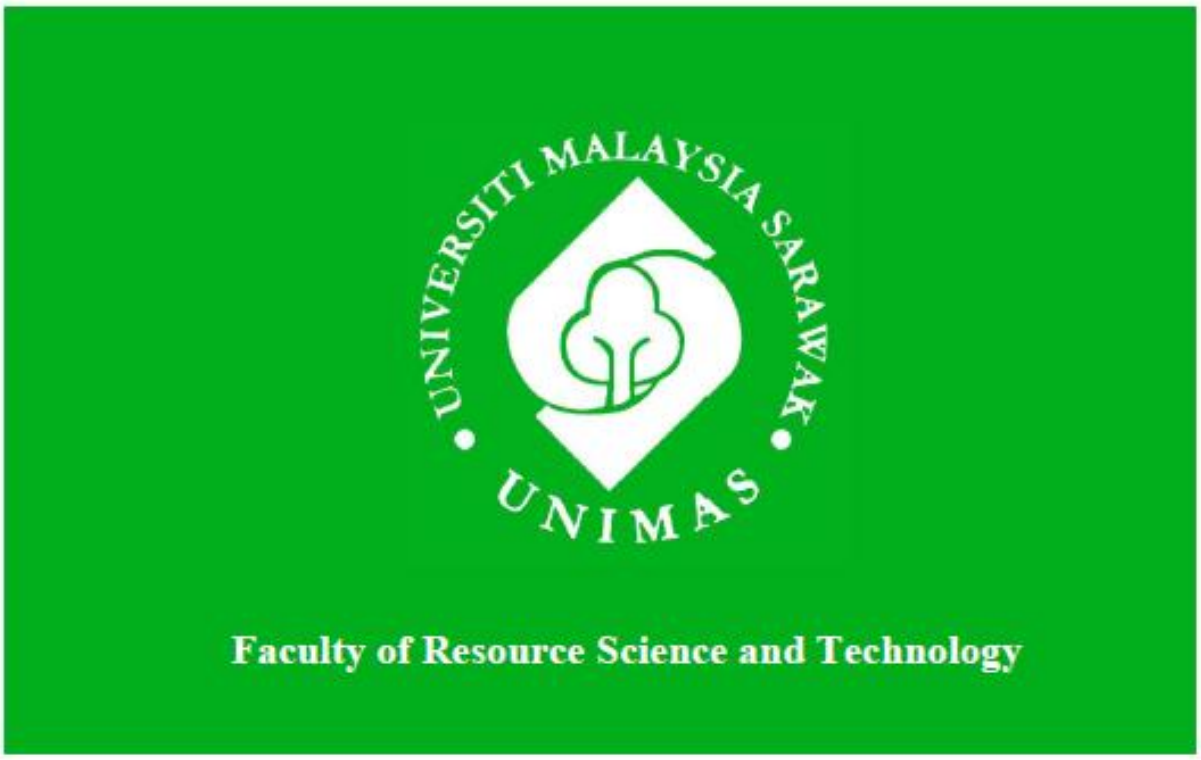

A MORPHOLOGICAL ANALYSIS OF DIADEM ROUNDLEAF BAT (HIPPOSIDERIDAE: *HIPPOSIDEROS DIADEMA*) IN MALAYSIA

Nurshilawati bt Abdul Latip (27774)

Bachelor of Science with Honours
Animal Resource and Management Programme
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A MORPHOLOGICAL ANALYSIS OF DIADEM ROUNDLEAF BAT (HIPPOSIDERIDAE: *HIPPOSIDEROS DIADEMA*) IN MALAYSIA

NURSHILAWATI BT ABDUL LATIP

This project report is submitted in partial fulfilment of the requirements for the Degree of Bachelor of Science with Honours
(Animal Resource Science and Management)

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UNIVERSITI MALAYSIA SARAWAK
2013

DECLARATION

I hereby declare that no portion of this dissertation has been submitted in support of an application for another degree of qualification of this or any other university or institution of higher learning.

