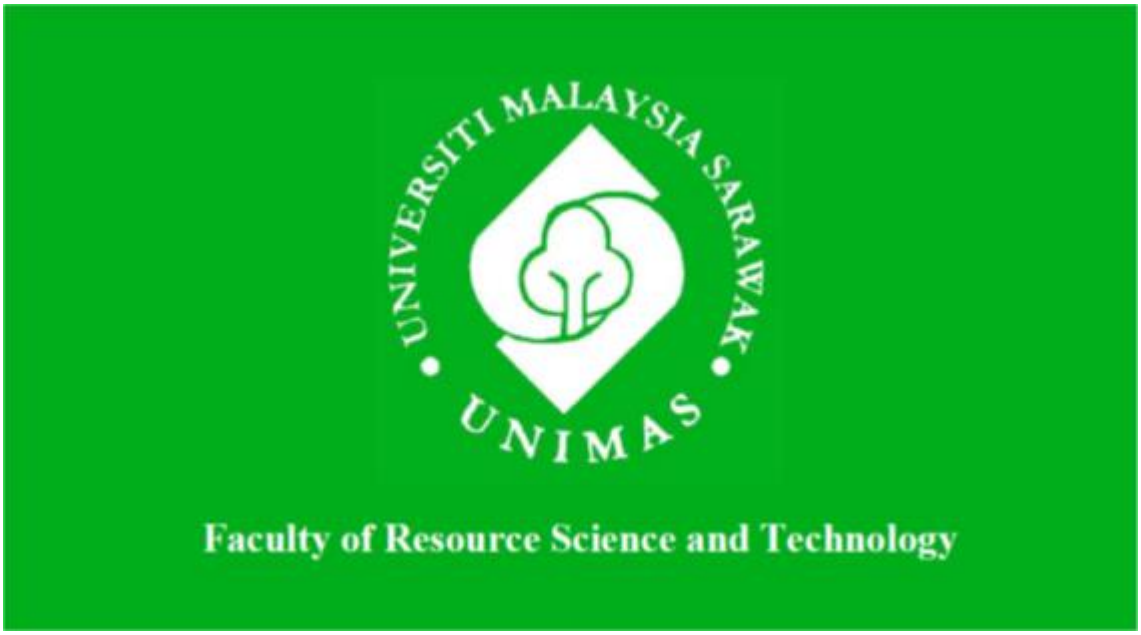

GENETIC, MORPHOLOGY AND ECHOLOCATION VARIATION OF EVENING BATS (GENUS *MYOTIS)* FROM BORNEO

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To my beloved mother, Putri Zalila Binti Megat Yaacob whom had passed on early of my age.

This is for you.

DECLARATION

I hereby declare that no portion of work referred to in 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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List of Abbreviations

NP	National Park
m	Meter
mm	Millimeter
TL	Total length
HB	Length of head body
TA	Length of tail
FA	Length of forearm
TB	Length of tibia
HF	Length of hind foot
E	Length of ear
g	Grams
kHz	Kilo Hertz
ms	Milliseconds
µl	Microlitres
°C	degree celcius
mins	minutes
secs	seconds
N	North
E	East
DNA	Deoxyribonucleic acid
Cytb	Cytochrome-b
RAG 2	Recombination Activating Gene 2
CTAB	Cetyl-Trimethylammonium Bromide

PCR	Polymerase Chain Reaction
MYA	Million Years Ago
EM3	Echo Meter 3
NCBI	National Centre for Biotechnology Information
MEGA	Molecular Evolutionary Genetics Analysis
SPSS	Statistical Package for the Social Sciences
DFA	Discriminant Function Analysis
FM	Frequency Modulated
Fmax	maximum frequency
Fmin	minimum frequency
FPeak	Peak frequency
Dur	Duration

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Genetic, Morphology and Echolocation Variation of Evening Bats (Genus *Myotis)* from Borneo

