UTILITY OF BATS (CHIROPTERA) AS ECOLOGICAL INDICATORS IN PENINSULAR MALAYSIA

KHAIRUNNISA BINTI SYARIPUDDIN

FACULTY OF SCIENCE
UNIVERSITY OF MALAYA
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KHAIRUNNISA BINTI SYARIPUDDIN

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Name of Candidate: **KHAIRUNNISA BINTI SYARIPUDDIN**

I/C/Passport No: **900201137446**

Regisration/Matric No.: **SGR130005**

Name of Degree: **MASTER OF SCIENCE**

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ABSTRACT

As ecologists we are tasked with studying the effects of environmental changes on ecosystems, but it is impossible to study every species and every interaction. Therefore, a relatively small group of species must be chosen as a proxy for monitoring general patterns of ecological change. Bats might be suitable for the role as ecological indicators because they have high species richness and abundance, represent several distinct feeding guilds, occupy high trophic positions, and perform key ecosystem services such as pollination, seed dispersal, nutrient recycling and arthropod control.

A critical prerequisite for an ecological indicator group is stable and accurate species recognition. In this regard, the first study was conducted on the changing perspectives of diversity of bats at Ulu Gombak, particularly looking at the role of DNA barcoding in species recognition. During the surveys, DNA barcodes were obtained from 45 bats which were assigned to seven species. Five of these were dark taxa, previously reported species which lack formal description. One bat belonged to a putative cryptic species which had not been reported previously. These five species were added to the cumulative checklist for Ulu Gombak taking the total to 57 species of bats. The high number of cryptic species uncovered supports the prediction that the number of bat species in Ulu Gombak is significantly underestimated. However, the findings showed that DNA barcoding can be employed easily and effectively to recognize well-characterized and stable species units within and across surveys.

The second study assessed the potential of bats as biodiversity indicators, *i.e.* bat diversity as a proxy measure for total biodiversity. For this study, four key criteria and a comparison with beetles and butterflies were used. Based on the four key criteria, bats

and butterflies showed good potential as bioindicators and should be given more prominence in the evaluation of biodiversity in Southeast Asia.

The third study assessed bats as indicators of environmental contamination with a case study looking at mercury contamination in hydroelectric reservoirs. Significantly higher concentrations of mercury were found in the fur of insectivorous bats than frugivorous bats suggesting mercury was being exported out of the reservoirs by aquatic insect prey. Ten bats (*H.* cf. *larvatus*) sampled at Kenyir Lake had mercury concentrations approaching or exceeding 10 mg/kg, which is the threshold at which detrimental effects occur in humans, bats and mice. Future hydroelectric projects should be aware that mercury contamination can occur due to construction of reservoirs and move through the ecosystem through trophic pathways.

The combined findings of the three studies suggest that bats can be effectively employed as ecological indicators. Therefore, bats are recommended to play a central role in monitoring ecological change in Peninsular Malaysia in the years to come.

ABSTRAK

Sebagai ahli ekologi, kita ditugaskan untuk mengkaji kesan perubahan persekitaran ke atas ekosistem, tetapi adalah mustahil untuk mengkaji setiap spesies dan setiap interaksi. Oleh itu, sekumpulan kecil spesies mesti dipilih sebagai proksi untuk memantau corak umum perubahan ekologi. Kelawar berkemungkinan sesuai sebagai petunjuk ekologi kerana mereka mempunyai kekayaan dan kelimpahan spesies yang tinggi, memiliki pelbagai cara pemakanan, berada pada aras trofik tinggi, dan melaksanakan perkhidmatan ekosistem penting seperti pendebungaan, penyebaran biji benih, kitar semula nutrien dan pengawalan artropoda.

Pra-syarat penting bagi kumpulan penunjuk ekologi adalah pengenalpastian spesies dengan tepat. Sehubungan itu, kajian pertama telah dijalankan terhadap perubahan perspektif kepelbagaian kelawar di Ulu Gombak, terutamanya melihat peranan "DNA barcoding" dalam pengenalpastian spesies. Kod bar DNA telah diperolehi daripada 45 kelawar yang dikumpulkan dalam tujuh spesies. Lima daripadanya adalah "taksa gelap" iaitu spesies yang dilaporkan sebelum ini yang mempunyai kurang penerangan formal dan "spesies samar" yang belum dilaporkan. Lima spesies ini telah ditambah kepada senarai semak terkumpul untuk Ulu Gombak menjadikan jumlah keseluruhan kelawar ialah 57 spesies. Bilangan tinggi "spesies samar" yang terbongkar menyokong ramalan bahawa bilangan spesies kelawar di Ulu Gombak lebih tinggi dari anggaran. Walau bagaimanapun, hasil kajian menunjukkan bahawa "DNA barcoding" boleh digunakan dengan mudah dan berkesan untuk mengenali spesies yang stabil.

Kajian kedua menilai keupayaan kelawar sebagai petunjuk biodiversiti, iaitu kepelbagaian spesies kelawar sebagai proksi untuk jumlah biodiversiti. Untuk kajian ini, empat kriteria utama dan perbandingan dengan kumbang dan rama-rama telah dibuat. Berdasarkan empat kriteria utama , kelawar dan rama-rama menunjukkan potensi yang baik sebagai petunjuk biodiversiti yang perlu diberikan keutamaan dalam penilaian kepelbagaian biologi di Asia Tenggara.

Kajian ketiga menilai kelawar sebagai petunjuk pencemaran alam sekitar dengan melihat pencemaran raksa dalam empangan hidroelektrik dengan melakukan perbandingan antara kepekatan raksa dalam kelawar buah dan kelawar serangga . Kepekatan raksa adalah lebih tinggi dalam bulu kelawar serangga. Sepuluh kelawar (*H.* cf. *larvatus*) disampel pada Tasik Kenyir mempunyai kepekatan raksa menghampiri atau melebihi 10 mg/kg, iaitu ambang di mana kesan memudaratkan berlaku pada manusia, kelawar dan tikus. Oleh itu, projek empangan hidroelektrik di masa hadapan harus dipantau sekiranya melibatkan pencemaran raksa.

Gabungan penemuan tiga kajian ini menunjukkan bahawa kelawar boleh berperanan sebagai petunjuk ekologi. Oleh itu, kelawar disyorkan memainkan peranan utama dalam memantau perubahan ekologi di Semenanjung Malaysia pada tahun-tahun mendatang.

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LIST OF SYMBOLS AND ABBREVIATIONS

%	Percentage
~	Approximate
<	Less than
>	More than
±	Plus-minus
≥	Greater than or equal to
ABGD	Automatic Barcode Gap Discovery
BOLD	Barcode of Life Datasystems
COI	Cytochrome c oxidase subunit I
ddH2O	Double distilled water
DNA	Deoxyribonucleic acid
e.g.	Latin phrase *exempli gratia* (for example)
et al.	Latin phrase *et alia* (and other)
g	Gram
h	Hour
ha	Hectare
Hg	Mercury
Hg^{+}	Mercurous
Hg^{2+}	Mercuric
i.e.	Latin phrase *id est* (that is)
m	Meter
MeHg	Methylmercury
mg/kg	Milligram per kilogram
ml	Milliliter
MOTU	Molecular operational taxonomic unit

mtDNA	Mitochondrial deoxyribonucleic acid
PCR	Polymerase chain reaction
ppm	Part per million
SD	Standard deviation
SE	Standard error
sp.	Species (singular)
spp.	Species (plural)
ß	Beta
UMKL	University of Malaya, Kuala Lumpur
vs.	Versus
α	Alpha

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CHAPTER 1

GENERAL INTRODUCTION

Four 'biodiversity hotspots' overlap in Southeast Asia: Indo-Burma, Sundaland, the Philippines and Wallacea (Sodhi *et al.,* 2004). Malaysia is a part of Sundaland and is recognized as one of the twelve mega-biodiversity countries in the world (Giri *et al.,* 2001; Jamadon *et al.,* 2007) with over 15000 species of flowering plants, 1500 species of terrestrial vertebrates and 150000 species of invertebrates (Fong *et al.,* 2006). Among the 290 mammal species known in Malaysia, over 125 are bats and these account for over 10% of the world's bat species (Kingston *et. al*., 2006). Malaysia has been reported as experiencing the highest percentage of forest loss in the world between 2000 and 2012; mostly attributed to logging for timber industries and forest conversion for oil palm plantations (Butler, 2013). This should raise flags among those concerned about global biodiversity hotspots, and especially Malaysia where there is a high number of endemic species. According to the IUCN Red List of Threatened Species (2013), there are 50 species of animals and 190 species of plants listed as critically endangered species in Malaysia. This includes charismatic mega fauna such as the Sumatran rhino (*Dicerorhinus sumatrensis*) which is now extinct in Peninsular Malaysia and represented by only a few surviving individuals in a sanctuary in Sabah. A loss of habitats for wildlife resulted in a loss of biodiversity as well as an increase of human-wildlife conflicts.

As ecologists, we are tasked with studying the effect of these environmental changes on ecosystems, but it is impossible to study every species and every interaction occurring in the ecosystem. Therefore, a relatively small group of species must be chosen as a proxy for monitoring general patterns of ecological change. Hence, this study examines the utility of bats as ecological indicators, focusing in particular on bats in Peninsular Malaysia.

Bats have high species richness and abundance, represent several distinct feeding guilds, occupy high trophic positions, and perform key ecosystem services such as pollination, seed dispersal, nutrient recycling and arthropod control. The human interest in bats, coupled with their unique biology, suggests that bats could be useful, yet currently underappreciated, models for ecology (Jones *et al*., 2009), particularly as conservation flagships, as indicators of the "total" biodiversity of a site, and as indicators of environmental contamination resulting from changes in land use.

Hence, the following objectives were set for this study:

i. To study the potential of DNA barcoding approach in yielding precise assessment of bat diversity.
ii. To examine the potential of bats as an indicator group for "total biodiversity" using assessment of four key criteria and comparison with beetles and butterflies.
iii. To investigate the role of bats as indicators of environmental pollution resulting from changes in land use.

CHAPTER 2

LITERATURE REVIEW

2.1 Bats

Bats (Order: Chiroptera) are the most ubiquitous group of mammals with 330 species described in Southeast Asia (Kingston, 2010) and 1,150 species in the world (IUCN, 2013). Due to high diversity of bat species in Malaysia, the country has become a centre for research on bats. Various research related to populations and assemblages, ecological, behavioral, and biological aspects of different groups of bats have been undertaken. Kingston *et al.* (2003) studied species richness of insectivorous bat in Krau Wildlife Game Reserve. Francis (1994) sampled the Krau Wildlife Game Reserve and Sepilok, Sabah to compare the abundance of fruit bats in the subcanopy and ground level at both sites. Francis (1990) also estimated the community trophic structure of primary lowland dipterocarp forest in Peninsular Malaysia and Sabah. Shafie *et al.* (2011) assessed the diversity of bats in two different habitats (*i.e.* secondary forest and oil palm plantation) along Kerian River, Perak. Campbell et al. (2006) performed a comparative study on the population structure of *Cynopterus* fruit bats in Peninsular Malaysia and southern Thailand.

However, detailed studies of bat diversity suggest that species richness within this mega-diversity region might be underestimated by at least 50%, as higher levels of endemism and greater intra-specific population structure were recognized than previously realized (Francis *et al*., 2010). The high number of overlooked taxa could be attributed to their cryptic behavior and morphology (Clare *et al*., 2007).

DNA barcoding has been shown to be a useful tool for identifying mammal species, particularly when morphological characters (e.g. cranial and dental characters) are not readily available or are unreliable (Borisenko, 2008). DNA samples taken from the animals can be analyzed to provide a standardized DNA sequence which can be used to identify the specimen to a species by comparing with a library of sequences of known species origin in the Barcode of Life Datasystems (www.barcodinglife.org).

Proper documentation of bat species is essential for conservation and bats are ecologically and economically important. Insectivorous bats play important role in regulating insect populations, especially nocturnal insect species. An insectivorous bat can consume between 20-50% of their total body weight of insects in each foraging session (Brunet-Rossinni & Austad, 2004). Since the insectivorous bats occupy a high trophic level, they are likely to show consequences of pollutants before organisms at lower trophic levels because accumulation of pollutants increases at higher positions in the food webs (Jones *et al*., 2009). Therefore, bats have been proposed as an indicator group for measuring pollution and environmental disturbances in the ecosystem (Jones *et al*., 2009). Frugivorous bats disperse seeds and replant forests while nectarivorous bats pollinate many forest flowers (Hodgkison *et al*., 2003). In Malaysia, fruit bats are important to the agriculture as pollinators and seed dispersal agents for at least 31 plant species with high commercial value including durian (*Durio* spp.) and petai (*Parkia speciosa* and *Parkia javanica*) (Kingston *et al*., 2006). The fecal matter of insectivorous bats, abundant on cave floors not only provides a source of nutrients for invertebrates in cave ecosystems but also is used by humans as a fertilizer for agricultural crops (Mildenstein & de Jong, 2011). Moreover, certain species of bats should be given more attention as some are capable of transmitting virus to humans and other animals (Calisher, 2006). For instance, the Nipah and Hendra viruses from fruit bats once caused

diseases in humans in Malaysia (Halpin *et al*., 2000; Yob *et al.*, 2001). Hence, bats are reservoirs for infectious diseases whose epidemiology may reflect environmental stress (Jones *et al*., 2009).

2.2 Bats as biodiversity indicators

The use of indicator taxa in biodiversity assessment overcomes the lack of human resources (e.g. time, money and trained personnel) as it acts as a 'proxy' for the entire biota or "total" biodiversity (Moreno *et al*., 2007). Collectively, these species must have stable taxonomy, be easily surveyed, widely distributed and show graded responses to habitat changes which correlate with the responses of other taxa (Spector & Forsyth, 1998; Moreno *et al*., 2007).

Fenton *et al.* (1992) suggested that the subfamily Phyllostominae is useful as a habitat indicator since they were captured more often in forested than unforested sites in Mexico. High species richness of Phyllostominae in a community indicates a healthy habitat, and the assessment of bat assemblages was suggested to provide sufficient data for decision-making in conservation (Medellin *et al*., 2000). Castro-Luna *et al*., (2007) were able to evaluate the responses of bats to habitat modification by comparing the richness, diversity and abundance of specific feeding guilds and intra-family levels. In contrast to the Neotropics, there has been a lack of studies assessing the potential of bats as a biodiversity indicator group in Southeast Asia.

2.3 Bats as ecotoxicology indicator

In addition to the potential role of bats as a biodiversity indicator, bats could also be employed as an indicator of ecological health in the field of ecotoxicology. The

position of insectivorous bats at a high trophic level could expose them to high levels of contaminants (e.g. heavy metals - lead, cadmium, mercury) through their diet (Alleva et al., 2006; Jones *et al.*, 2009). Bats that feed on insects emerging from aquatic systems can show accumulation of heavy metals such as mercury consumed through their insect prey (Wada *et al.,* 2010). However, relatively little attention has been paid to the concentration of contaminants in bats or other insectivorous animals (Hickey *et al.*, 2001).

In the case of mercury, if it is present in the aquatic insect prey, there should be accumulation of mercury in the fur of insectivorous bats. For instance, measurements for hair taken from insectivorous bats captured in the South River, Virginia, USA, exceeded the specified adverse mercury effect levels of 10 ppm (Nam *et al.,* 2012). Individuals with mercury levels >10 ppm can experience significant and detrimental changes to brain neurochemistry (Wada *et al.,* 2010, Nam *et al.,* 2012). Despite the protected status of bats and their role as bioindicators of general ecosystem health (Jones *et al.,* 2009) the group has not previously been used as a model in ecotoxicology studies in Malaysia.

CHAPTER 3

CHANGING PERSPECTIVES ON THE DIVERSITY OF BATS (CHIROPTERA) AT ULU GOMBAK SINCE THE ESTABLISHMENT OF THE FIELD STUDIES CENTRE IN 1965

3.1 Introduction

In Southeast Asia, the nineteenth century saw a dramatic increase in the rate of discovery of bat species, a trend that leveled off during the first half of the twentieth century (Kingston, 2010). However, over the last two decades, as a result of intensive and new surveying approaches, 14 new species of bats have been described from Southeast Asia, not only from new study sites, but also from well-studied areas (e.g., Bates *et al.*, 2000; Hendrichsen *et al.*, 2001; Matveev, 2005). Peninsular Malaysia supports more than 100 bat species (Simmons, 2005), representing approximately 40% of the native mammal species (Medway, 1982). The species richness of bats at Ulu Gombak, reported as 50 species (Heller & Volleth, 1995), was the highest recorded bat species for a single locality in the Old World until an intensive sampling effort uncovered 65 species at Krau Wildlife Reserve, Pahang (Kingston *et al.*, 2003).