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NURSHILAWATI BT ABDUL LATIP

Animal Resource Science and Management Programme

Department of Zoology

Faculty of Resource Science and Technology

Universiti Malaysia Sarawak

The project entitled ‘A Morphological Analysis of Diadem Roundleaf Bat (Hipposideridae: *Hipposideros diadema*) in Malaysia’ was prepared by Nurshilawati bt Abdul Latip and submitted to the Faculty of Resource Science and Technology in partial fulfilment of the requirements for the Degree of Bachelor of Science (Honours) in Zoology.

Received for examination by:

_____

(Roberta Chaya Tawie Tingga)

Date:

_____

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LIST OF ABBREVIATIONS

External morphological characters

D3MCL = third digit metacarpal length
D5MCL = fifth digit metacarpal length
EL = ear length
PES = pes length
TVL = tail to ventral length
D4MCL = fourth digit metacarpal length
D3P1L = third digit first phalanx length
FA = forearm length
TL = tibia length

Craniodental characters

BL = bullae length
C1M3L = canine molar length or maxillary tooth row length
CW = cranial width
DL = dentary length
GSL = great skull length
M3M3B = breadth across both third molar teeth outside surfaces
M2W = second molar tooth crown width
PPL = postpalatal length
ZW = zygomatic width
C1C1B = breadth across both canine outside surfaces
C1WB = canine tooth basal width
DBC = distance between cochleae
GBPL = greatest basal pit length
IOW = interorbital width
M2L = second molar tooth crown length
MW = mastoid width
PL = palatal length

Forest type

LDF = Lowland dipterocarp forest

MDF = Mixed dipteropcarp forest

PSF = Peat swamp forest

Place

M = UNIMAS Zoological Museum

NP = National park

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Universiti Malaysia Sarawak

ABSTRACT

The morphological study was carried out to record and analyse the morphological characters of *H. diadema* from four different populations in Malaysia (Peninsular Malaysia, South and North Sarawak, Sabah). Nine external, 17 craniodental characters were measured and analysed using the multivariate analysis and discriminant function analysis (DFA) using the SPSS Version 20.0 software. Cluster analysis was done using Paleontological Statistic (PAST) version 2.17. The external and craniodental characters were analysed separately. For external character, the highest character loading for both Function 1 and 2 was forearm (FA), whereas for Function 3 it was tibia (TL). For internal character, the highest character loading for Function 1, 2, and 3 were great skull length (GSL), palatal length (PL), and postpalatal length (PPL). These internal characters are cranial characters which two of them are related to palatal length. These findings establish the morphological variation between four geographically separate populations of *H. diadema* within Malaysia.

Key words: Morphometric, *H. diadema*, discriminant function, polymorphism, cranial

Abstrak

Kajian morfologi telah dijalankan untuk merekod dan menganalisa ukuran morfologi H. diadema daripada empat populasi yang berlainan di Malaysia (Semenanjung Malaysia, Selatan dan Utara Sarawak, Sabah). Sembilan ukuran morfologi luaran, 17 ukuran kraniodental telah diambil dan dianalisis dengan menggunakan analisis multivariat dan analisis fungsi diskriminan (DFA) menggunakan perisian SPSS Versi 20.0. Selain itu, klaster analisis telah dibuat dengan menggunakan perisian statistik paleontologi (PAST) Versi 2.17. Ukuran-ukuran luaran dan dalaman dianalisis secara berasingan. Untuk ciri luaran, ukuran tertinggi bagi kedua-dua Fungsi 1 dan 2 adalah panjang lengan (FA), manakala untuk Fungsi 3 adalah panjang tibia (TL). Untuk ukuran dalaman, ukuran tertinggi bagi Fungsi 1,2, dan 3 adalah panjang krania (GSL), panjang lelangit (PL), dan panjang pasca lelangit (PPL). Dua daripada ukuran dalaman, berkaitan dengan ukuran bagi panjang lelangit. Kajian ini menunjukan terdapat variasi morfologi antara keempat-empat populasi H. diadema mengikut geografi yang berasingan dalam Malaysia.