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ABSTRACT

The island of Borneo is considered as the biodiversity hotspot with a high number of endemicity. The species of bats represent a significant component of the terrestrial mammals on the island. The genus *Myotis* is an insectivorous bat which is a diverse and widespread group from the family Vespertilionidae. Their mouse-eared structure and distinct dentition characterised the genus. The genus has high interspecific diversity that potentially represent cryptic diversity. Therefore, it is important to study the taxonomical differentiation of the members from this genus to better describe their diversity. This is especially important in Borneo where studies on this group is lacking and further hampered by species misidentifications. Hence, three parameters of species identifications in insectivorous bats were explored which is genetic analysis, morphological measurements and echolocation variations. Through genetic analysis on the sequences of mtDNA at *cytb* region, three phylogenetic trees were constructed showing a similar topology and clade members with the Pairwise Distance computation to support the differences. The analysis of morphological measurements using Discrimination Function Analysis (DFA) showed an overlap of the character functions in the scatter plot of this analysis for different species of *Myotis*. Echolocation calls from several species of *Myotis* were described and compared on its call parameters. However, it is not sufficient in determining the species as there is a variety of call structure according to the surrounding during the call's recordings. Hence, it is important in combining these three parameters in order to have a more reliable method in species identification.

Keywords : genus *Myotis*, Borneo, genetic, morphology, echolocation, taxonomy, phylogenetic relationship

ABSTRAK

Pulau Borneo merupakan sebuah pusat kepada jumlah kepelbagaian biologi dan keendemikan yang tingggi. Spesies kelawar mewakili sebahagian penting komponen mamalia darat di pulau ini. Genus Myotis adalah kelawar pemakan serangga yang pelbagai dan mempunyai taburan yang luas dari keluarga Vespertilionidae. Genus ini dikenalpasti dari telinganya yang menyerupai tikus dan struktur gigi yang berbeza. Kepelbagaian interspesies yang tinggi dalam genus ini boleh menyebabkan pelbagai spesis kriptik. Oleh itu, ia amat ditekankan untuk mempelajari perbezaan taksonomi antara spesis dalam genus tersebut supaya kepelbagaian mereka dapat dijelaskan dengan lebih mendalam. Ini menjadi satu keutamaan, lebih-lebih lagi di Borneo, di mana terdapat kekurangan kajian ke atas kumpulan ini dan lebih disukarkan dengan kesalahan identifikasi spesis. Jadi, tiga parameter untuk identifikasi spesis dalam kelawar pemakan serangga telah diguna pakai iaitu kajian genetik, ukuran morfologi dan variasi ekolokasi. Melalui kajian genetik dalam jujukan DNA mitokondria di kawasan cytb, tiga pokok filogenetik telah dibina yang mempunyai topologi dan ahli klad yang sama dengan komputasi jarak Pairwise *untuk menyokong perbezaan. Ukuran morfologi menggunakan Fungsi Diskriminasi analisis menunjukkan menunjukkan pertindihan dalam fungsi karakter dalam graf taburan dari spesis Myotis yang berbeza. Panggilan ekolokasi dari spesis Myotis yang berbeza telah digambarkan dan dibandingkan mengikut parameter panggilan mereka. Namun begitu, ia tidak mencukupi untuk menentukan spesis kerana terdapat pelbagai struktur panggilan mengikut persekitaran semasa rakaman panggilan. Oleh itu, ia adalah penting untuk menggabungkan tiga parameter tersebut untuk mendapat cara yang lebih dipercayai dalam identifikasi spesies.*

Kata kunci : *genus Myotis, Borneo, genetik, morfologi, ekolokasi, taksonomi, hubungan filoginetik*

1.0 Introduction

1.1 General Introduction

Borneo rainforest is renowned worldwide as a tropical rainforest with a high level of species diversity and endemism. According to Mackinnon *et al.* (1996), the hot spot for diversity and endemism in Borneo are found in the north of the island particularly in the northern East Kalimantan, Sabah and eastern Sarawak as well as in Brunei Darussalam (Catullo *et al.,* 2008; Struebig *et al.,* 2012). Species diversity and richness of the volant mammal, bats, in Borneo represent one third of the terrestrial mammals. The species of bats that are dominating the island Borneo typically belonged to the insectivorous bats especially from the families of Hipposideridae, Rhinolophidae and Vespertilionidae (Struebig *et al.,* 2012).

Genus *Myotis* is one of the most widely distributed groups of bats from the family Vespertilionidae. This genus of insect bats is characterised by their mouse-eared structure and distinct dentition among other genera of insectivorous bats as shown in Figure 1. Genus *Myotis* are known to have over 120 extant species which can be found in every geographical area except the South and North Poles and some oceanic islands (Gunnell *et al.,* 2017). There is a high number of morphological similarities within the extant species in the genus *Myotis* that requires a combination of other datasets such as genetic components and echolocation call parameters to better classify the organisms in their own species further.