Bats have been proposed as important indicators of the state of ecological communities, and bat surveys are often used for conservation planning on the assumption that the protection of bats will protect key habitat for many other taxa (Francis *et al.*, 2010). However, rapid changes in land use and deforestation in Malaysia in recent decades have put many of the bat species at risk of extinction (Sodhi *et al.*, 2004). Accurate species identifications are important to assess bat diversity but due to the presence of hidden species within cryptic species complexes, the identity of many

Malaysian bats appears to be uncertain (Kingston, 2010). It has been suggested that the real number of bat species is at least twice that currently recognized (Francis *et al*., 2010). The increased use of molecular methods, particularly DNA barcoding (Wilson *et al*., 2014), for bat species identification is proving invaluable in differentiating cryptic taxa overlooked by morphological methods. In the present ethical climate, the fact that accurate species identification can be achieved from small wing tissue punches without the need to sacrifice individuals is another significant advantage (Wilson *et al*., 2014).

Ulu Gombak Field Studies Centre, founded by Medway in 1965 (Medway, 1966), occupies approximately 120 ha of the 17,000 ha Ulu Gombak Forest Reserve. Several pioneering studies in ecology have been conducted at the field centre and a multitude of new species from diverse taxonomic groups have been described from Ulu Gombak by various researchers from all over the world (e.g., Macdonald & Mattingly, 1960; Ballerio & Maruyama, 2010; Nuril Aida & Idris, 2011). The objective of the present study was to investigate the changing perspectives on bat diversity at Ulu Gombak since the establishment of the field study centre, and particularly how estimates of species richness have changed very recently due to the inclusion of DNA barcoding into surveys.

3.2 Materials and methods

3.2.1 Study site

Ulu Gombak Forest Reserve is located at the southern border of the old highway from Kuala Lumpur to Bentong, Pahang. It was selectively logged in 1960s and has very little seasonal variation in temperature (Medway, 1966). Ulu Gombak Field Study Centre of the University of Malaya is situated at the western edge of the reserve (3°20'N, 101°45'E) (**Figure 3.1**). This site is of considerable biological importance in Malaysia and several surveys of bats have been conducted over the past 50 years (e.g. Medway, 1966; Hill, 1972; Sly, 1975; Yenbutra & Felten, 1983; Heller & Volleth, 1989; Yusof, 2005; Syaripuddin, 2012).

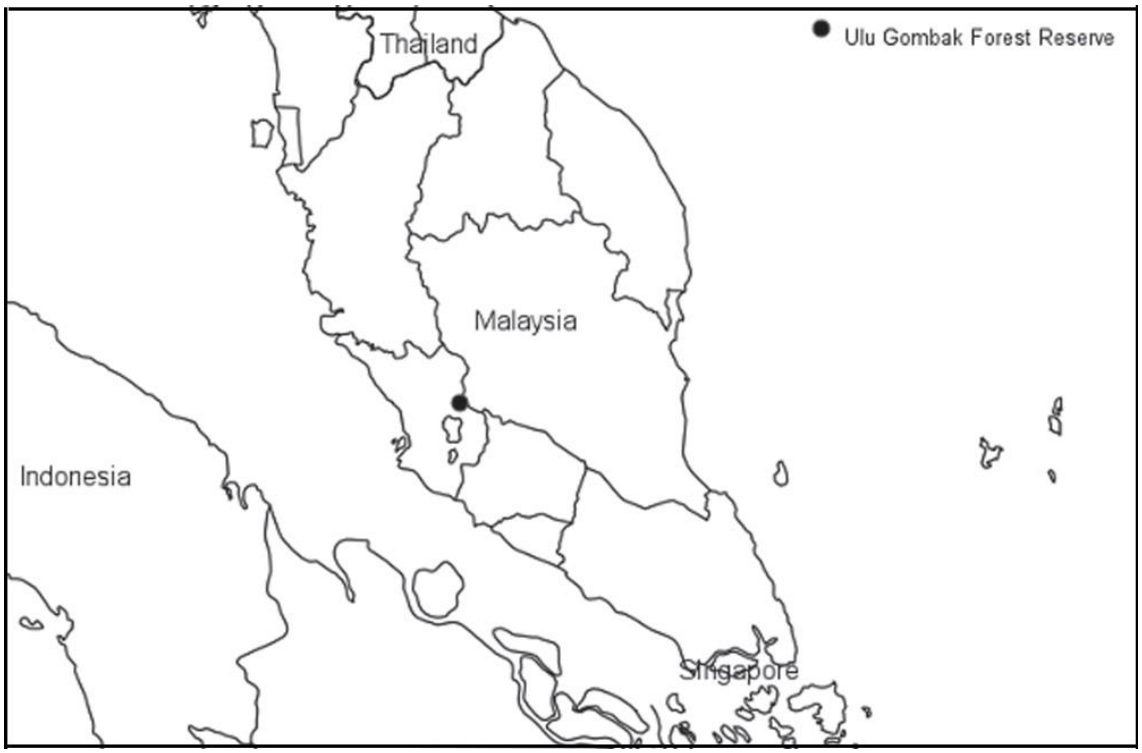

Figure 3.1: Location of Ulu Gombak Forest Reserve and Ulu Gombak Field Studies Centre.

3.2.2 Literature review and museum specimens

Records of bat species recorded at Ulu Gombak since 1966 were extracted from literature (**Table 3.1**). The collection of the Museum of Zoology, University of Malaya (UMKL) was examined for preserved bat specimens collected from Ulu Gombak.

3.2.3 DNA barcoding

Ten mist nets (9 × 4 m) and four harp traps were set at ten locations within Ulu Gombak Forest Reserve from 11–15 November 2012 and 11–14 March 2013. The nets and traps were checked hourly from sunset (19:30) to late night (22:00) and again at sunrise (07:30). The protocols for tissue sampling, DNA extraction, amplification and sequencing of bat DNA barcodes followed Wilson (2012) and Wilson *et al*. (2014) using the universal vertebrate primer pair VF1d_t1 and VR1d_t1 (Ivanova *et al*., 2012). The resulting DNA barcodes were uploaded to BOLD (Ratnasingham & Hebert, 2007) and are available (with GenBank Accessions) in the public dataset DS-MEDWAY. DNA barcodes were assigned to species using the 'Full Database' (see Wilson *et al*., 2014).

3.3 Results

One hundred and sixty records of bats at Ulu Gombak were extracted from literature and the UMKL collection resulting in 52 traditional species records between 1962 and 2012 (**Table 3.1**; **Figure 3.2**). This represents an increase of one species every two years between the initial checklist of Medway (1966), based on an Institute for Medical Research report and our study.

Table 3.1: Checklist of bats species recorded in Ulu Gombak.

	Sources		Sources
PTEROPODIDAE		*Hipposideros bicolor*142[d]	14
Balionycteris maculata	1,10,11,12,13	*Hipposideros cervinus*[E]	8,10,11,13
Chironax melanocephalus[A]	1,10,11,	*Hipposidero cervinus*CMF02[e] *s*	14
*Chironax melanocephalus*GOM01[a]	14	*Hipposideros cineraceus*	1,3,11
Cynopterus brachyotis	1,10,11,12,13,14	*Hipposideros diadema*	1,3,10,11,13
Cynopterus horsfieldi	1,3,10,11,12,13	*Hipposideros galeritus*[e]	1
Cynopterus JLE sp. A	14	*Hipposideros larvatus*	1,11,13
Dyacopterus spadiceus	13	*Hipposideros sabanus*	10,11
Eonycteris spelaea	1,10,11,13	**VESPETILIONIDAE**	
Macroglossus lagochilus[b]	1	*Eptesicus circumdatus*	10,11
Macroglossus minimus[b]	1	*Glischropus tylopus*	10,11,13
Macroglossus sobrinus[B]	10,11	*Hesperoptenus blanfordi*	10,11
Megaerops ecaudatus	9,11,13,14	*Hesperoptenus doriae*	4,10,11
Penthetor lucasi	1,10,11	*Hesperoptenus tomesi*	10,11
Pteropus vampyrus	1,11	*Kerivoula papillosa*[F]	2,11,13
Rousethus amplexicaudatus	10,11,12	*Kerivoula* sp.[f]	1
EMBALLONURIDAE		*Miniopterus schreibersii*	10,11
Emballonura monticola	1,3,10,11	*Murina aenea*	7,11
Taphozous melanopogon	1,11	*Murina cyclotis*	11,13
Taphozous saccolainus	10,11	*Murina suilla*	10,11,13
NYCTERIDAE		*Myotis horsefieldii*	11
Nycteris javanica[C]	10,11	*Myotis montivagus*	3,10,11
Nycteris tragata[c]	13	*Myotis muricola*[G]	3,10,11
MEGADERMATIDAE		*Myotis mystacinus*[g]	1
Megaderma lyra	2	*Myotis ridleyi*	10,11
Megaderma spasma	1,10,11	*Philetor brachypterus*	6,10,11,13
RHINOLOPHIDAE		*Phoniscus atrox*	1,3,4,10,11
Rhinolophus affinis	3,13	*Pipistrellus* sp.[h]	1
Rhinolophus luctus	1,10,11,13	*Pipistrellus stenopterus*[H]	11
Rhinolophus refulgens	11	*Scotophilus kuhlii*[I]	10,11
Rhinolophus sedulus	1,3,10,11,13	*Scotophilus temminckii*[i]	1
Rhinolophus stheno	10,11,13	*Tylonycteris pachypus*	1,3,10,11
Rhinolophus trifoliatus	3,10,11,13	*Tylonycteris robustula*	1,10,11,13
HIPPOSIDERIDAE		**MOLOSSIDAE**	
Coelops frithii	5,11	*Chaerephon* sp.	1,11
Hipposideros bicolor[D]	1,3,10,11,13	*Cheiromeles torquatus*	1,11
*Hipposideros bicolor*131[d]	14		

Species names with same alphabetical superscript have been considered by some researchers to be the same species or synonyms, in such cases, the capital letters are used to denote the valid name.

Sources: 1. Medway, 1966; 2. Medway, 1967; 3. UMKL, 1963-1969; 4. Medway et. al., 1983; 5. Hill, 1972; 6. Hill, 1974; 7. Sly, 1975; 8. Jenkins & Hill, 1981; 9. Yenbutra & Felten, 1983; 10. Heller & Volleth, 1989; 11. Heller & Volleth, 1995; 12. Yusof, 2005; 13. Syaripuddin, 2012; 14. This study.

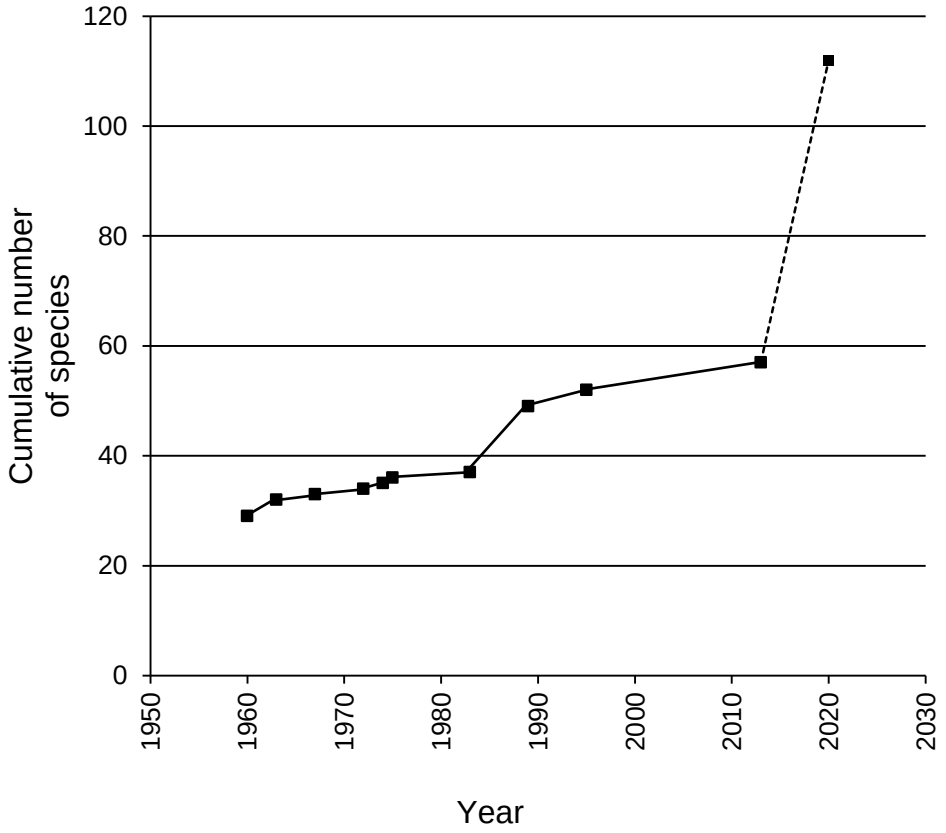

Figure 3.2: Cumulative number of bat species recorded at Ulu Gombak Forest Reserve and the projected number (dashed line) of bat species after intensive DNA barcoding.

DNA barcodes were successfully amplified and sequenced from 45 specimens sampled in our surveys during 2012/2013. The DNA barcodes were assigned into seven taxa (**Table 3.2**). Of these seven, four species were dark taxa (Maddison *et al*., 2012; Wilson *et al*., 2014) in the genera *Cynopterus* (**Figure 3.3**) and *Hipposideros* (see Francis *et al*., 2010; Wilson *et al*., 2014). One DNA barcode matched to *Chironax melanocephalus* but with only 95.8% similarity (**Table 3.2**; **Figure 3.3**) suggesting this belonged to a cryptic species which was annotated as *C. melanocephalus*GOM01. Therefore, of the seven species sampled in our surveys, five (71%) were dark or cryptic taxa. This value and the tally of 52 traditional species were used to extrapolate that the species richness of Ulu Gombak could be 89 bat species (**Figure 3.2**).

Table 3.2: Taxonomic name, similarity (%) and BOLD BIN of the closest matching DNA barcodes to our 45 specimens collected at Ulu Gombak in 2012/2013. The name in parentheses has also been used for the dark taxon.

Field ID	Name of the closest match	Similarity with closest match (%)	BOLD BIN
BGH-1	*Cynopterus* JLE sp. A	99.7	BOLD:AAA9308
BGM-10	*Cynopterus brachyotis*	99.3	BOLD:AAA9800
BGM-11	*Cynopterus brachyotis*	99.5	BOLD:AAA9800
BGH-12	*Hipposideros cervinus*CMF02	100.0	BOLD:AAB6249
BGM-14	*Megaerops ecaudatus*	99.4	BOLD:ABA9836
BGM-15	*Cynopterus brachyotis*	99.7	BOLD:AAA9800
BGM-16	*Megaerops ecaudatus*	98.7	BOLD:ABA9836
BGM-17	*Cynopterus brachyotis*	99.8	BOLD:AAA9800
BGM-18	*Megaerops ecaudatus*	99.3	BOLD:ABA9836
BGM-19	*Chironax melanocephalus* (*Chironax melanocephalus*GOM01)	95.8	BOLD:AAE9045
BGM-20	*Cynopterus* JLE sp. A	99.3	BOLD:AAA9308
BGM-21	*Cynopterus brachyotis*	98.7	BOLD:AAA9800
BGM-22	*Cynopterus brachyotis*	99.5	BOLD:AAA9800
BGM-23	*Megaerops ecaudatus*	98.7	BOLD:ABA9836
BGM-24	*Megaerops ecaudatus*	99.7	BOLD:ABA9836
BGM-25	*Cynopterus brachyotis*	99.7	BOLD:AAA9800
BGM-26	*Megaerops ecaudatus*	98.4	BOLD:ABA9836
BGM-27	*Cynopterus brachyotis*	99.5	BOLD:AAA9800
BGM-2	*Hipposideros cervinus*CMF02	99.8	BOLD:AAB6249
BGM-3	*Cynopterus brachyotis*	99.5	BOLD:AAA9800
BGH-4	*Hipposideros cervinus*CMF02	100.0	BOLD:AAB6249
BGM-5	*Cynopterus brachyotis*	99.7	BOLD:AAA9800

Table 3.2, continued.