Kata kunci: Morfometrik, H. diadema, fungsi diskriminan, polimorfisme, krania

CHAPTER ONE

INTRODUCTION

1.1 Chiroptera

Bats belong to the order of Chiroptera with two suborders of Megachiroptera (Old World fruit bats) and Microchiroptera (echolocating bats) (Kate *et al*., 2002). Microchiropterans are insectivorous bats and possess a highly specialised echolocation system that allows them to recognise flying insects based on the pattern of their wing-beats (Neüweiler, 2000). Old World leaf-nosed bats (Hipposideridae) are among the most widespread and ecologically diverse groups of insectivorous bats in the Old World tropics (Murray *et al*., 2012). Diadem Roundleaf bat *Hipposideros diadema* is one of the most widely spread species from this family and can be found in variety of forested habitats and cave roosts. They are usually roosts in large colonies in caves and sometimes, with other species namely *H. larvatus* (Payne *et al*., 1985).

H. diadema has dark brown upperparts fur with pale bases, white patches on the shoulders and sides; underparts greyish white (Payne *et al*., 1985). There is no recent studies conducted concerning this species in terms of the morphological variations between different populations in Sarawak. This study will assess the morphological variation of *H.*

diadema from different geographical localities in Sarawak by using morphometric characters.

Besides, *H. diadema* is known to be sexually dimorphic for skull, dentary and dental characters; for many measurements males average slightly larger than females (Kitchener *et al*., 1992). This morphological study therefore can also be used to study sexual dimorphism within species.

Moreover**,** this study should be able to determine the ecology of *H. diadema* because different ecological forces between populations, such as feeding behaviour, breeding, foraging behaviour, crowding effects and resource availability, might be the moulding factors if the morphological characters in the different populations study show wide range of variation. In this study, feeding ecology for this species can be determined. Predictions about the insects eaten by bats also can be made from the morphology of teeth, jaws, and cranial (Bongdanowicz *et al*., 1999).

1.2 Objectives

To assess the morphological variation of *H. diadema* populations from four different geographical localities in Malaysia by using their morphometrical characters.

CHAPTER TWO

LITERATURE REVIEW

2.1 Distribution and Ecology

H. diadema is one of the biggest roundleaf bat (Hipposideroidae) in Borneo. Its forearm measurement range from 76-86 mm (Payne *et al.,* 1985). It was known to occur in Borneo, Malaya Peninsular, and Lesser Sunda region (Corbet and Hill, 1992; Kitchener *et al.*, 1992). *H. diadema* is exhibit carnivory in preying on invertebrates such as coleoptera, lepidoptera and various orthopteroid. This species have a constant frequency and slightly longer duration than other *Hipposideros* spp (Parvey and Burvel, 1997).

2.2 Previous Study

The study on taxonomy and geographic morphological variation of *H. diadema* in the Lesser Sunda Islands, Indonesia has been done by Kitchener *et al*. (1992). They carried out the morphological study using multivariate statistical analyses on 157 specimens of *H. diadema* from the Lesser Sunda Is, Borneo, Java, Thailand, Peninsula Malaysia, Philippines, New Guinea, Solomon Is, Bismark Is, and Australia. According to Kitchener *et al*. (1992), *H. diadema* from the Lesser Sunda Islands showed considerable morphological variation and appeared to include several distinct forms.

Recent study of morphological variations of Malaysian *Hipposideros* species was also has been done by Sazali *et al.* (2012). In the study, 22 individuals have been used to study the morphological variations of *Hipposideros* species in Malaysia, but none of the selected species used were *H. diadema.* The four species from Hipposideros that were used by Sazali *et al*. (2012) includes *H. ater*, *H. bicolor*, *H. cineraceus* and *H. dyacorum*. From the study, they found that the diagnostic parameters were the third digit second phalanx length and palatal length.