Figure 1. *Myotis ridleyi* from Bukit Kana National Park

Genetic data provide a better overview of studying the diversity of animals. It is also critical in discovering new mammalian species particularly in diverse groups like the genus *Myotis.* The species of the genus *Myotis* are karyotypically conserved and possessed similar morphology among them (Bickham *et al.,* 2004; Ruedi *et al.,* 2015). Previous molecular studies have shown that the morphology-based subdivision of the genus *Myotis* does not match the phylogeny which indicate, the high level of adaptive convergences (Loso *et al.,* 1998; Stadelmann *et al.,* 2007). With this strong evidence, it is clear that molecular studies should be the main focus for the analysis of species in genus *Myotis*.

Morphological characteristics, on the other hand, is a more traditional method in identifying the genera. It is a fast method in identifying the species of the genus *Myotis* especially in the field. The colouration and morphometric measurements of forearm and hindfoot of the sample caught, can provide a non-invasive way of species identification. In recent studies, species of genus

Myotis were found to have obtained its evolution through various convergent morphological traits by their mode of food acquisition compared to its phylogeny (Stadelmann *et al.,* 2007). Hence, it is suggested that morphological observations of samples paired with molecular analyses will significantly improve the knowledge of these species in tropical areas (Ruedi *et al.,* 2017). Another method that can reinforce the classification of microbats is using its echolocation frequencies.

Echolocation calls in bats had been useful in species identification as well as in the studies of bat's behaviour and ecology (Barclay, 1999; Russo and Voigt, 2016). All bats emit echolocation calls in order to navigate in the dark and detect prey or food items. The echolocation frequencies reflection will allow the organisms to perceive their surrounding by elaborating echo acoustic images. These calls are species-specific concerning their phylogeny, habitat use and biology of the species (Russo and Voigt, 2016). Studies done by Voight-Heucke *et al.* (2010) suggested that, echolocation calls in bats has a dual role, not only for orientation and foraging, it is also used as a communication on individual identity, gender and group affiliation. Hence, utilising the echolocation calls of bats in the genus *Myotis* will be a golden approach in differentiating them.

Fundamentally, the usage of few field-based methods might not be enough in identifying morphologically similar species in bats. This shows that the discoveries of genetic, morphology and echolocation variation among the genus *Myotis* from the family Vespertilionidae is a great initiative to ensure a correct placement of genus *Myotis* organisms found in Borneo are done thoroughly.

1.2 Problem Statement

There is a lack of understanding on the study of genetics, morphology and echolocation frequencies among the morphologically cryptic evening bats. The taxonomic division of the genus *Myotis* had been difficult as there are high plesiomorphic and undifferentiated morphological characters (Ruedi and Mayer, 2001). Genetic studies have also shown that there are multiple lineages of genus *Myotis* currently unrecognised (Larsen *et al.*, 2012). There are also a handful of individuals which are wrongfully classified with other morphologically cryptic *Myotis* among them (Juste *et al.*, 2019) which calls for a clear method in identifying and differentiating the evening bats species Therefore, it is an excellent opportunity to study the genetic, morphology and echolocation variation among genus *Myotis* in hopes of rectifying the confusion of the bats in the genus.

1.3 Objectives

To assess the diversity of genus *Myotis* using genetic analysis and the support of morphological characteristics and echolocation variation of evening bats (Genus *Myotis*) in Borneo.

1.4 Hypothesis

H_o: There is no significant diversity of genus *Myotis* in its genetic components when combined with morphological characteristics and echolocation variations.

H_a: There is a significant diversity of genus *Myotis* in its genetic components when combined with morphological characteristics and echolocation variations.

2.0 Literature Review

2.1 Genus *Myotis* in Borneo

The Borneo island is located at the southeast of Malay Peninsula and the southwest of Philippines. It is the third largest island in the world with a concentrated rainforest area covering approximately 287,000 square miles. Borneo island possessed one of the most unique identities as it comprises of four political territories; Sabah and Sarawak, Kalimantan and Brunei Darussalam (Sada *et al.*, 2019). The most significant identity of the Borneo island is of its species diversity and endemicity (Struebig *et al.*, 2012). There are over 100 species of bats recorded from Borneo (Rosli *et al.*, 2018). Among the species of Chiropterans, there are a number of 10 species of *Myotis* in Borneo (Phillipps and Phillipps, 2016).