Sample	Species	Similarity with closest match (%)	BIN URI
BGM-7	*Megaerops ecaudatus*	99.2	BOLD:ABA9836
BGM-6	*Hipposideros cervinus*CMF02	99.6	BOLD:AAB6249
BGM-8	*Hipposideros cervinus*CMF02	99.5	BOLD:AAB6249
BGM-9	*Cynopterus* JLE sp. A	99.0	BOLD:AAA9308
TF-5	*Cynopterus brachyotis*	99.1	BOLD:AAA9800
TF-6	*Cynopterus* JLE sp. A	100.0	BOLD:AAA9308
TF-8	*Cynopterus brachyotis*	98.2	BOLD:AAA9800
TF-9	*Hipposideros cervinus*CMF02	100.0	BOLD:AAB6249
TF-15	*Hipposideros cervinus*CMF02	100.0	BOLD:AAB6249
TF-20	*Hipposideros cervinus*CMF02	100.0	BOLD:AAB6249
TI-10	*Hipposideros cervinus*CMF02	97.5	BOLD:AAB6249
TI-13	*Hipposideros bicolor*131	99.7	BOLD:AAD3329
TI-14	*Hipposideros cervinus*CMF02	99.8	BOLD:AAB6249
TI-16	*Hipposideros cervinus*CMF02	99.5	BOLD:AAB6249
TI-18	*Hipposideros cervinus*CMF02	100.0	BOLD:AAB6249
TI-21	*Hipposideros* cf. *bicolor* (*H.* bicolor142)	100.0	BOLD:AAC0445
TI-22	*Hipposideros cervinus*CMF02	99.8	BOLD:AAB6249
TI-23	*Hipposideros cervinus*CMF02	100.0	BOLD:AAB6249
TI-24	*Hipposideros cervinus*CMF02	100.0	BOLD:AAB6249
TI-7	*Hipposideros cervinus*CMF02	100.0	BOLD:AAB6249
TI-8	*Hipposideros cervinus*CMF02	100.0	BOLD:AAB6249
TF-7	*Cynopterus brachyotis*	98.5	BOLD:AAA9800
TI-12	*Hipposideros* cf. *bicolor* (*H. bicolor*142)	100.0	BOLD:AAC0445

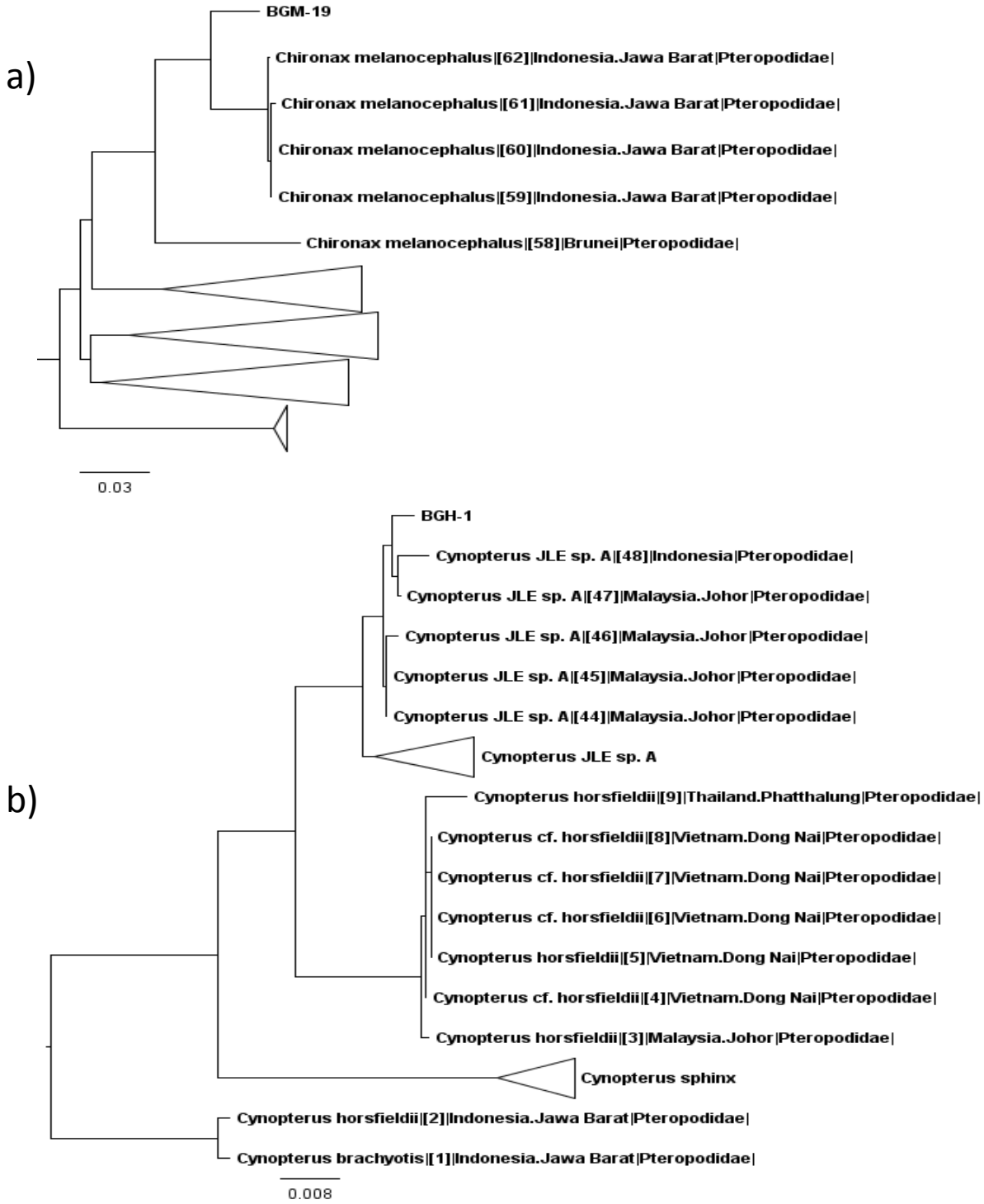

Figure 3.3: Neighbor-joining trees produced by BOLD identification engine for the identification of DNA barcodes a) BGM-19 and b) BGH-1 from bats sampled at Ulu Gombak.

3.4 Discussion

Ulu Gombak has been recognized as the home of one of the most diverse community of bats in the Old World based on species richness (Kingston *et al*., 2003). The literature review and examination of the UMKL collection revealed a total of 52 traditional species records with several taxa missed or omitted in previous compilations. For example, one specimen of *Rhinolophus affinis* in UMKL, collected at Ulu Gombak in 1963; was not included in the checklists of Medway (1966) or Heller & Volleth (1995). This highlights the importance of museum collections as historical records of biodiversity that are relevant and accessible to contemporary research projects. Overall, 28 new records for bat species were documented at Ulu Gombak since the establishment of Ulu Gombak Field Study Centre in 1966, equivalent to one additional species record every two years.

All the previous checklists reviewed in the present study have relied upon morphological identification of species. However, the reported presence of cryptic taxa within morphological species makes diversity assessment using morphological criteria questionable. For example, "*Hipposideros bicolor*" includes two morphologically similar species (*H. bicolor*131 and *H. bicolor*142) (Kingston *et al*., 2001), both present at Ulu Gombak. Cryptic taxa like these can only be recognized by acoustic and/or molecular methods such as DNA barcoding (Kingston *et al*., 2001; Francis *et al*., 2010). Recently a cryptic species from the genus *Kerivoula* with extremely similar morphology (but possibly an unusual fur coloration) to *K. hardwickii* has been described as *K. krau* from Krau Wildlife Reserve after being confirmed by an 11% divergence in DNA barcodes (Francis *et al*., 2007).

When DNA barcoding was incorporated into a survey of bats at Ulu Gombak, DNA barcodes from this survey were found to match the DNA barcodes in BOLD belonging to documented species (e.g., Francis *et al*., 2010) that do not yet have formal species names. These have come to be known as "dark taxa" (Maddison *et al*., 2012; Wilson *et al*., 2014). As a result of this survey, five species (dark taxa) were added to the cumulative checklist for Ulu Gombak taking the total to 57 species. *Chironax melanocephala*GOM01 had not been reported in prior studies, but the deep DNA barcode divergence (4.2%) from conspecifics from Indonesia strongly suggests this is a cryptic species newly uncovered by this survey. Which one is the valid *C. melanocephala* and whether the species are allopatric or both present at Ulu Gombak remain to be seen. The high proportion of cryptic species sampled during relatively small-scale surveys suggests that bat diversity at Ulu Gombak is not yet completely known and is significantly underestimated.

The DNA barcodes from this survey were assigned a species identification with high probability using the BOLD identification engine. This was also the case for the dark taxa due to the extensive DNA barcode reference library for Southeast Asian bats in BOLD (largely from Francis *et al*., 2010). DNA barcodes for *H. bicolor* fell into two distinct clusters (see Francis *et al*., 2010; Wilson *et al*., 2014). Similarly, the deep DNA barcode variation within morphological species in *Cynopterus* had been encountered in prior DNA barcode surveys conducted at other locations. *C.* JLE sp. A is also known as "*C.* cf. *brachyotis* Forest" (Francis *et al*., 2010) and has recently been subject to morphometric cluster analysis (Jayaraj *et al*., 2012). These results support the view that DNA barcoding provides an accurate, rapid and cost-effective approach for identification of bats at Ulu Gombak. The high number of cryptic complexes in this survey supports the suggestion of Francis *et al*. (2010) that the number of bat species in

Southeast Asia is significantly underestimated. The projected number of 89 bat species for Ulu Gombak (**Figure 3.2**) provides a benchmark for future, more intensive surveys using multiple trapping methods and covering a larger area of the reserve, but critically, incorporating DNA barcoding for species recognition.

3.5 Conclusion

This study recorded five added bat species to the cumulative checklist for Ulu Gombak taking the total to 57 species of bats. This suggested that bat diversity in the site is not yet completely well studied and is significantly underestimated. The importance of historical records of biodiversity from museum collections was highlighted to be relevant to contemporary research projects. Also, the presence of cryptic taxa which can only be recognized by acoustic and/or molecular methods such as DNA barcoding makes diversity assessment utilizing morphological criteria questionable. Hence, further intensive surveys using multiple trapping techniques which cover larger part of the reserve should be conducted in the future, taking into account the importance to incorporate DNA barcoding as well as access of museum collections for more precise species inventories. The presence of cryptic species would need the consideration to reexamine the total biodiversity of other forest reserves as well.

CHAPTER 4

ARE BUTTERFLIES, BATS AND BEETLES GOOD BIODIVERSITY INDICATORS IN TROPICAL SOUTHEAST ASIA? AN ASSESSMENT USING FOUR KEY CRITERIA AND DNA BARCODES

4.1 Introduction

The world is facing rapid growth of the human population and widespread urbanization (United Nations, 2004; Bongaarts, 2009). In Asia in particular, the human population has doubled over the last 40 years (Jones, 2013). Consequently, the availability of habitats for wildlife is diminishing, resulting in extinction of species (McKinney, 2002; Kowarik, 2011). Protecting habitats is vital to conserve populations of species in decline. However, the designation of all remaining wild land as protected areas is unrealistic. In an effort to conserve the most species, sites with the highest total biodiversity should be selected to receive complete protection (Mittermeier *et al*., 1998). Informed decision-making requires assessment of the biodiversity of a site (α-diversity) and comparisons of biodiversity between sites (ß-diversity) (Martin *et al*., 2005), yet, due to limited time and resources performing an inventory of all the species present at a site is an impossibility. Thus, a relatively small group of species, sometimes even a single species (Spitzer *et al*., 2009), is frequently used as a proxy for "total" biodiversity (Ferris & Humphrey, 1999, Kerr *et al*., 2000, Koch *et al*., 2013).

Various criteria have been suggested for the selection of an ideal biodiversity "indicator" group (e.g. Pearson, 1994; Ferris & Humphrey, 1999; Fleishman *et al*., 2000; Cleary, 2004). The attributes commonly regarded as essential for a bioindicator group can be synthesized under four key criteria:

(i) Tractable taxonomy – The component species must be easy to identify even by non-specialists, facilitating comparisons between surveys conducted at different times, different locations and by different researchers. DNA barcoding, the use of short standardized DNA sequences for species identification, can impact on this criterion, allowing rapid evaluation of species diversity by non-experts (Laforest *et al*., 2013; but see Krishnamurthy & Francis, 2012).

(ii) Easily surveyed – A well-known ecology allows for the design of effective sampling protocols that can be standardized and deployed in a cost- and time-efficient manner.

(iii) Broadly distributed higher taxa; specialized and habitat-sensitive lower taxa – The group must be present at all sites with stable population sizes, but exhibit different species composition at different sites.

(iv) Patterns of biodiversity reflected in other groups – The group should be a biodiversity "umbrella", meaning conservation of the group would benefit numerous co-occurring species from other groups (Fleishman *et al*., 2000).

Various animal groups have been advocated as useful bioindicators including: butterflies (Lepidoptera), due to their intimate relationship with plants (e.g. Thomas, 2005; Spitzer *et al*., 2009); bats (Chiroptera), due to their high diversity, top-predator and conservation status (e.g. Pineda *et al*., 2005; Jones *et al*., 2009); and dung beetles (Coleoptera), due to their ecological specialization and relationship with mammals (e.g. Spector, 2006; Novelo *et al*., 2007). In this study butterflies, bats and beetles were assessed against the four key criteria above to determine their potential as bioindicator groups in tropical Southeast Asia.

4.2 Materials and methods

4.2.1 Field sites

Standardized surveys of the three target groups (butterflies, bats and dung beetles) were conducted at Rimba Ilmu Botanic Garden, University of Malaya, Kuala Lumpur (N 03° 7', E 101° 39') and Ulu Gombak Forest Reserve, Selangor (N 03° 19', E 101° 44') (**Figure 4.1**). Rimba Ilmu is an 80 ha tropical botanical garden, formerly a rubber plantation, which houses over 1,600 species of tropical plants (Jussof, 2010). Ulu Gombak Forest Reserve is a 17,000 ha selectively logged forest reserve (Sing *et al*., 2013). The surveys were conducted at each site over three days and three nights and were completed during two consecutive weeks in March 2013. The days were all dry and sunny and the nights also clear and dry, with the exception of a small amount of rain on the second night at Rimba Ilmu (<2 h).

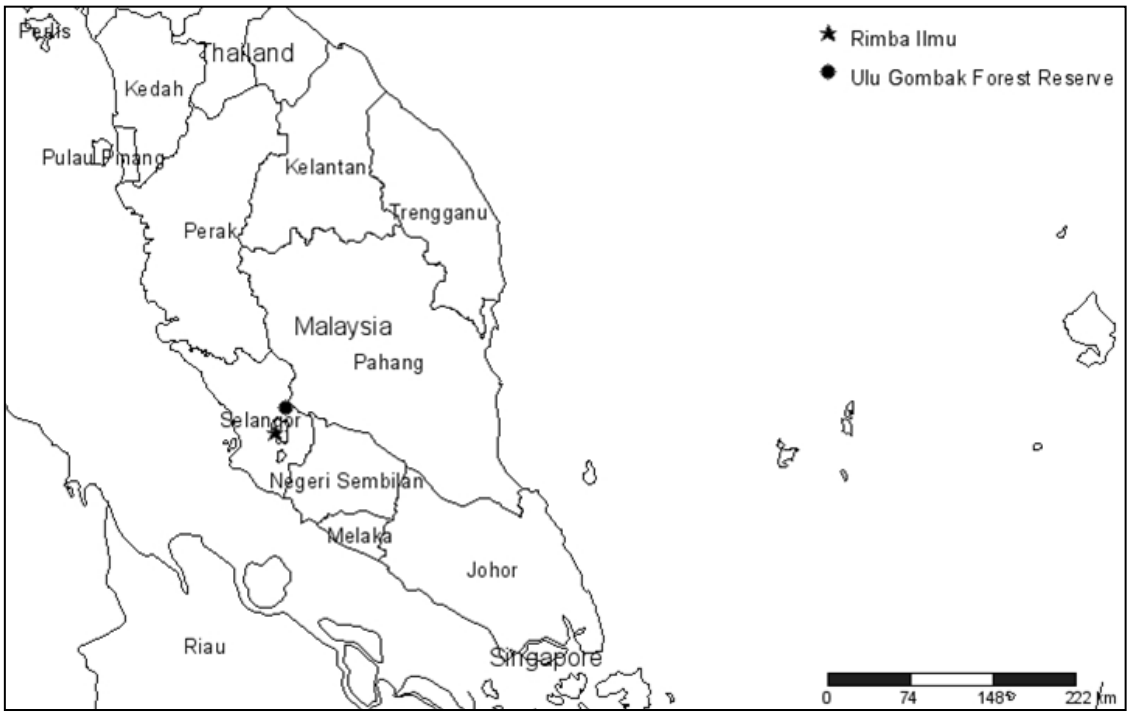

Figure 4.1: The two study sites in Peninsular Malaysia.