Another recent study on morphometric analysis of the fawn roundleaf bat, *H. cervinus* from several populations in Sarawak has also been done by Sazali and Juary (2012). They found that two characters were identified as the best predictors to distinguish the three populations of *H. cervinus* were canine molar length or maxillary tooth row length and ear length.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study sites

The study was conducted at several localities in Malaysia. The sampling sites were Kubah National Park, Sebangkoi Resort Park, Wind Cave Nature Resort, and Bako National Park. All locality of species studied has been divided into Peninsular Malaysia, Sabah, and Sarawak for three different populations. For Peninsular Malaysia and Sabah, all the specimens were retrieved from the voucher specimens deposited in UNIMAS Zoological Museum, FRST. Whereas, for Sarawak population of the species studied, sample collection was done at the proposed sampling localities and voucher specimens were also used in this study.

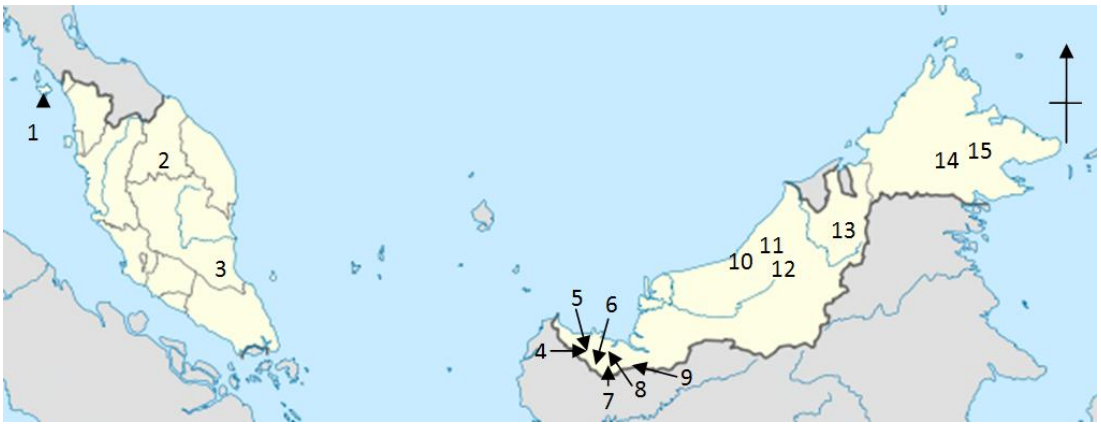

Figure 1. Localities of the specimens used in this study. Localities were as follows: 1, Lubuk Sembilang, Pulau Langkawi, Kedah; 2, Banjaran Titiwangsa, Gua Musang, Kelantan; 3, Merapoh, Pahang; 4, Bau, Sarawak; 5, Matang Wildlife Centre, Sarawak; 6, Kubah National Park, Sarawak; 7, Kampung Giam, Padawan, Sarawak; 8, UNIMAS, Kota Samarahan, Sarawak; 9, Sabal Forest Reserve, Sarawak; 10, Lambir National Park; 11, Niah National Park, Sarawak; 12, Kuala Besar, Niah, Sarawak; 13, Mulu National Park; 14, Imbak Canyon, Sabah; 15, Gomantong Cave, Sabah (Source: Google image).

3.2 Sample collection

Four harp traps (four bands) and ten mist nets was deployed at each study sites in the cave roosts and forested areas as well as open areas. Sampling was conducted for at least three consecutive nights. Nets and traps was set by 1730h and closed between 0200h and 0700h on the following day. Nets were checked for every one hour interval before closing them the next morning. Harp traps were check for three to four times each night. Wet specimens of *H. diadema* were also included from UNIMAS Zoological Museum, in addition to the specimens collected needed in this morphological study (See appendix 1).

