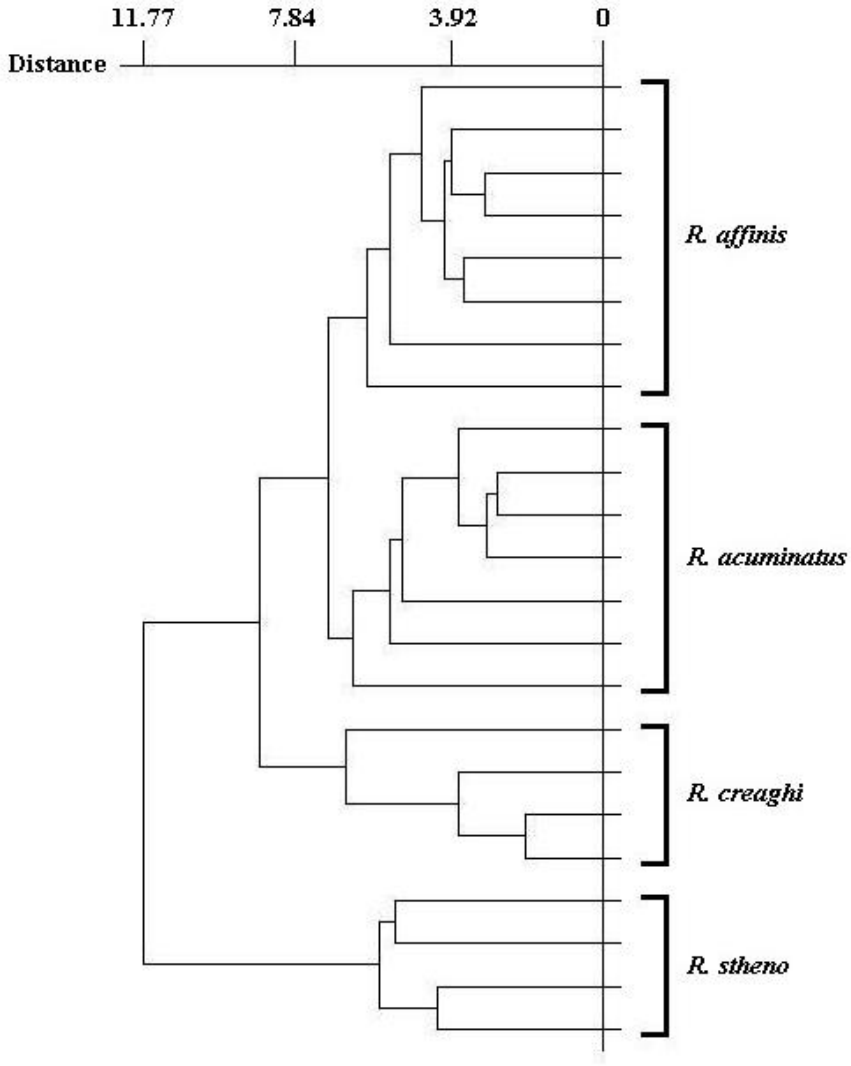


Figure 3**:** UPGMA Cluster Analysis of four selected *Rhinolophus* species.

## CONCLUSION AND RECOMMENDATION

Overall, the morphometric analysis has a potential for species identification within its genus as each species is well separated into each cluster. Besides that, correct field identification of species is very important in order to infer accurate biological diversity and ecological information on the study taxa. Further analyses using more species should be conducted to completely review the morphometric relationships among Malaysian *Rhinolophus* as well as with other species and compared with molecular studies for better understanding.

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