4.2.2 Standardized sampling protocols

Butterflies were sampled using sweep nets by two experienced butterfly catchers walking continuously at a standardized pace along two 1000 m transects, 500 m apart, between 10:00 and 12:00 (following Pollard, 1977). The right hind leg (when viewed dorsally) of each captured butterfly was collected into a 1.5 ml tube using forceps before the butterfly was released. If a butterfly with no right hind leg was captured it was released as a probable re-capture.

Ten mist nets and four harp traps were set along two transects 500 m apart between 19:00-07:30 to sample bats. The nets and traps were checked every hour until 22:00 and then checked again at 07:30. A small wing punch was collected from each captured bat into a 1.5 ml tube following AMNH (2013). If a bat with a wing punch was captured it was released without re-sampling as a probable re-capture.

Beetles were sampled overnight using the standardized trapping protocol of Inward *et al*. (2011) with slight modifications. In brief, 20 baited pitfall traps were set 10 m apart along two 90 m transects, 500 m apart. On each transect, five traps were baited with fresh cow dung and five with raw chicken liver. Traps were emptied each morning. Beetles were rinsed in ddH2O then complete specimens in the case of small beetles, and single legs of large beetles, were placed individually into 1.5 ml tubes.

4.2.3 DNA barcoding

DNA extraction from bats and beetles was performed using a Nucleospin kit (Machery-Nagel, Germany) and from butterflies using a XytXtract™ Animal kit (Xytogen, Australia) following the manufacturers' instructions. A first attempt was made to PCR amplify the DNA barcode region of COI mtDNA following standard protocols (Wilson, 2012) using the primer pairs LepF1/LepR1 for butterflies and beetles and VF1d_t1/VR1d_t1 for bats (Wilson *et al*., 2014). If the first PCR failed PCR troubleshooting was conducted using the primer pairs MLepF1/LepR1 (Wilson, 2012) for butterflies and beetles and RonM/VF1d_t1 (Wilson *et al*., 2014) for bats. PCR products were sequenced using LepR1 or the M13R (t1) tail. The DNA barcodes were edited and aligned (Wilson, 2012) and sorted into molecular operational taxonomic units (MOTU) using the online Automatic Barcode Gap Discovery (ABGD) system (Puillandre *et al*., 2011). Previous studies have shown that there is typically a distinct pattern to intra- and interspecies DNA barcode genetic distances, a "barcode gap", but that this pattern can be unique to a dataset. ABGD uses an automatic recursive procedure to converge on the best patterns for the dataset and arranges DNA barcodes into clusters accordingly. The median number of ABGD clusters was used as the basis for the MOTU as this has produced good correspondence with traditional species in empirical studies. Representatives of each MOTU were submitted to the full database of the BOLD identification engine (Ratnasingham & Hebert, 2007) to assign a taxonomic name to the MOTU. Species names were assigned using a >98% sequence similarity threshold. When there was no match >98%, family names were assigned using the strict tree-based method of Wilson *et al*. (2011) based on the "Tree Based Identification" of the BOLD identification engine (Ratnasingham & Hebert, 2007). This method requires

the unknown DNA barcode to be nested within a cluster of sequences from the same family.

4.2.4 Assessment of the groups against key criteria

(i) Tractable taxonomy – This criterion was assessed based on DNA barcoding success. Successful PCR amplification on the first pass, and the number of MOTU assigned taxonomic names, were quantified.

(ii) Easily surveyed – The number of individuals and MOTU sampled were divided by the total number of person-hours required for surveying the group.

(iii) Broadly distributed higher taxa; specialized and habitat-sensitive lower taxa –

The similarity between sites in terms of higher taxa (families) and species (MOTU) was assessed using the Sorenson Similarity index. The index has values between 0 and 1 with 1 indicating identicalness. For families, values closer to 1 are preferable, whereas for species, values closer to 0 are preferable.

(iv) Patterns of biodiversity reflected in other groups – The relationship between the species richness of each group was analyzed using Pairwise Spearman's Rank Correlation (following Koch *et al*., 2013).

4.3 Results

4.3.1 Tractable taxonomy

The PCR success rate on the first pass was high for both bats and butterflies (>70%) but low for beetles (36%) (**Table 4.1**). After troubleshooting, eighteen butterfly DNA barcodes were discarded as likely contaminants as they were either messy sequences or failed to match target taxa in BOLD. Two bat samples failed to PCR amplify after several attempts. Two beetle samples also failed to PCR amplify after several attempts while a further three were likely contaminants as they showed high similarity with non-target taxa. The DNA barcodes produced for this study are available on BOLD in the public dataset DS-MBIO. The number of MOTU assigned a species and family name was high for butterflies and bats (>82%) compared with beetles (**Table 4.1**).

Table 4.1: DNA barcoding success for butterflies, bats and beetles.

Group	n[a] (Rimba Ilmu/ Ulu Gombak	PCR success on first pass (%)	Number of MOTU	Number of families	MOTU assigned a species name (%)	MOTU assigned a family name (%)
Butterflies	125/138	71	78	6[b]	82	99
Bats	16/27	81	7	3	86	100
Beetles	123/93	36	40	10	8	68

[a]Includes samples which failed to amplify and likely contaminants.
[b]There are only six families of butterflies, but one specimen of the Lepidoptera family Callidulidae, which contains day flying moths, was also sampled as part of this indicator group.

4.3.2 Easily surveyed

This study required 24 person-hours for sampling butterflies, 216 for sampling bats and 14 for sampling beetles. Bats accounted for an order of magnitude fewer individuals and species sampled per person-hour than the butterflies and beetles (**Figure 4.2**).

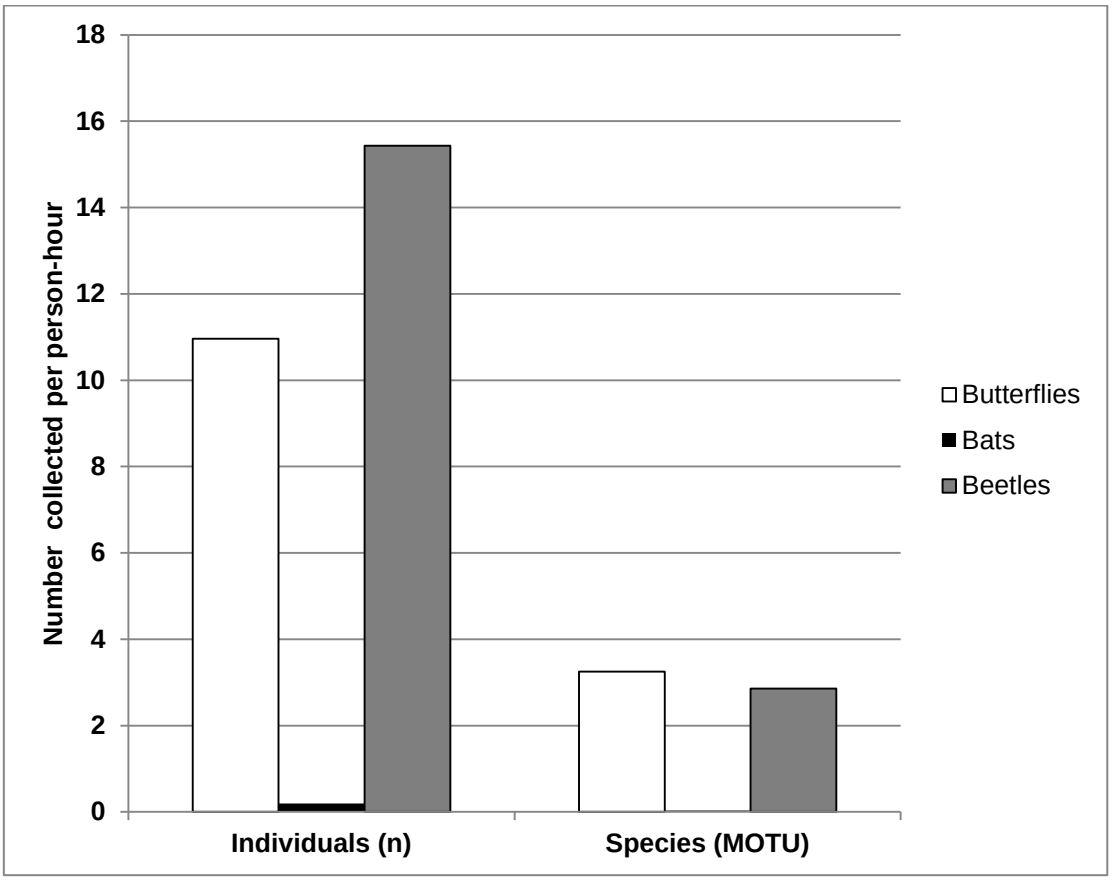

Figure 4.2: Comparison of effort required to sample the three potential bioindicator groups.

4.3.3 Broadly distributed higher taxa; specialized and habitat-sensitive lower taxa

The two sites showed high similarity (≥80% shared between the two sites) in terms of the butterfly and bat families sampled. All groups were relatively habitat-sensitive at species level with less than 15% overlap of MOTU between the two sites (**Figure 4.3**).

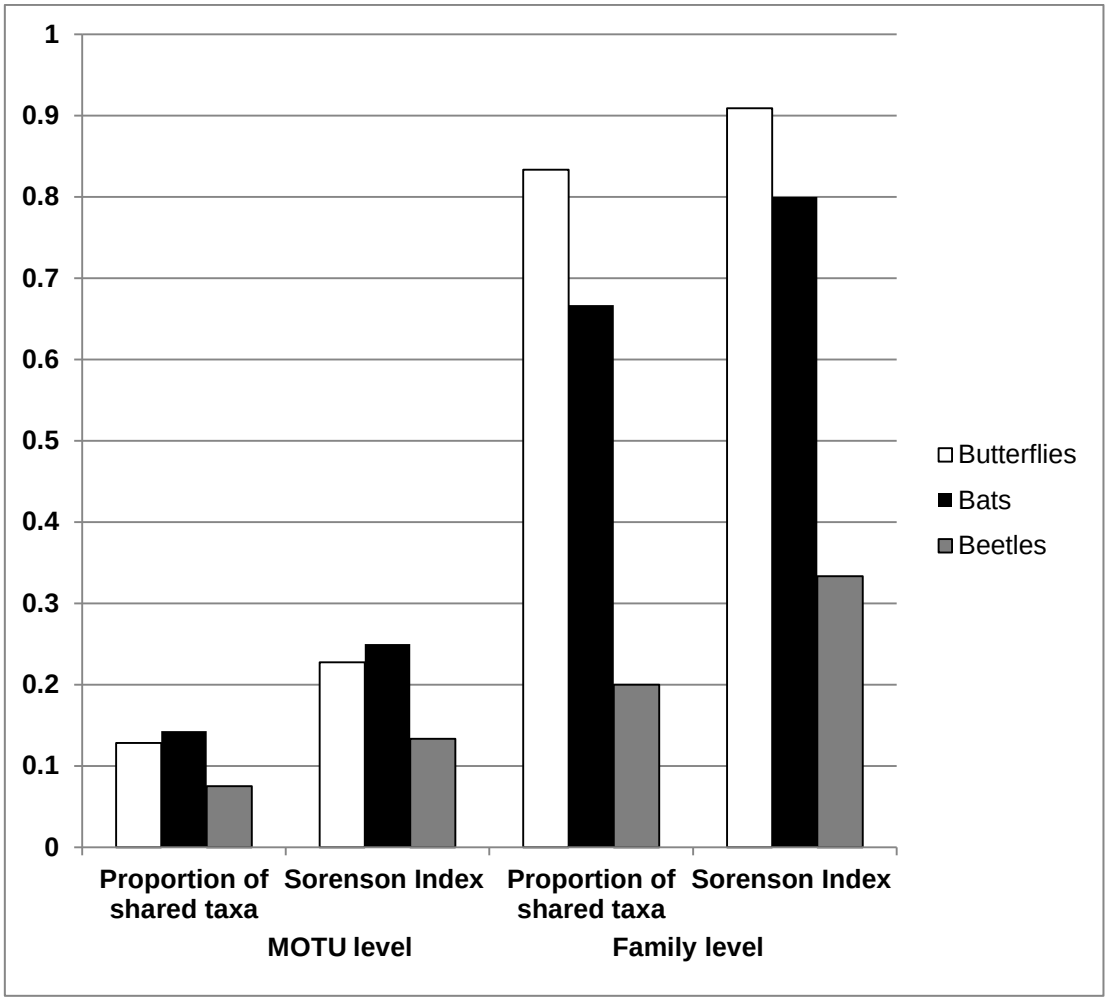

Figure 4.3: The distribution of butterfly, bat and beetle taxa between two sites, Rimba Ilmu and Ulu Gombak.

4.3.4 Patterns of biodiversity reflected in other groups

The species richness of all three groups were positively correlated with each other (**Table 4.2**; **Figure 4.4**). The species richness of butterflies and bats were strongly correlated and statistically significant ($p < 0.02$). Both bat and beetle species richness and beetle and butterfly species richness were weakly correlated and not statistically significant (**Table 4.2**).

Table 4.2: Pairwise comparisons using Spearman's Rank Correlation between species diversity of butterflies, bats and beetles during six sampling events at Rimba Ilmu and Ulu Gombak. Values below the diagonal are the Spearman's Rank Correlation coefficient; values above the diagonal are p-values.

	Butterflies	Bats	Beetles
Butterflies		<0.02	<0.32
Bats	0.88		<0.82
Beetles	0.49	0.12	

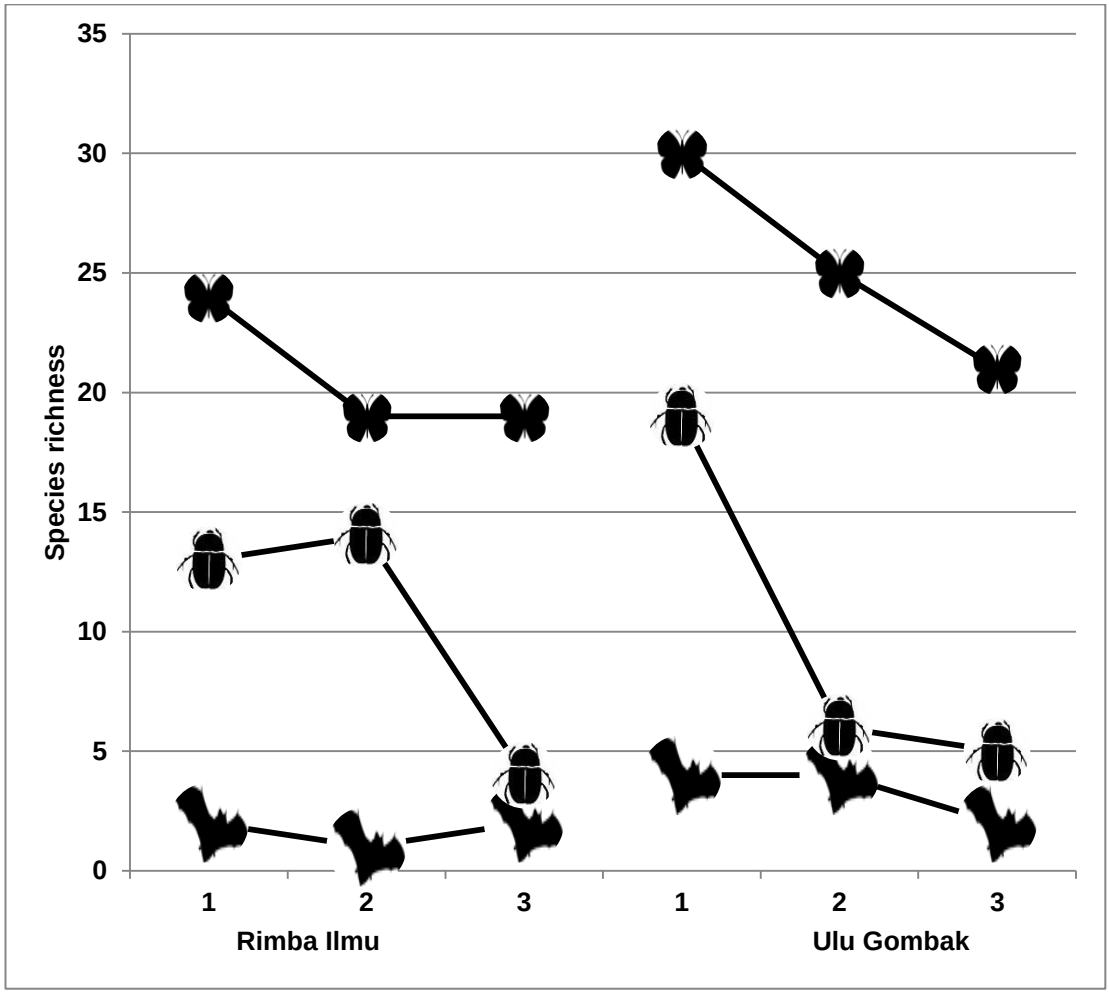

Figure 4.4: Patterns of species richness of butterflies, bats and beetles during six sampling events at Rimba Ilmu and Ulu Gombak. The relationship between the species richnesses of each group was analyzed using pairwise Spearman's rank correlation.