3.3 Identification, external measurement, and preservation of samples

The sample was identified following Payne *et al*. (1985). Information such as body measurements, gender and age (juvenile or adult) was recorded in a data form (Appendix 2). Only adults were used in the analysis of morphometrics. Other than that such as adults female with lactating young or any pregnant female and the exceed number of individual

wanted was released and excluded in the study. According to Kunz (1988), development changes in cartilaginous growth plates can also be used to generate quantitative estimates of age in bats. Immature are much duller in colour, grey or grey-brown and if visualised under bright light, the wing joints of immature appear banded because of the cartilage is still not changing to bone (Payne *et al*., 1985). For external measurements, nine morphological characters were measured following Sazali and Juary (2012). The measurements include characters of third digit metacarpal length (D3MCL), third digit first phalanx length (D3P1L), fourth digit metacarpal length (D4MCL), fifth digit metacarpal length (D5MCL), ear length (EL), forearm length (FA), pes length (PES), tibia length (TL), tail to ventral length (TVL). All of the samples were preserved in 70% ethanol.

3.4 Skull extraction

Skulls were extracted. In order to prevent any bias measurements due to tissue intact on the skull, the skull was cleaned from all tissues for more precise measurements. After skulls were extracted and cleaned, appropriate labelling and tagging were used. There were 28 skull extracted (Appendix 2). Ten measurements of external morphology (Appendix 3) and 17 craniodental have been measured (Appendix 4).

3.5 Skull and dental measurements

Eleven skull measurements were taken for this morphometric study following Sazali and Juary (2012). The measurements are great skull length (GSL), interorbital width (IOW), cranial width (CW), mastoid width (MW), zygomatic width (ZW), postpalatal length (PPL), palatal length (PL), distance between cochleae (DBC), bulla length (BL), greatest basal pit

length (GBPL), and also dentary length (DL). Whereas for dental, six measurements were included namely canine tooth basal width (C1BW), breadth across both canine outside surfaces (C1C1B), breadth across both third molar teeth outside surfaces (M3M3B), canine molar length or maxillary tooth row length (C1M3L), second molar tooth crown length (M2L), and also second molar tooth crown width (M2W) following Sazali and Juary (2012) and Sazali *et al*., (2012).

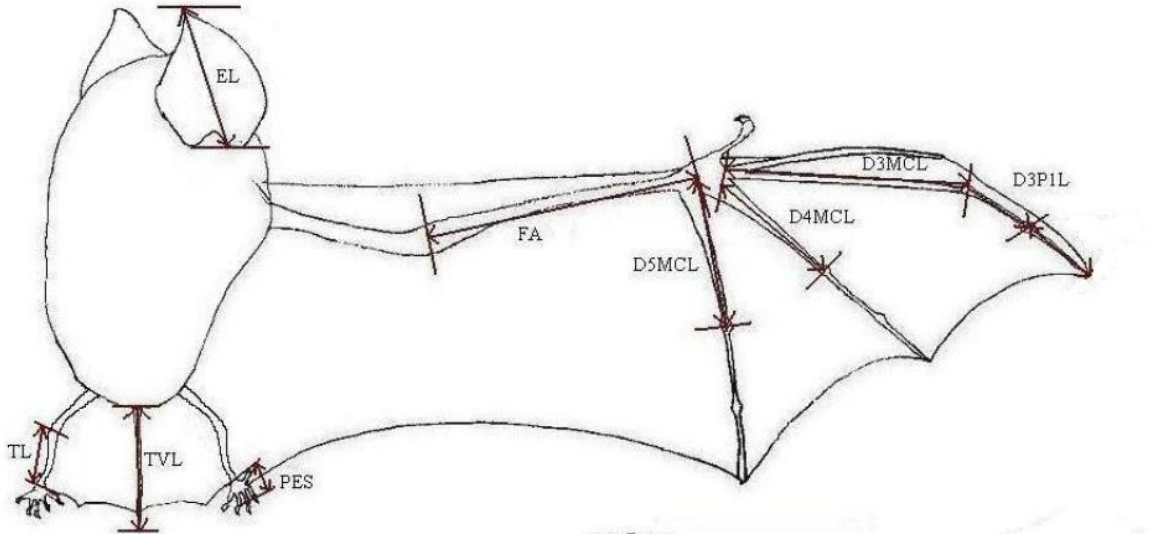

Figure 2. External morphological characters measurement (modified from Sazali et *al*., 2012)