The newly recognised species of *M. borneoensis* were found endemic to Borneo. This finding arises from the research on specimens of *M. montivagus* from different localities in the study done by Gorfol *et al.* (2013). The results obtained proved that *M. montivagus borneoensis* have the largest cranial form compared to the specimens from South China and North Myanmar. The species *M. borneoensis* was previously known as the subspecies of *M. montivagus borneoensis* in Hill and Francis (1984), Corbet and Hill (1992) and in the writings of Koopman (1994). Other than that, the specific study of *M. adversus* done by Kitchener *et al.* (1995) found the subspecies of *M. adversus* in Borneo namely, *M. a. carimate* has similarities to the *M. moluccarum* found in Australia, however limited material were found to support the comparison (Gorfol *et al.*, 2013).

The ecology of genus *Myotis* in Borneo were mentioned in several studies. The species habitat of *M. borneoensis* was recorded alongside forest streams and in cave with karst area (Gorfol *et al.,* 2013). Rosli *et al.* (2018) in its recent study observed the roosting behaviour of *Myotis horsfieldii*, where it is one out of 11 species found roosting inside the Wind Cave Nature Reserve. Other than that, the study by Struebig *et al.* (2012) showed that *M. ater, M. muricola* and *M. ridleyi* are all tree roosters with the addition of cave roosting ecology for *M. ater*. In the study done at Bako National Park, Sarawak by Khan *et al.* (2005), two females of *M. ater* were caught in the mangrove and mixed dipterocarp forests. While roosting behavior of the species *M. ater,* were observed to roost as an individual or in small colonies in caves. A single female of *M. muricola* was also found in the riverine forest of Bako National Park (Khan *et al.,* 2005)

2.2 Distribution recordings of genus *Myotis* in Borneo

Recordings on the genus *Myotis* distribution were reported at several locations in Borneo. The Bornean endemic species of *Myotis*, *M. borneoensis* was found occurring in Sabah, Sarawak and also Kalimantan. It was recorded that the species habitat is alongside forest streams while Kalimantan specimens was collected emerging from a cave in karst area (Gorfol *et al.*, 2013). The small bats collection in western Sabah that was done by Benda (2010) proved that an individual of *Myotis muricola* was recorded in Sapulut, Sabah. Despite the statement on the common abundance of the species done by Payne and Francis (1998), Francis and Hill (1998), Suyanto and Struebig (2007) as well as Abdullah *et al.* (2007) and Kofron (2002), only one specimen of *M. muricola* was present among the almost a hundred bats collection in the study (Benda, 2010).

The discoveries of three species of genus *Myotis* in Brunei areas were conducted in a study by Struebig *et al.* (2012). Few individuals of *M. ridelyi* were recorded in Ingei, Peradayan and Temburong. This study also recorded few individuals of *M. muricola* at several locations such as Andulau, Merimbun, Peradayan and Temburong. Additionaly, an individual of the species *M. ater* was also observed in Peradayan (Struebig *et al.*, 2012).

The distribution of the species of *M. ater* in Sarawak was previously recorded only in Kelabit uplands (Payne *et al.*, 1985; Corbet and Hill, 1992; Khan *et al.*, 2005) However, new findings by Khan *et al.* (2005) recorded two individuals of *M. ater* were caught in Bako National Park. While *M. ater* distribution in Sabah is at Gomantong, Baturong and Gunung Kinabalu. Another

new recorded species of the genus *Myotis* in Bako National Park that was done in the same study is *M. muricola*. The species is a new recorded of species in the park and one of the least captured species in the study at only 0.34% or one individual recorded (Khan *et al.*, 2005).

Another survey of *Myotis* distribution in Borneo was done by Abdullah *et al*. (2007) on the large bat caves of Malaysian Borneo recorded an endemic species of *M. gomantongensis* inside the Gomantong Cave situated in the east of the state capital of Sabah, Kota Kinabalu. Phillips and Phillips (2018) mentioned that the species *M. gomantongensis* was only recorded from Gomantong and Baturong Caves in East Sabah in small numbers. Other species of the genus *Myotis* recorded in the study is *M. horsfieldii* that was found in Niah Cave at Niah NP, Sarawak (Abdullah *et al.*, 2007).