4.4 Discussion

In light of rapid habitat loss in Southeast Asia, there is a pressing need for a standardized system of rapid, yet meaningful, measures of biodiversity. Considering that performing a complete inventory of species at a site is impossible, it is not surprising that research on the use of limited groups of species as indicators of biodiversity has a long history (e.g. Pearson, 1994; Lawton *et al*., 1998; Jonsson & Jonsell, 1999; Rainio & Niemelä, 2003). However, the choice of such indicator groups still remains largely intuitive rather than evidence-based (Gillison *et al*., 2013). In this study, a model for assessment of the bioindicator potential of a group based on four key criteria was presented, which was then used to provide quantitative data on the bioindicator potential of three groups (butterflies, bats and beetles) surveyed at two sites in Peninsular Malaysia.

The first criterion used to assess bioindicator potential of a group was "taxonomic tractability", the ease of identifying component species by non-specialists. For this study, this criterion was evaluated through DNA barcoding success. DNA barcoding is the use of short standardized DNA sequences for species identification (Kress & Erickson, 2012). Butterflies and bats have been the target of large DNA barcoding campaigns (e.g. Clare *et al*., 2007; Janzen *et al*., 2009; Dincă *et al*., 2011) including recently in Southeast Asia (Francis *et al*., 2010; Wilson, 2013). Consequently the protocols for DNA barcoding these groups are well-optimized (Ivanova *et al*., 2012; Wilson, 2012). Therefore it was not surprising that bats had high PCR amplification success in this study (81% on the first pass) but the lower success (71%) for butterflies was unexpected.

The DNA barcode reference libraries for Lepidoptera and Chiroptera are well-developed with more than 700,000 DNA barcodes (75,000 species) for Lepidoptera and 20,000 DNA barcodes (700 species) for Chiroptera in BOLD. The large reference libraries resulted in a high number (>82%) of assignments of species and family names to the MOTU from these groups, after blasting representatives against BOLD. An advantage of the DNA barcoding approach is that it can assign samples to "dark taxa" adding precision to species inventories. "Dark taxa" are species that have been previously recognized and reported by researchers, often through DNA barcoding, but which have not (yet) been formally described (Maddison *et al*., 2012). For example, *Cynopterus* cf. *brachyotis* Forest also known as *Cynopterus* JLE sp.A, reported by Francis *et al*. (2010) was recorded in this study. Unlike butterflies and bats, beetles have relatively poor coverage in BOLD and the lack of optimization for beetle DNA barcoding is a major drawback to the use of beetles as a bioindicator group. DNA barcoding studies of beetles have tended to target the 3' end of COI mtDNA (Baselga *et al*., 2013a) and the commonly used 'Lep' insect primers targeting the 5' "barcode region" (Wilson, 2012), as used in this study, seem to have low success for beetles. However, new primers have recently been designed to target the 5' region in beetles (Baselga *et al*., 2013b) which could impact on the rating of beetles for this criterion (**Table 4.3**).

Table 4.3: Ranking of groups for bioindicator potential according to four key criteria.

Criterion	Butterflies	Bats	Beetles
Tractable taxonomy	2	1	3
Easily surveyed	2	3	1
Taxonomic distribution	1	2	3
Diversity patterns reflected in other groups	1	2	3
Overall Rank	**1**	**2**	**3**

The second criterion used to assess bioindicator potential of a group was "easily surveyed" and was quantified through cost- and time-efficiency of the sampling protocol. In terms of person-hours required for the sampling protocols, beetles required the least hours while bats required the most. Bat sampling requires at least two people to set up and disassemble mist nets and harp traps, as well as to attend to the catch regularly to avoid injury to the bats and escapees. Conversely, beetle traps can be set by an individual and left unattended overnight. In terms of cost, bat sampling requires expensive specialized equipment, while butterflies and beetles can be sampled with inexpensive homemade devices. However, it is also worth considering the ease of achieving a precisely comparable sampling protocol. For example, we can easily imagine a tendency for keen butterfly collectors to target any "rare" or unusual species they encounter during their walks rather than collecting randomly from among the butterfly assemblages. An alternative approach to sweep net sampling could be to use Malaise or light traps to sample lepidopterans and this may also reduce the required person-hours. The pitfall traps were raided by dogs at Ulu Gombak but left undisturbed at Rimba Ilmu. Ants were also a confounding factor, constructing nests over the traps at Ulu Gombak and probably contributing to the lower number of beetles sampled at this site. The choice of location for setting mist nets and harp traps can have an effect on the efficiency of bat trapping, being influenced by vegetation and microclimate (Larsen *et al*., 2007). Other factors affecting the ability to generate comparable survey data include 'tourists', and the seasonality of species (New, 1997).

The third criterion used to assess bioindicator potential of a group was "broadly distributed higher taxa; specialized and habitat-sensitive lower taxa". There was a high similarity (>66%) of family composition at Rimba Ilmu and Ulu Gombak Forest Reserve for both bats and butterflies. Bats are generally widespread in term of distribution but each species occupies a specific habitat (e.g. caves, bamboos, hollow barks, foliages etc.)

(Francis, 2008). Butterflies are ubiquitous in vegetated terrestrial ecosystems yet specialized, e.g. to disturbed or primary forest areas, as their caterpillars have strict dependence on specific host plants (Kunte *et al*., 1999). The sampling included ten families of beetles and the group exhibited a pattern of specialization at both low and high taxonomic levels, although Scarabaeidae dominated the samples at both sites, indicating their high preference for the baits (Larsen & Forsyth, 2005).

The fourth criterion used to assess bioindicator potential of a group was "patterns of biodiversity reflected in other groups". A good bioindicator group should be able to be used to predict the diversity patterns of other unrelated groups at the site. The species richness of all three groups were found to be correlated with each other. The species richness of butterflies had a significant correlation with the species richness of bats suggesting that the species richness of butterflies is useful to predict the species richness of bats and vice versa. Harvey *et al*. (2006) likewise, found a significant correlation between species richness of bats and nectarivorous butterflies in Rivas, Nicaragua. However, in another study in the Neotropics, butterfly species richness was found to be strongly correlated with species richness of birds but not with mammals (Robbins & Opler, 1997). Under this criterion butterflies were ranked higher than bats as the butterflies-beetles correlation was marginally stronger than the bats-beetles correlation (**Table 4.2**).

4.5 Conclusions

In this study a model for the assessment of the bioindicator potential of a group based on four key criteria was presented, which was then used to provide quantitative data on the bioindicator potential of three groups, butterflies, bats, and beetles, surveyed at two sites in Peninsular Malaysia. Butterflies had the most potential as a bioindicator group ranking first in two of the criteria, taxonomic distribution and reflection of diversity patterns in other groups, and second in another two, taxonomic tractability and ease of surveying (**Table 4.3**).

DNA barcoding protocols for butterflies are well-optimized and there is a well-developed DNA barcode reference library available with which to assign butterfly DNA barcodes a precise taxonomic name. Furthermore, butterfly sampling requires only a few hours per day with simple apparatus. Butterfly families are few and widespread, but species are habitat specific due to a strict dependence on certain host plants. Butterfly species richness showed a significant correlation with the species richness of another unrelated animal group, bats. The ability to generate comparable survey data is an important factor in the establishment of bioindicator groups as well as the development of optimized DNA barcoding protocols and DNA barcode reference libraries. Despite easy surveying, beetles suffered in the overall ranking due to inefficient PCR amplification and low representation in BOLD.

The results suggest that out of the three animal groups assessed, butterflies has the most potential as an indicator of biodiversity and surveys of butterflies should be given more prominence in evaluation of biodiversity at sites in Southeast Asia.

CHAPTER 5

MERCURY ACCUMULATION IN BATS NEAR HYDROELECTRIC RESERVOIRS IN PENINSULAR MALAYSIA

5.1 Introduction

Mercury (Hg) contamination has become a well-known global issue (Pacyna *et al*., 2006; Selin *et al*., 2007) as the burning of coal, creation of hydroelectric dams, metal mining and municipal waste incineration have increased and augmented the amount of inorganic mercury entering the atmosphere and water sources (Chan *et al*., 2003). Extensive deforestation and agricultural land use also release mercury from soils creating point sources of local, acute contamination (Barbosa *et al*., 2003). Lake-sediment records suggest locations distant from point source contamination can also receive significant inputs of anthropogenically released mercury due to transcontinental and global distribution of highly volatile, atmospheric mercury (Fitzgerald *et al*., 1998; Chan *et al*., 2003).

In aquatic systems, relatively harmless inorganic mercuric (Hg^{2+}) or mercurous (Hg^{+}) forms of mercury are naturally present in the substrate, but can be transformed by sulphate-reducing and iron-reducing bacteria to methylmercury (MeHg) (Chan *et al*., 2003; Poulain & Barkay, 2013). Significant amounts of mercury can be introduced into aquatic foodwebs during the flooding of forests (Barbosa *et al*., 2003), such as during the construction of hydroelectric dams (Bodaly *et al*., 1984; Stokes & Wren, 1987; Ikingura & Akagi, 2003). When a reservoir is created, submerged vegetation and organic material start to slowly decompose (Rodgers *et al*., 1995), leading to a rise in the dissolution rate of organic car- bon, increased release of mercury bound to organic

material and higher net mercury methylation rates (Chan *et al*., 2003). A deeper water column and increased decomposition creates anoxic conditions which are ideal for mercury methylation (Hylander *et al*., 2006). A study of reservoirs up to 67 years old suggested that it may take 20–30 years before mercury concentrations return to pre-dam levels (Hylander *et al*., 2006).

Methylmercury has been shown to be a potent neurotoxin in humans (Mergler *et al*., 2007) and other mammals including bats and otters (Basu *et al*., 2005; Nam *et al*., 2012). Central nervous system damage caused by methylmercury toxicity in mammals includes motor and sensory deficits and behavioral impairment (Wolfe *et al*., 1998). Increased levels of methylmercury in vertebrates have been shown to impair reproductive system function (Wada *et al*., 2010; Nam *et al*., 2012). Methylmercury is readily transferred across the placenta and can concentrate selectively in the fetal brain, causing developmental alterations leading to fetal death (Wolfe *et al*., 1998). Infants can also be exposed to methylmercury during lactation (Mergler *et al*., 2007).

Mercury biomagnifies as it moves up the food chain, with high trophic level species, such as top predators showing higher concentrations of mercury in their tissues than primary consumers, which absorb mercury (Barbosa *et al*., 2003, Stewart *et al*., 2008). Insects that have aquatic larval stages could act as biovectors, exporting methylmercury from aquatic systems upon emergence (Benoit *et al*., 2013; Mogren *et al*., 2013). The biomass of aquatic insects can reach 190kg/ha per day in productive lake systems (Mogren *et al*., 2013).

Most studies of environmental mercury contamination have been conducted in temperate regions (e.g. Baxter, 1977; Tweedy *et al*., 2013), have measured total mercury in fish (e.g. Barbosa *et al*., 2003), aquatic insects (e.g. Hall *et al*., 1998; Benoit *et al*., 2013); or fish-eating birds and mammals (see Chan *et al*., 2003 and references

therein). In Malaysia, studies have examined mercury levels in fish and seafood (e.g. Bloom, 1992; Agusa *et al*., 2005; Hajeb *et al*., 2009) and in humans living in coastal communities, or in fishing communities near lakes (e.g. Sivalingam & Sani, 1980; Hajeb *et al*., 2008).

Sixty reservoirs have been created as the result of hydroelectric damming over the past 80 years in Malaysia (ICOLD, 2014). Twelve more dams, slated for construction by 2020, have been planned for Malaysian Borneo alone (Herbertson, 2013; Thin, 2013). Despite the increasing concern regarding mercury contamination in this global biodiversity hotspot, no studies currently exist of methylmercury accumulation in non-human mammals.

One mammalian group showing potential as a model for the study of mercury contamination and bioaccumulation through trophic levels is bats (Chiroptera) (Nam *et al*., 2012; Yates *et al*., 2014). Bat assemblages occupy high and low trophic levels, are species rich and abundant, and represent several distinct feeding guilds including frugivorous and insectivorous species (Rojas *et al*., 2013). Insectivorous bats eat 20–50% of their pre-feeding body mass in insects every night (Brunet-Rossinni & Austad, 2004) including insects with an aquatic larval life stage (e.g. Megaloptera, Trichoptera, certain Diptera, certain Coleoptera, Neuroptera, Ephemeroptera, and Odonata) and/or insects without an aquatic larval life stage (e.g. most Lepidoptera, and certain Coleoptera) (Bogdanowicz *et al*., 1999; Fukui *et al*., 2006). The limited studies of the diet of insectivorous bat species found in Malaysia (*Hipposideros*, and *Rhinolophus*) suggest 1-4% of the insects consumed have an aquatic larval stage (Thabah *et al*., 2006; Jiang *et al*., 2008). If mercury is present in aquatic insect prey, there should be accumulation of mercury in the tissues of insectivorous bats. Hair and blood mercury concentration are closely correlated (Yates *et al*., 2014) and both are accepted as valid

biomarkers of methylmercury exposure (US EPA, 2001). Hair generally has a 250-300 fold higher mercury concentration than blood (Mergler *et al*., 2007; Wada *et al*., 2010) and mercury fixed in the hair at the time of collection is stable and can give a longitudinal history of blood mercury levels (US EPA, 2001). To our knowledge no studies have compared mercury concentration in insectivorous and frugivorous bat species and there is only a single unpublished report of Hg concentrations in bats from Malaysia (Yates, unpublished).

The overall objective of this study was to investigate whether the concentration of total mercury in the fur of insectivorous bat species was significantly higher than that in the fur of frugivorous bat species sampled near reservoirs created by hydroelectric damming in Peninsular Malaysia.

5.2 Materials and methods

5.2.1 Study area

Bats were sampled on the shores of two major hydroelectric reservoirs in Peninsular Malaysia: Temenggor Lake, Perak (N 05° 31', E 101° 26') and Kenyir Lake, Terengganu (N 05° 08', E 102° 46') (**Figure 5.1**) between 16-25 July 2013. Temenggor Lake was created in 1979 and is the second largest man-made lake in Peninsular Malaysia, covering 15,200 ha (Lin, 2006) with an average depth of 127 m and average width of 537 m (Davidson *et al*., 1995). The reservoir is filled by two major river systems in the north, two in the east and one in the west (Norizam & Ali, 2000). The lake is used as a supply for domestic water consumption (Khalik & Abdullah, 2012) and is also fished by the local aboriginal community. Bats were sampled along the eastern edge of Temenggor Lake (**Figure 5.1a**). Kenyir Lake was created in 1986 and is the largest man-made lake in Peninsular Malaysia covering 36,900 ha with an average depth of 37 m and a maximum depth of 145 m (Kamaruddin *et al*., 2011). The lake receives water from two main rivers– the Terengganu River and the Terengan River (Rouf *et al*., 2010). Bats were sampled along the northeastern edge of Kenyir Lake (**Figure 5.1b**).