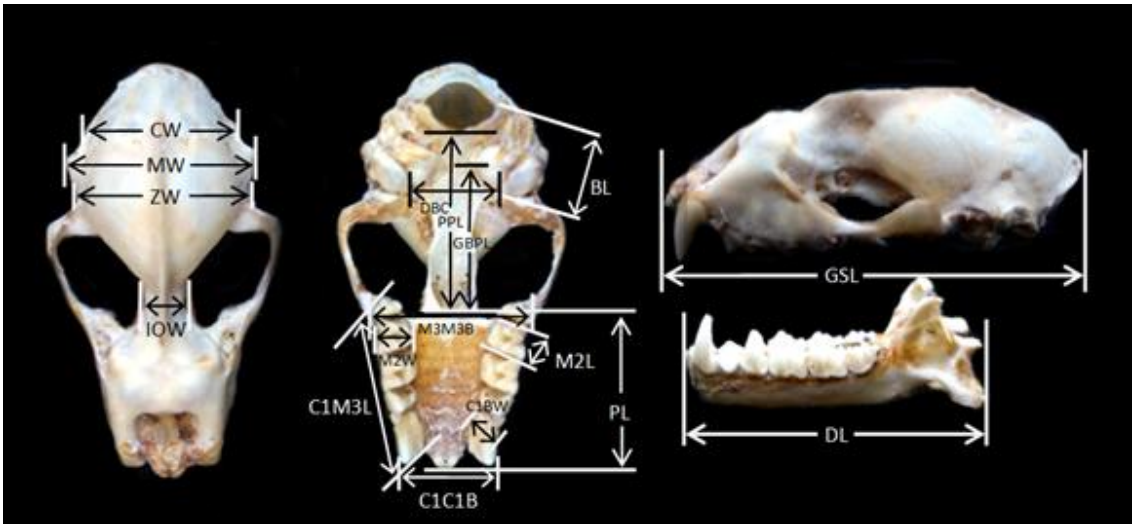

Figure 3. Cranial measurements taken from lateral, dorsal, and ventral view (measurements based on Sazali and Juary, 2012).

3.6 Morphometric analysis

All measurements were taken using a digital calliper with the aid of microscope following methods of Sazali and Juary (2012). Multivariate analysis was used to explain the relationship of the specimens, the similarity among species. For sex dimorphism, multiple regression analysis was used. For this morphological study, the data was analysed using Discriminant Function Analysis (DFA) with the stepwise procedure using the Statistical Package for Social Sciences (SPSS) version 20.0 software (SPSS Inc., 2010) to find the significant characters for discriminating the three populations. Normality and data transformation was included in the analysis. All data were standardised using $\log_{10}$ transformation. Cluster analysis was done using Paleontological Statistic (PAST) version 2.17 software (Hammer *et al.*, 2001).

CHAPTER FOUR

RESULTS

4.1 Field sampling

Cumulative of 17 trapping night in sampling localities which were Sebangkoi Recreational Park, Kubah National Park, Wind Cave Nature Reserve, and Bako National Park was done to collect sample for this study. A total of 525 individuals bats were recorded at all sampling localities, represent 31 species from seven families (Appendix 1). There were 10 species caught as singletons, namely *H. dyacorum, Glischropus tylopus, Pipistrellus petersi, Tylonycteris robustula, Myotis muricola, Eonycteris spelaea, Rhinolophus borneensis, M. horsfieldii, Murina suilla,*

4.2 Analysis

4.2.1 Univariate analysis for external morphology.

The descriptive statistics for *H. diadema* used in this study can be summarised in Table 1. Nine characters were screened on the separate sexes for any sex-dependent polymorphism (sexual dimorphism). None of the nine examined characters were found to be sexually dimorphic (Table 2).

Table 1 Mean ± standard deviation, minimum and maximum of external morphological values of *H. diadema* for each locality.