2.3 Morphological characteristics of genus *Myotis*

Diversity of organisms can be primarily identified by morphological characteristics and it can be further analysed by morphometric methods for description and comparison with other organisms (Rohlf, 1990). Comparison of morphometric analysis is an easy and useful method for species identification. This can be done by performing discriminant analysis for the functions or the sex of each individual (Ghazali, 2009). A number of morphological characteristics can be utilised in species identification. The tragus shape of bats in genus *Myotis* is a useful identification key in distinguishing interspecific morphological differences among *Myotis* species and intraspecific differences such as from bats of the genus *Kerivoula*. This can be seen in the species of *M. muricola* that has a slender and blunter tragus in compare to other *Myotis* species (Phillipps and Phillipps, 2018). Identification of a few species in genus *Myotis* can be done by observing the bat's limb. (Morgan *et al.*, 2019). According to Phillipps and Phillipps (2018), *M. ater* can be identified by the attached wing membrane to its toes. The wing membrane in *M. adversus* on the other hand, is attached at the ankle.

Research done by Fenton and Bogdanowicz (2002) found that the morphological characteristics of species in genus *Myotis* are able to provide foraging style information which can be associated with the bat's external features. For example, *M. adversus* which are characterised by their large foot are trawlers, which is a type of foraging behaviour that will fly close to water or flat surface where they will take prey with their feet. Other than that, water bats which are aerial feeders, have short calcars, short ears, small hind feet and narrow tragi (Fenton and Bogdanowicz, 2002).

In a study done by Benda (2010), a specimen of *M. muricola* found in Sepulut, Sabah was compared with other specimens from other parts of Asia and Sumatra. The comparison showed that the Bornean samples of *M. muricola* possessed a larger skull size than the specimen found from North-Eastern India, Indochina and Sumatra. It is also shown to be considerably larger than specimens of North Western India. Because of this comparison, Benda (2010) suggested that Bornean populations of *M. muricola* might represent a separate subspecies from NW Indian populations. The study then compared the skull dimensions and ratios to samples of other localities and had deduced that the Sumatran, Indochinese samples of *M. muricola* are similar to each other, while it has distinct skull features to those in NW India (Benda, 2010).

The morphological features of *M. borneoensis* was assessed in the study done by Gorfol *et al.* (2013). It is recorded that the specimen has a dense and long fur at the upperpart of the body, with blackish brown bases and dark brown tips. When compared with other specimens of *M. montivagus*, where the species was previously placed as a subspecies, it shows differences in cranial characteristics and measurements. A presence of high sagittal and lambdoid crests in the Bornean species was observed, whereas other subspecies of *M. montivagus* are poorly developed in the parts. A smaller premolar (P3) was also measured in the Bornean and Peninsular Malaysia specimens compared to those of other Asia regions as shown in Figure 2 (Gorfol *et al.,* 2013). In short, the Bornean species of genus *Myotis* have significant difference between *Myotis* from other localities. The differences of their characters are useful in classifying species of genus *Myotis* according to their geography.

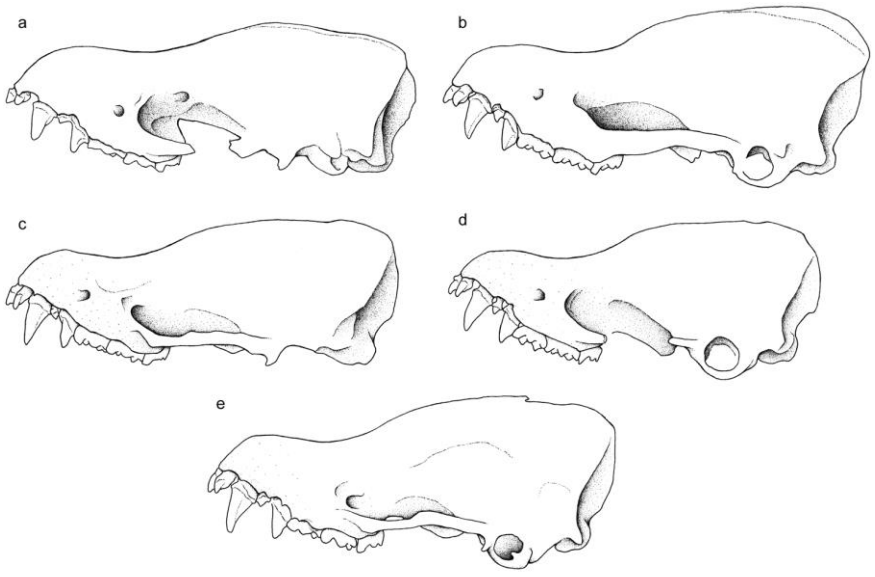

Figure 2. A lateral view of the skulls of a= *M. annectans* from Thailand, b= *M. borneoensis*, c= *M. federatus*, d= *M. montivagus*, e= *M. peytoni*. (adapted from Gorfol *et al.*, 2013)