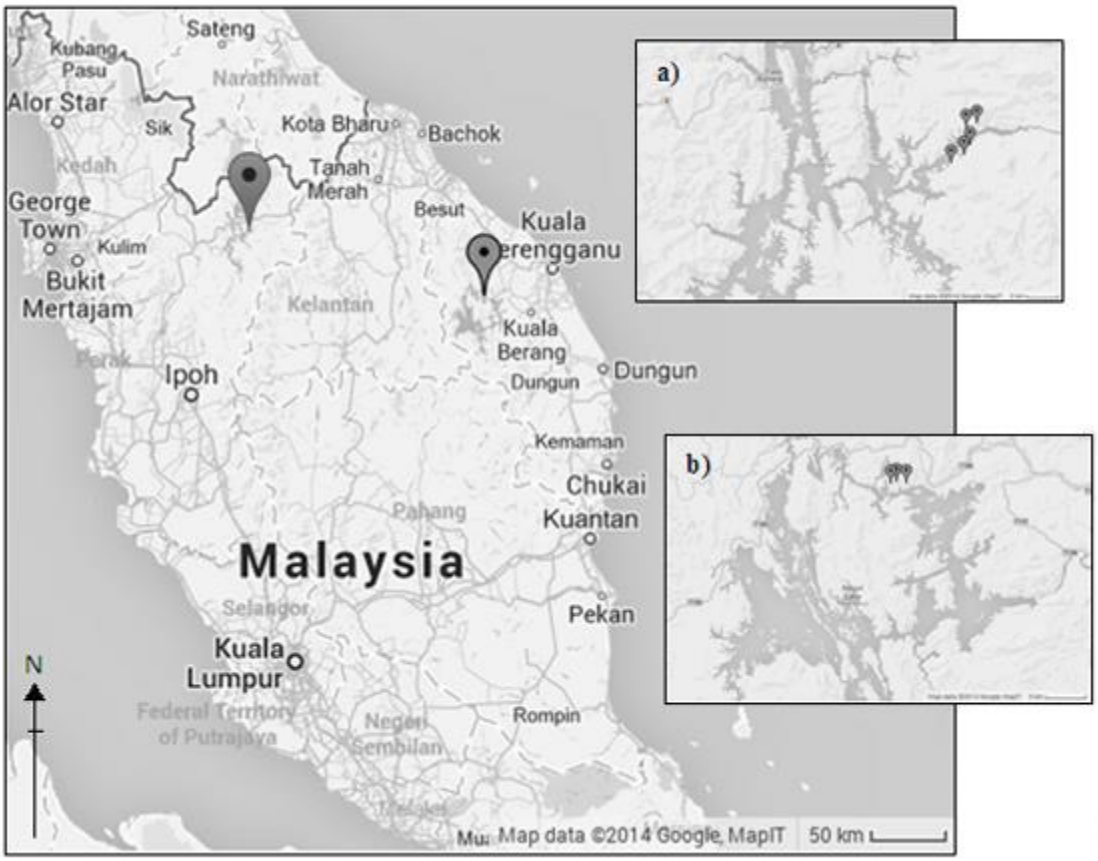

Figure 5.1 Study sites in Peninsular Malaysia where bat fur was sampled for mercury analysis (2013): **a)** Temenggor Lake and **b)** Kenyir Lake.

5.2.2 Capture, sample collection and handling

Bats were captured using 4 four-bank harp traps positioned across flight paths (trails, logging skids or streams) and ten mist nets set near the lake edge. Traps were set at 19:00 until 07:30 and were checked at 30 minutes intervals with sampling continuing until morning unless it rained. A small wing punch was collected from each captured bat into a 1.5 ml microcentrifuge tube following AMNH (2013). Hair samples were taken from each captured bat by snipping a small amount of hair (0.02g) from the upper part of the body using stainless steel scissors. Hair was stored in a 1.5 ml microcentrifuge tube. Scissors and forceps were cleaned with alcohol and sterile tissues between bats to avoid cross-contamination. If a bat with a wing punch was captured it was treated as a re-capture (Faure *et al*., 2009) and not subjected to another wing punch or further hair sampling. Sex and lifestage of the captured bats were recorded. Bats were identified in the field using morphological guides (Kingston *et al*., 2006; Francis, 2008), but given the prevalence of cryptic bat species in Malaysia (Sing *et al*., 2013; Wilson *et al*., 2014) species identification was confirmed using DNA barcoding (Francis *et al*., 2010), following standard methods used in previous studies (see Sing *et al*., 2013; Wilson *et al*., 2014).

5.2.3 Mercury analysis

Hair samples from adults of the most abundant genera of the two feeding guilds - insectivorous (*Hipposideros*, *Rhinolophus*) and frugivorous (*Cynopterus*, *Megaerops*) were selected for analysis of total mercury. We measured total mercury concentration in fur which is a standard approach, and is directly proportional to the concentration of methlymercury in the fur (Yates *et al*., 2014). Total mercury concentration was

measured using a Milestone Direct Mercury Analyzer (DMA-80) following US EPA Method 7473 (US EPA, 2007). The total mercury detection limit is 0.001 mg/kg.

Quality control included the use of standard reference materials DORM-3 (percentage recovery = 89.2-101.1%) and DOLT-4 (percentage recovery = 96.1-106.2%), running method blanks, sample blanks, and sample duplicates (percentage recovery = 83.5-103.6%), initially and then every 20 samples. The percentage recovery of spiked material was 92.7%.

5.2.4 Statistical analysis

Mercury concentration was compared between bats of different genera and species (with singleton species omitted from the test) using one-way ANOVA with a post-hoc Tukey HSD test; and between feeding guilds (frugivorous vs. insectivorous), sites (Temenggor Lake vs. Kenyir Lake) and sexes using two-way ANOVA with a post-hoc Tukey HSD test. All statistical analyses were performed using JMP 11.1.1 (SAS Institute 2013).

5.3 Results

Forty-one samples (9 frugivorous bats and 32 insectivorous bats) from Temenggor Lake and 87 samples (22 frugivorous bats and 65 insectivorous bats) from Kenyir Lake were analyzed for mercury concentration, comprising 12 species (two genera) of insectivorous bats and three species (two genera) of frugivorous bats. Note that bat species in Malaysia are often "dark taxa", species which have been recognized and recorded previously but which have not yet been formally described (Sing *et al*., 2013; Wilson *et al*., 2014), so a few of the species are referred to using non-Linnaean species names (**Table 5.1**).

Table 5.1: Total mercury concentrations in fur (mg/kg) for bat species sampled near Temenggor Lake and Kenyir Lake, Peninsular Malaysia.

Guild	Genus	Species	n	Mean / Value	Standard deviation	Statistical significance *
Frugivorous	*Cynopterus*	*C. horsfieldii*	10	0.012	0.004	a
		C. JLE sp. A	7	0.015	0.007	a
	Megaerops	*M. ecaudatus*	14	0.023	0.009	a
Insectivorous	*Hipposideros*	*H.* cf. *bicolor*	11	2.293	0.856	a, b
		H. cf. *larvatus*	47	7.136	2.546	d
		H. cervinus	1	8.988	0	
		H. diadema	1	3.789	0	
		H. doriae	1	5.135	0	
		H. dyacorum	1	9.525	0	
	Rhinolophus	*R. affinis*	23	2.686	1.985	b
		R. chiewkweeae	2	7.393	1.793	c, d
		R. trifoliatus	7	3.969	1.987	b, c
		R. acuminatus	1	0.627	0	
		R. lepidus	1	1.760	0	
		R. luctus	1	3.132	0	

* Species that share common letters do not differ significantly. Singleton species were omitted from the post hoc Tukey HSD test

Insectivorous genera (5.13 ± 3.10 SD mg/kg) had significantly higher concentrations of mercury than frugivorous genera (0.02 ± 0.01 SD mg/kg) ($F(3,124) = 48.64$, $p < 00001$). The post-hoc Tukey HSD test indicated that the genera *Hipposideros* (6.26 ± 2.98 SD mg/kg) and *Rhinolophus* (3.14 ± 2.22 SD mg/kg) had significantly higher concentrations of mercury than the two genera, *Megaerops* (0.023 ± 0.009 SD mg/kg) and *Cynopterus* (0.013 ± 0.006 SD mg/kg) (**Figure 5.2**).

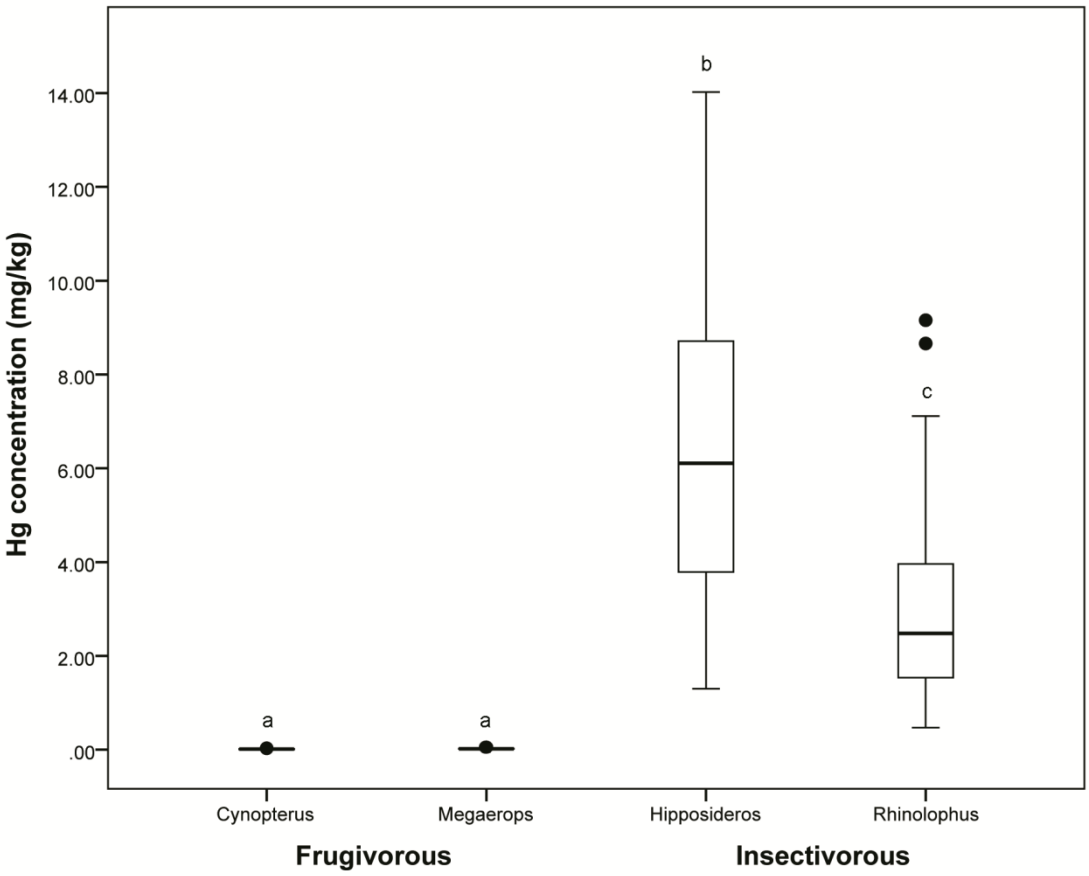

Figure 5.2: Mean mercury concentration (mg/kg) in fur from bats of different feeding guilds (Frugivorous or Insectivorous) grouped by genus with standard deviation bars. Different letters above the bars indicate significant differences between the means based on post hoc Tukey HSD test. Black circles are outliers.

Hipposideros cf *larvatus* (7.136 ± 2.546 SD mg/kg) and *Rhinolophus chiewkweeae* (7.393 ± 1.793 SD mg/kg) both had significantly higher mercury concentrations than the other insectivorous species (with singleton species omitted). There were no significant differences in mercury concentration among the frugivorous bat species ((F (7,113) = 40.29, p < 0.0001); **Table 5.1**).

Mercury concentrations in insectivorous bats at Kenyir were significantly higher than insectivorous bats at Temenggor (F (1,124) = 10.41, p = 0.0016). Grouped separately by site, mercury concentrations in insectivorous bats were significantly higher than frugivorous bats at both sites. The interaction between guild and site was significant (F (1,124) = 10.50, p = 0.0015) (**Figure 5.3**).

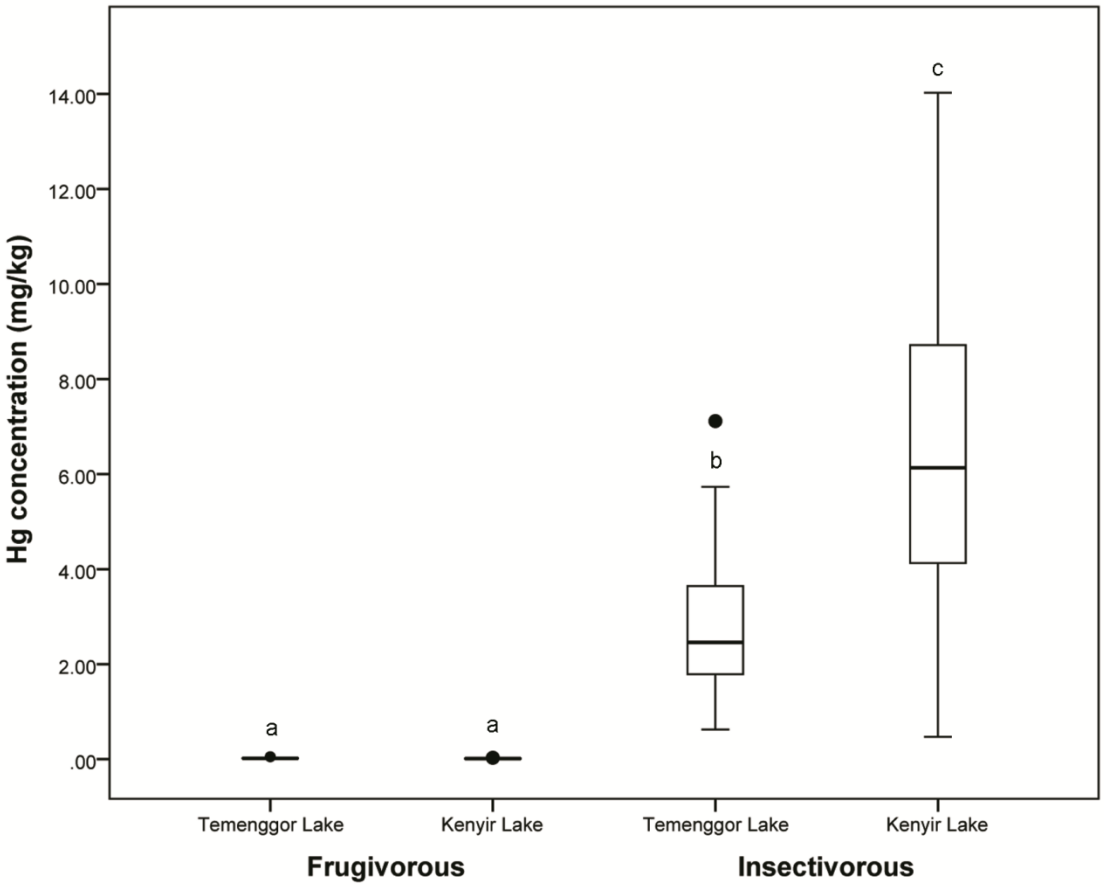

Figure 5.3: Mean mercury concentration (mg/kg) in fur from bats of different feeding guilds (Frugivorous or Insectivorous) grouped by study site (Temenggor Lake or Kenyir Lake) with standard deviation bars. Different letters above the bars indicate significant differences between the means based on post hoc Tukey HSD test. Black circles are outliers.

Comparison of mercury concentrations between sex was not significant ($F_{(1,124)} = 0.0006$, $p = 0.9810$). On average, females exhibited slightly lower mercury concentrations (3.412 ± 3.669 SD mg/kg) than males (4.347 ± 3.261 SD mg/kg) (**Figure 5.4**).

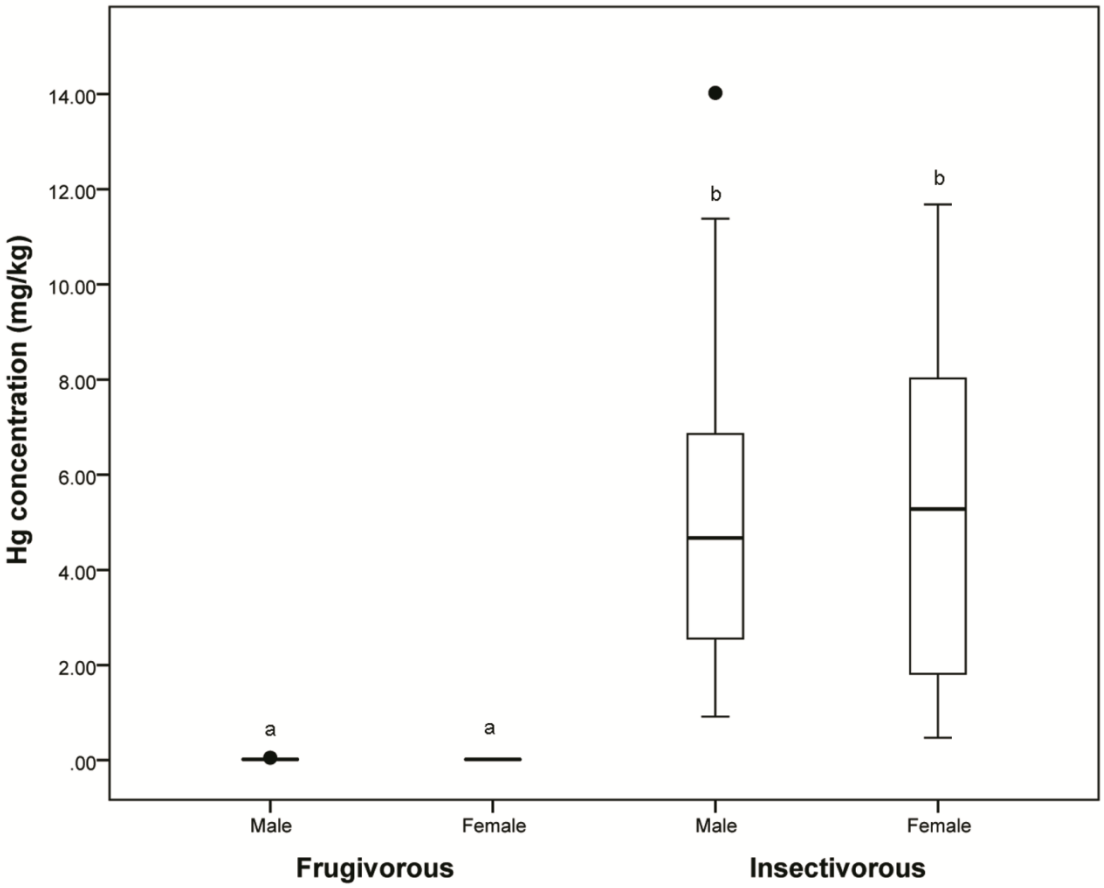

Figure 5.4: Mean mercury concentration (mg/kg) in fur from bats of different feeding guilds (Frugivorous or Insectivorous) grouped by sex with standard deviation bars. Different letters above the bars indicate significant differences between the means based on post hoc Tukey HSD test. Black circles are outliers.