Character	Peninsular Malaysia (n=4)	South Sarawak (n=10)	North Sarawak (n=10)	Sabah (n=4)
TL	32.89±2.219 (29.95-35.34)	33.61±1.754 (29.65-35.56)	32.71±1.274 (30.69-34.42)	33.90±1.645 (32.23-36.01)
TVL	42.58±6.919 (37.33-52.33)	47.70±4.371 (42.67-54.67)	44.30±5.061 (38.00-54.00)	48.42±3.035 (45.67-51.67)
PES	14.54±0.618 (13.94-15.38)	14.48±1.009 (12.72-15.78)	14.05±0.977 (12.83-15.87)	14.19±0.412 (13.65-14.62)
EL	23.26±0.973 (21.82-23.94)	25.16±1.153 (22.66-27.16)	24.27±1.797 (22.06-27.03)	24.25±1.022 (23.19-25.64)
FA	82.81±5.021 (76.57-88.84)	85.32±2.549 (80.60-89.59)	82.33±2.524 (79.18-87.29)	83.43±2.602 (80.90-87.00)
D5MCL	56.33±4.977 (49.53-61.23)	58.83±1.190 (56.69-60.29)	56.94±1.297 (55.25-59.04)	57.90±2.051 (55.48-60.47)
D4MCL	59.46±5.980 (51.29-65.34)	62.91±1.372 (60.66-65.08)	61.29±1.698 (59.78-63.25)	61.87±1.906 (59.87-64.41)
D3MCL	61.23±6.433 (52.06-66.81)	65.50±1.673 (61.77-67.35)	63.44±1.698 (61.03-66.28)	64.57±2.094 (61.93-67.03)
D3P1L	27.62±1.992 (24.79-29.41)	28.04±0.779 (26.70-29.04)	27.66±0.968 (25.22-28.57)	27.48±0.944 (26.68-28.9)

Table 2 F values from multiple regression of external characters of *H. diadema* on sex.

Character	Sex	
	F value	**Sig.**
TL	0.286	0.597
TVL	0.358	0.555
PES	0.002	0.969
EL	1.132	0.297
FA	0.264	0.612
D5MCL	0.062	0.806
D4MCL	0.019	0.892
D3MCL	0.145	0.706
D3P1L	0.211	0.650

4.2.2 Normality test and data transformation

Most of the analysed characters using SPSS software show abnormal data distribution. The following external measurements showed a normal data distribution with $p > 0.05$ are TL, TVL, PES, EL, FA, and D5MCL (Table 3). Log_{10} transformation was applied to standardised all data and to avoid any occurrence of bias in response to some none-normality distribution (Krebs,1999).

Table 3 Normality test for each external character.

Character	Kolmogorov-Smirnov		
	Statistic	df	Sig.
TL	0.109	28	0.200*
TVL	0.087	28	0.200*
PES	0.087	28	0.200*
EL	0.084	28	0.200*
FA	0.086	28	0.200*
D5MCL	0.109	28	0.200*
D4MCL	0.179	28	0.022
D3MCL	0.159	28	0.069
D3P1L	0.157	28	0.076

* indicate not significant at $p > 0.05$.

4.2.3 Discriminant function analysis for external morphology.

The DFA for external measurements successfully extracted three significant functions; Function 1, 2, and 3 explained 51.5%, 32.6% and 15.8% of the variance respectively (Table 4). Function 1 with higher character loadings has higher variability of characters in the analysis. The Wilk's lambda statistic (Table 5) for the tests of Function 1 through 3 function (Wilk's lambda = 0.372) have a probability of $p = 0.101$, whereas the Function 2 through 3 function (Wilk's lambda = 0.603) have probability of $p = 0.181$. For the test of Function 3 (Wilk's lambda = 0.840) have probability of $p = 0.269$. None from the three Functions has a significance level of $p < 0.05$. All Functions were insignificant (Table 5).

The accuracy rate of correctly classifying each population for external morphology analyses of five characters (Table 6) was only 42.9%. The highest character loadings observed in both Function 1 and 2 is forearm (FA) with a canonical discriminant function standardize coefficient value of -1.657 and 1.506 respectively, whereas in Function 3, it was tibia length (TL) with the value of 1.513 (Table 6).