5.4 Discussion

It was found that mercury concentration was significantly higher in the hair of insectivorous bats than of frugivorous bats sampled at two hydroelectric lakes in Peninsular Malaysia. This suggests that insectivorous bats could be accumulating methylmercury through their diet. The diet of insectivorous bats includes emergent aquatic insects (Fukui *et al*., 2006) that are, plausibly, contaminated with mercury from the lakes (Tremblay & Lucotte, 1997; Tweedy *et al*., 2013). Other studies have demonstrated that aquatic insects can act as biovectors transferring sedimentary mercury from lakes into terrestrial predators on the shoreline (Haro *et al*., 2013; Tweedy *et al*., 2013). Interestingly, Reidinger (1972) argued that mercury contamination in bats probably occurred from their free water drinking source rather than through their insect prey, however, this idea has largely been abandoned (Yates *et al*., 2014).

Of the two insectivorous genera analyzed for mercury concentration, *Hipposideros* made up the largest proportion of analyzed samples (37%) and showed significantly higher mercury concentrations than the *Rhinolophus* bats. This could be due to a larger proportion of aquatic insects in the *Hipposideros* diet; however, records on the diet of bat species in Malaysia are limited. One study conducted in various secondary forests in Malaysia reported that 17% of the diet of *H. larvatus s.l.* consisted of Coleoptera with the rest comprising unidentifiable insect fragments (Muda, 1991). Thabah *et al.* (2006) reported that the diet of *H. larvatus s.l.* collected from 11 sites in the Indo-Malayan region (India, China, Myanmar, Malaysia) comprised more than 80% coleopterans. Alternatively, the diet of *Rhinolophus affinis* from a cave in Jiangxi Province, China contained more than 50% of Lepidoptera (Jiang *et al*., 2008). While the diet of insectivorous bats is likely to be opportunistic, relying on the presence and density of prey species in the bats particular foraging area, this could suggest a larger

proportion of aquatic insect species in the diet of *Hipposideros* compared to *Rhinolophus* bats, and may explain the increased exposure to mercury contamination of *Hipposideros* bats in the study areas. It is also a possibility that *Hipposideros* bats could be foraging more frequently and cover a larger area including over water bodies. But no studies have documented this yet. The use of next generation DNA sequencing (NGS) to establish the taxonomic identity of prey fragments - "DNA metabarcoding" - in feces could help resolve this question (Razgour *et al*., 2011). Both the frugivorous bat genera sampled, *Cynopterus* and *Megaerops*, showed significantly lower concentrations of mercury (~99% lower than the insectivorous genera). The frugivorous bats were expected to exhibit low concentrations of mercury because their diet likely contains little mercury. Plant roots absorb small amounts of mercury from soils and the mercury is not directly translocated from root tissues to the tissues at the top of plants (Patra & Sharma, 2000) where the bats are feeding.

The comparison between the mercury concentrations in insectivorous bats collected at the two lakes showed a significantly higher concentration of mercury in bats sampled at Kenyir Lake. There was no known point source or intense agricultural activities near the study area at the lake. Kenyir Lake is shallower than Temenggor Lake, based on average depth, allowing rapid erosion of soils which increases bioavailability of mercury-rich particles to filter feeding invertebrates (Lucotte *et al*., 1999). Limitations on methylmercury production in Temenggor Lake might include low total mercury concentrations in the flooded soils and sediments and rapid oxidation and decay of organic matter leading to low total organic carbon in the reservoir (Ikingura & Akagi, 2003). The study area in Temenggor Lake can be considered pristine without human encroachment except for small-scale fishing and collecting of forest resources by aborigines. Ikingura and Akagi (2003) reported it was a common phenomenon for fish

mercury concentration to be negatively correlated with age of reservoirs even with a difference of only five years. Therefore, the age of the reservoirs could potentially be a partial explanation for the differences in mercury concentration in bats from the two lakes in our study, as Kenyir Lake is seven years younger than Temenggor Lake.

There was no significant variation in mercury concentration among sexes within each feeding guild. Similarly, no significant difference in mercury concentration between sexes was observed for both adult and juvenile bats from Oneida Lake, New York, USA (Yates *et al*., 2014) and Southwest England (Walker *et al*., 2007). Mercury contamination would not be expected to vary significantly between males and females of the same species as they live in colonies and most likely have a very similar diet.

Comparing mercury concentration in bats on a global scale, mean mercury concentrations in the fur of *Myotis lucifugus*, *M. septentrionalis*, *M. leibii* and *M. grisescens* from non-point source sites in Quebec exceeded the threshold for mercury concentration in hair (10 mg/kg) (Hickey *et al*., 2001) at which detrimental effects occur in humans (Murata *et al*., 1999) and neurobehavioral disorders occurred in rodents (Burton *et al*., 1977). Mercury concentrations in fur from bats at point source sites in North America have been reported as 28 mg/kg to 132 mg/kg (Wada *et al*., 2010; Nam *et al*., 2012; Yates *et al*., 2014) 5-30 times higher than the values for insectivorous bats in the present study. However the values in this study are similar to the mean mercury concentration in fur from bats at 69 non-point source sites in North America (6.44 mg/kg) (Yates *et al*., 2014).

5.5 Conclusion

This is the first study comparing mercury concentrations in frugivorous and insectivorous bats at hydroelectric reservoirs. Fur from ten bats (*H.* cf. *larvatus*) sampled at Kenyir Lake had mercury concentrations approaching or exceeding 10 mg/kg which is the threshold at which harmful effects occur in mammals (Murata *et al*., 1999; Burton *et al*., 1977). Insectivorous bats consuming large numbers of prey emerging from new reservoirs could be exposed to increased, and potentially harmful, levels of mercury as has been shown previously in insectivorous songbirds (Gerrard & St Louis, 2001). A reduction in bat populations due to neurological problems as a result of mercury toxicity could have serious consequences for the local ecosystem: insectivorous bats are important for controlling insect populations and for nutrient recycling (Jones *et al*., 2009). Malaysia has created 60 reservoirs as a consequence of hydroelectric damming since 1920; however, the ecological consequences of hydroelectric damming have never received serious consideration. Likewise, many other countries have embraced hydroelectricity as a renewable energy resource resulting in the creation of thousands of reservoirs around the world (Barros *et al*., 2011).

CHAPTER 6

GENERAL DISCUSSION

A critical criterion in selecting a suitable indicator group is stable and accurate species recognition which is achievable through a molecular approach. DNA barcoding is a reliable identification tool by comparing DNA sequences generated from small initial samples against the barcode reference libraries (Wilson *et al.,* 2014). Bats have been a target of a large DNA barcoding campaign in Southeast Asia (Francis *et al.*, 2010), facilitating the incorporation of DNA barcoding in identification to species level. The use of field guides for bat species identification is well-known but is unable to recognize dark taxa and cryptic species which are achievable through molecular approach, adding precision to the species inventory. This was supported in the first study, investigating the impact of DNA barcoding to the changing perspectives on bat species diversity in Ulu Gombak Field Centre. The results were able to uncover a high number (71%) of cryptic species, supporting the prediction that the number of bat species in Ulu Gombak is significantly underestimated. DNA barcoding also was applied in the second study to assess the first proposed key criterion: tractable taxonomy. The result showed that bats had a high PCR success rate (81%) and a high number (>82%) of assignments of species and family names to the MOTU after blasting representatives against BOLD. In the third study, DNA barcoding was also incorporated and allowed the recognition of 12 bat species including three "dark taxa". Hence, this suggested that DNA barcoding is able to enhance the effectiveness and efficiency of employing bats as ecological indicator groups in Southeast Asia.

The second study suggested bats as good biodiversity indicator when assessed using four key criteria. As discussed, the bats have tractable and stable taxonomy for species recognition which could be enhanced with the inclusion of DNA barcoding. Compared to butterflies and beetles, bats are relatively not easily surveyed due to high person-hours and more complex equipment needed. However, the increased time and equipment investment may be justifiable as they show variety of feeding guilds in comparison to the other assessed taxa (butterflies and dung beetles). Bats have both strong connections with other plants and animals because they are frugivorous/nectarivorous and insectivorous/carnivorous bats, thus their richness and abundance data may more accurately reflect the quality of habitat. Also, bats are widely-distributed across wide range of habitats but each species inhabits a specific roosting site. Hence, high species richness within a site would indicate a healthy habitat which offers various niches for many groups of biota. The second study also showed significant relationship between species richness of bats and butterflies, suggesting bat diversity can reflect other co-occurring taxa which also include plant taxa. Results from previous studies have shown a significant relationship between bat communities and vegetation diversity and structure, suggesting bats as good disturbance indicators and this encouraged at least three reserves in tropical Mexico to adopt bats in evaluation of conservation status in each reserves (Medellin *et al*., 2000).

Other than being a suitable biodiversity indicator group, bats have the potential to be environmental indicators as well. In addition to the four key criteria exhibited by bats, they are also sensitive towards human-induced disturbances in the ecosystems and positioned at various trophic levels in food webs (Jones *et al*., 2009). Insectivorous bats occupy high trophic levels, consume large portion of insects per night, and travel great distances each night, thus increasing the capacity of bioaccumulation and

biomagnification of contaminants through their diet intake (Zukal *et al*., 2012). In addition, they would incorporate the contaminants into their hairs as a valid and stable biomarker (US EPA, 2001). Therefore, bats are suitable to be used as indicators of certain environmental pollutions. This was supported by the third study which assessed mercury contamination in bats near hydroelectric reservoirs in Peninsular Malaysia. As hypothesized, the result showed significantly high mercury concentrations in insectivorous bats compared to frugivorous bats that were sampled near the reservoirs, supporting the view that the intake of mercury in bats was through their diet. In Kenyir Lake, bats were observed showing higher mercury concentrations than bats in Temenggor Lake, which exceeded the threshold at which the concentrations could cause lethal effects to humans and mammals. This highlighted the urgent need to reconsider the construction plan of hydroelectric reservoirs in future, especially in Malaysia, where several dams are scheduled for construction in the coming decades (Herbertson, 2013; Thin, 2013). Heavy metal contamination in the current 60 reservoirs in the country (ICOLD, 2014) should be studied to monitor the effects of land-use to the ecosystems by employing bats as the indicator group.

The combined findings of these three studies suggested that bats can be effectively employed as ecological indicators. For instance, other countries like United Kingdom have already recognized the importance of bats as bioindicators and the government has adopted bats into their suite of biodiversity indicators since May 2008 (Jones *et al*., 2009). Even an international symposium had been held in Barcelona (2012) to bring together experts to discuss the importance of bats as bioindicators and to open up new ideas for developing successful bat monitoring schemes. Therefore, it is recommended that bats play a central role as a bioindicator group in monitoring ecological change in Peninsular Malaysia in years to come.

CHAPTER 7

CONCLUSION AND RECOMMENDATION

Bats were found to fit the criteria as a good ecological indicator group for Peninsular Malaysia. First of all, they are taxonomically stable, supported by the inclusion of DNA barcoding in recognizing the species accurately. Secondly, they are suitable to be chosen as an indicator in biodiversity monitoring. This study proposed four key criteria to select a small group of species to be used as a proxy for "total" biodiversity. These are, i) easily surveyed, ii) tractable taxonomy, iii) broadly distributed higher taxa but specialized species, and iv) diversity patterns reflected in other groups. Based on these criteria, bats showed good potential as a bioindicator group although this study revealed butterflies to be a better group compared to bats. Nonetheless, these could be taken as evidences for bats having good potential as a bioindicator group and should be given more attention in the evaluation of biodiversity of sites in Southeast Asia. Thirdly, bats are positioned at various trophic levels in food webs and sensitive towards human-induced disturbances in the ecosystems, making them as good environmental indicators. Significantly higher concentrations of total mercury were found in the fur of insectivorous bats caught near hydroelectric reservoirs with ten bats sampled at Kenyir Lake had mercury concentrations approaching or exceeding 10 mg/kg, which is the threshold at which detrimental effects occur in humans, bats and mice. Therefore, it is good to acknowledge the potential of bats as environmental indicators and more studies should be done to evaluate the impact of employing bats in other type of environmental contamination monitoring.

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2. **Syaripuddin, K.**, Sing, K. W. & Wilson, J. J. (2013). Are butterflies, bats and beetles good biodiversity indicators in Southeast Asia? An assessment using four key criteria and DNA barcodes. Paper presented at the 18th Biological Sciences Graduate Congress.

3. **Syaripuddin, K.**, Kumar, A., Sing, K. W., Halim, M. R. A., Nursyereen, M. N., & Wilson, J. J. (2014). Mercury accumulation in bats near hydroelectric reservoirs in Peninsular Malaysia. *Ecotoxicology*, *23*(7), 1-8.

APPENDIX

APPENDIX A: Paper published.

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CHANGING PERSPECTIVES ON THE DIVERSITY OF BATS (MAMMALIA: CHIROPTERA) AT ULU GOMBAK SINCE THE ESTABLISHMENT OF THE FIELD STUDY CENTRE IN 1965

Kong-Wah Sing
Museum of Zoology, Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia
Email: gary.singkw@gmail.com (Corresponding author)

Khairunnisa Syaripuddin
Museum of Zoology, Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia
Email: knisa.syar12@gmail.com

John-James Wilson
Museum of Zoology, Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia
Email: johnwilson@um.edu.my

ABSTRACT. — Ulu Gombak Forest Reserve is a selectively logged forest located at the Pahang-Selangor border. A field studies centre was established at the western edge of the reserve by Medway in 1965. Ulu Gombak had previously been reported as the single locality with the highest species richness of bats in the Old World. In light of recent studies demonstrating extensive numbers of cryptic bat species, diversity assessments at Ulu Gombak would benefit from reexamination. In this study we examine changing perspectives on bat diversity at Ulu Gombak since the establishment of the Field Study Centre, and particularly, how assessments of species richness change with the incorporation of DNA barcoding into bat surveys. One hundred and sixty records of bats at Ulu Gombak were extracted from literature and from the Museum of Zoology, University of Malaya collection. Fifty-two morphological species of bats had been recorded at Ulu Gombak between 1962 and 2012 which was equivalent to one additional species record every two years throughout this period. During surveys at Ulu Gombak in 2012/2013 DNA barcodes were obtained from 45 bats. The DNA barcodes were assigned to seven species. Four of these were dark taxa, previously reported species which lack formal description, in the genera *Cynopterus* and *Hipposideros*. Additionally, a deep DNA barcode divergence (4.2%) from conspecifics from Indonesia strongly suggested the presence of a cryptic species of *Chironax* which had not been reported previously. These five species were added to the cumulative checklist for Ulu Gombak taking the total to 57 species of bats. The high number of cryptic species uncovered supports the prediction that the number of bat species in Ulu Gombak is significantly underestimated. The projected number of 89 bat species provides a benchmark for future, more intensive, surveys using multiple trapping methods and covering a larger area of the reserve, but critically, incorporating DNA barcoding for species recognition.

KEY WORDS. — *Chironax*, cryptic species, dark taxa, DNA barcoding, Malaysia, museum collections

INTRODUCTION

In Southeast Asia, the nineteenth century saw a dramatic increase in the rate of discovery of bat species, a trend that leveled off during the first half of the twentieth century (Kingston, 2010). However, over the last two decades, as a result of intensive and new surveying approaches 14 new species of bats have been described from Southeast Asia, not only from new study sites, but also from well-studied areas (e.g., Bates et al., 2000; Hendrichsen et al., 2001; Matveev, 2005). Peninsular Malaysia supports in excess of 100 bat species (Simmons, 2005) representing approximately 40% of the native mammal species (Medway, 1982). The species richness of bats at Ulu Gombak, reported as 50 species (Heller & Volleth, 1995), was the highest recorded for a single locality in the Old World until an intensive sampling effort uncovered 65 species at Krau Wildlife Reserve, Pahang (Kingston, 2003; Kingston et al., 2003).

Bats have been proposed as important indicators of the state of ecological communities, and bat surveys are often used for conservation planning on the assumption that the protection of bats will protect key habitat for many other taxa (Francis et al., 2010). However, rapid changes in land-

use and deforestation in Malaysia in recent decades have put many of the bat species at risk of extinction (Sodhi et al., 2004). Although the distribution and taxonomy are better known for bats than for most other taxa (Francis et al., 2010) a lack of data on distributions and populations has hampered conservation efforts. Accurate species identifications are important to assess bat diversity but due to the presence of hidden species within cryptic species complexes, the identity of many Malaysian bats appears to be uncertain (Kingston, 2010). It has been suggested that the real number of bat species is at least twice that currently recognised (Francis et al., 2010). The increased use of molecular methods, particularly DNA barcoding (Wilson et al., 2013), for bat species identification is proving invaluable in differentiating cryptic taxa overlooked by morphological methods. In the present ethical climate, the fact that accurate species identification can be achieved from small wing tissue punches without the need to sacrifice individuals is another significant advantage (Wilson et al., 2013).

Ulu Gombak Field Studies Centre, founded by Medway in 1965 (Medway, 1966), occupies approximately 120 ha of the 17,000 ha Ulu Gombak Forest Reserve. Several pioneering studies in ecology have been conducted at the field centre and a multitude of new species from diverse taxonomic groups have been described from Ulu Gombak by various researchers from all over the world (e.g., Macdonald & Mattingly, 1960; Ballerio & Maruyama, 2010; Nuril Aida & Idris, 2011). The objective of the present study was to investigate the changing perspectives on bat diversity at Ulu Gombak since the establishment of the field studies centre, and particularly how estimates of species richness have changed very recently due to the inclusion of DNA barcoding into surveys.

MATERIAL AND METHODS

Ulu Gombak. — Ulu Gombak Forest Reserve is located at the southern border of the old highway from Kuala Lumpur to Bentong, Pahang. It is a selectively logged forest with very little seasonal variation in temperature (Medway, 1966). Ulu Gombak Field Study Centre of the University of Malaya is situated at the western edge of the reserve (3°20′N, 101°45′E) (Fig. 1). This site is of considerable biological importance in Malaysia and several surveys of bats have been conducted over the past 50 years.

Literature review and museum specimens. — Records of bat species recorded at Ulu Gombak since 1966 were extracted from literature (Table 1). The collection of the Museum of Zoology, University of Malaya (UMKL) was examined for preserved bat specimens collected from Ulu Gombak.

DNA barcoding. — Ten mist nets (9 × 4 m) and four harp traps were set at ten locations within Ulu Gombak Forest Reserve from 11–15 Nov.2012 and 11–14 Mar.2013. The nets were checked hourly from sunset to midnight and again at sunrise. Our protocols for tissue sampling, DNA extraction, amplification and sequencing of bat DNA barcodes followed Wilson (2012) and Wilson et al. (2013) using the primer pair VF1d_t1 and VR1d_t1 (Ivanova et al., 2012). The resulting DNA barcodes were uploaded to BOLD (Ratnasingham & Hebert, 2007) and are available (with GenBank Accessions) in the public dataset DS-MEDWAY. DNA barcodes were assigned to species using the 'Full Database' (see Wilson et al., 2013).

RESULTS

One hundred and sixty records of bats at Ulu Gombak were extracted from literature and the UMKL collection resulting in 52 traditional species records between 1962 and 2012 (Table 1; Fig. 2). This represents an increase of one species every two years between the initial checklist of Medway (1966), based on an Institute for Medical Research report and our study.

DNA barcodes were successfully amplified and sequenced from 45 specimens sampled in our surveys during 2012/2013.

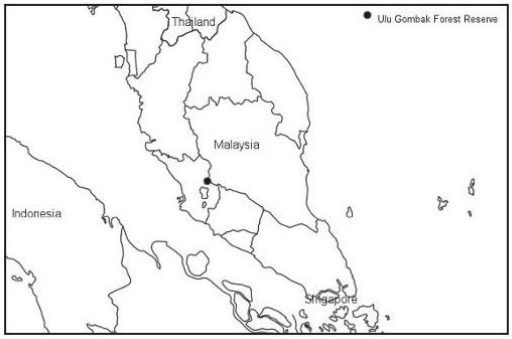

Fig. 1. Location of Ulu Gombak Forest Reserve and Ulu Gombak Field Studies Centre.

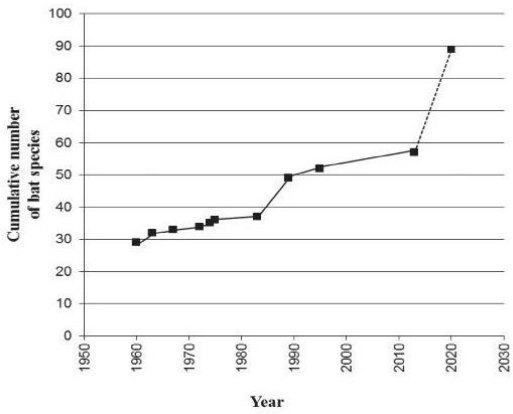

Fig. 2. Cumulative number of bat species recorded at Ulu Gombak Forest Reserve and the projected number (dashed line) of bat species after intensive DNA barcoding.

Table 1. Checklist of bats species recorded in Ulu Gombak. Species names with same alphabetical superscript have been considered by some researchers to be the same species or synonyms. In such cases, the capital letters are used to denote the valid name. References: 1, Medway, 1966; 2, Medway, 1967; 3, UMKL, 1963–1969; 4, Medway, 1983; 5, Hill, 1972; 6, Hill, 1974; 7, Sly, 1975; 8, Jenkins & Hill, 1981; 9, Yenbutra & Felten, 1983; 10, Heller & Volleth, 1989; 11, Heller & Volleth, 1995; 12, Yusof, 2005; 13, Syaripuddin, 2012; 14, This study.

Bat Species	Reference Source(s)
PTEEROPODIDAE	
Balionycteris maculata	1,10,11,12,13
Chironax melanocephalus[A]	1,10,11
Chironax melanocephalusGOM01[a]	14
Cynopterus brachyotis	1,10,11,12,13,14
Cynopterus horsfieldi	1,3,10,11,12,13
Cynopterus JLE sp. A	14
Dyacopterus spadiceus	13
Eonycteris spelaea	1,10,11,13
Macroglossus lagochilus[b]	1
Macroglossus minimus[b]	1
Macroglossus sobrinus[B]	10,11
Megaerops ecaudatus	9,11,13,14
Penthetor lucasi	1,10,11
Pteropus vampyrus	1,11
Rousettus amplexicaudatus	10,11,12
EMBALLONURIDAE	
Emballonura monticola	1,3,10,11
Taphozous melanopogon	1,11
Taphozous saccolaimus	10,11
NYCTERIDAE	
Nycteris javanica[C]	10,11
Nycteris tragata[c]	13
MEGADERMATIDAE	
Megaderma lyra	2
Megaderma spasma	1,10,11
RHINOLOPHIDAE	
Rhinolophus affinis	3,13
Rhinolophus luctus	1,10,11,13
Rhinolophus refulgens	11
Rhinolophus sedulus	1,3,10,11,13
Rhinolophus stheno	10,11,13
Rhinolophus trifoliatus	3,10,11,13
HIPPOSIDERIDAE	
Coelops frithii	5,11
Hipposideros bicolor[D]	1,3,10,11,13
Hipposideros bicolor131[d]	14
Hipposideros bicolor142[d]	14
Hipposideros cervinus[E]	8,10,11,13
Hipposidero cervinusCMF02[e]	14
Hipposideros cineraceus	1,3,11
Hipposideros diadema	1,3,10,11,13
Hipposideros galeritus[e]	1
Hipposideros larvatus	1,11,13
Hipposideros sabanus	10,11
VESPETILIONIDAE	
Eptesicus circumdatus	10,11
Glischropus tylopus	10,11,13
Hesperoptenus blanfordi	10,11
Hesperoptenus doriae	4,10,11
Hesperoptenus tomesi	10,11
Kerivoula papillosa[F]	2,11,13
Kerivoula sp.[f]	1
Miniopterus schreibersii	10,11
Murina aenea	7,11

Table 1.Cont'd.

Bat Species	Reference Source(s)
Murina cyclotis	11,13
Murina suilla	10,11,13
Myotis horsefieldii	11
Myotis montivagus	3,10,11
Myotis muricola[G]	3,10,11
Myotis mystacinus[g]	1
Myotis ridleyi	10,11
Philetor brachypterus	6,10,11,13
Phoniscus atrox	1,3,4,10,11
Pipistrellus sp.[h]	1
Pipistrellus stenopterus[H]	11
Scotophilus kuhlii[I]	10,11
Scotophilus temminckii[i]	1
Tylonycteris pachypus	1,3,10,11
Tylonycteris robustula	1,10,11,13
MOLOSSIDAE	
Chaerephon sp.	1,11
Cheiromeles torquatus	1,11

The DNA barcodes were assigned into seven taxa (Table 2). Of these seven, four species were dark taxa (Maddison et al., 2012; Wilson et al., 2013) in the genera *Cynopterus* (Fig. 3) and *Hipposideros* (see Francis et al., 2010; Wilson et al., 2013). One DNA barcode matched to *Chironax melanocephalus* but with only 95.8% similarity (Table 2; Fig. 3) suggesting this belonged to a cryptic species which we annotated as *C.* melanocephalusGOM01.

Therefore, of the seven species sampled in our surveys, five (71%) were dark or cryptic taxa. We used this value and the tally of 52 traditional species to extrapolate that the species richness of Ulu Gombak could be 89 bat species (Fig. 2).

DISCUSSION

Ulu Gombak has been recognised as the home of one of the most diverse community of bats in the Old World based on species richness (Kingston et al., 2003). Our literature review and examination of the UMKL collection revealed a total of 52 traditional species records with several taxa missed or omitted in previous compilations. For example, we have one specimen of *Rhinolophus affinis* in UMKL, collected at Ulu Gombak in 1963; this species was not included in the checklists of Medway (1966) or Heller & Volleth (1995). This highlights the importance of museum collections as historical records of biodiversity that are relevant and accessible to contemporary research projects. Overall, we documented 28 new records for bat species at Ulu Gombak since the establishment of Ulu Gombak Field Studies Centre in 1966, equivalent to one additional species record every two years.

All the previous checklists reviewed in the present study have relied upon morphological identification of species. However, the reported presence of cryptic taxa within morphological species makes diversity assessment using morphological criteria questionable. For example, "*Hipposideros bicolor*"

includes two morphologically similar species (*H.* bicolor131 and *H.* bicolor142) (Kingston et al., 2001), both present at Ulu Gombak. Cryptic taxa like these can only be recognised by acoustic and/or molecular methods such as DNA barcoding (Kingston et al., 2001; Francis et al., 2010). Recently a cryptic species from the genus *Kerivoula* with extremely similar morphology (but possibly an unusual fur colouration) to *K. hardwickii* has been described as *K. krau* from Krau Wildlife Reserve after being confirmed by an 11% divergence in DNA barcodes (Francis et al., 2007).

When we incorporated DNA barcoding into a survey of bats at Ulu Gombak, we found DNA barcodes from our survey matched to DNA barcodes in BOLD belonging to documented species (e.g., by Francis et al., 2010) that do not yet have formal species names. These have come to be known as "dark taxa" (Maddison et al., 2012; Wilson et al., 2013). As a result of our survey, five species (dark taxa) were added to the cumulative checklist for Ulu Gombak taking the total to 57 species. *Chironax* melanocephalaGOM01 had not been reported in prior studies, but the deep DNA barcode

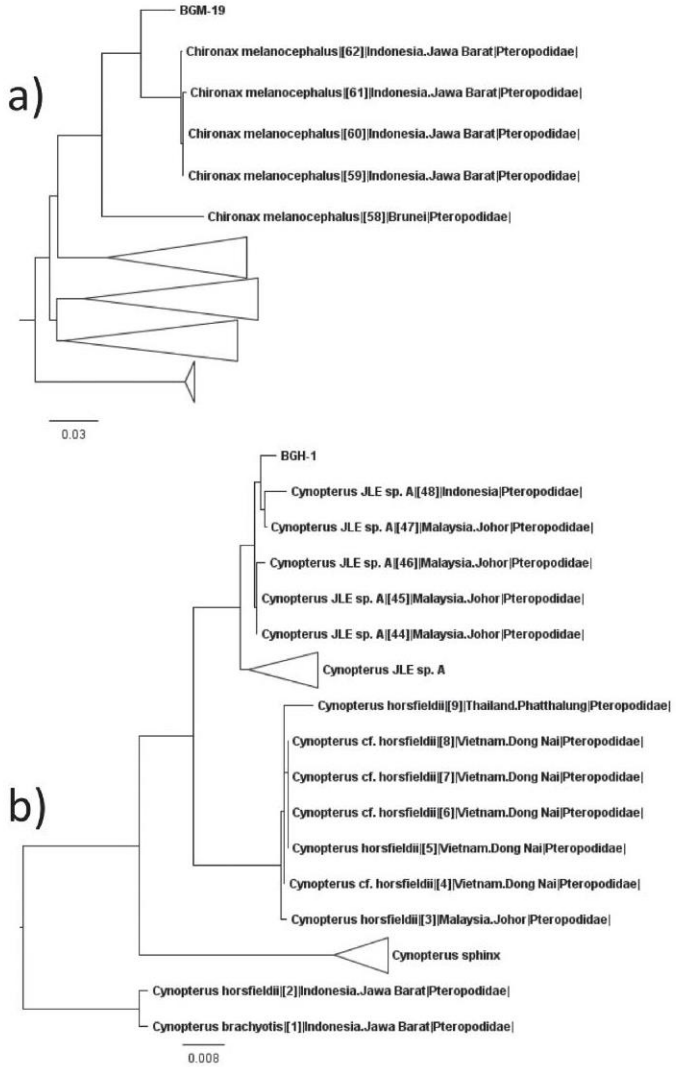

Fig. 3. Neighbour-joining trees produced by BOLD identification engine for the identification of DNA barcodes (a) BGM-19 and (b) BGH-1 from bats sampled at Ulu Gombak. Triangles represent clusters of multiple barcodes; height being proportional to the number of barcodes and width proportional to the genetic distance within the cluster. The scale bar indicates the genetic distance as a proportion.

Table 2. Taxonomic name, similarity (%) and BOLD BIN of the closest matching DNA barcodes to our 45 specimens collected at Ulu Gombak in 2012/2013.

Field ID	Name of the closest match	Similarity with closest match (%)	BOLD BIN
BGH-1	*Cynopterus* JLE sp. A	99.7	BOLD:AAA9308
BGM-10	*Cynopterus brachyotis*	99.3	BOLD:AAA9800
BGM-11	*Cynopterus brachyotis*	99.5	BOLD:AAA9800
BGH-12	*Hipposideros* cervinusCMF02	100.0	BOLD:AAB6249
BGM-14	*Megaerops ecaudatus*	99.4	BOLD:ABA9836
BGM-15	*Cynopterus brachyotis*	99.7	BOLD:AAA9800
BGM-16	*Megaerops ecaudatus*	98.7	BOLD:ABA9836
BGM-17	*Cynopterus brachyotis*	99.8	BOLD:AAA9800
BGM-18	*Megaerops ecaudatus*	99.3	BOLD:ABA9836
BGM-19	*Chironax melanocephalus* (*C.* melanocephalusGOM01)	95.8	BOLD:AAE9045
BGM-20	*Cynopterus* JLE sp. A	99.3	BOLD:AAA9308
BGM-21	*Cynopterus brachyotis*	98.7	BOLD:AAA9800
BGM-22	*Cynopterus brachyotis*	99.5	BOLD:AAA9800
BGM-23	*Megaerops ecaudatus*	98.7	BOLD:ABA9836
BGM-24	*Megaerops ecaudatus*	99.7	BOLD:ABA9836
BGM-25	*Cynopterus brachyotis*	99.7	BOLD:AAA9800
BGM-26	*Megaerops ecaudatus*	98.4	BOLD:ABA9836
BGM-27	*Cynopterus brachyotis*	99.5	BOLD:AAA9800
BGM-2	*Hipposideros* cervinusCMF02	99.8	BOLD:AAB6249
BGM-3	*Cynopterus brachyotis*	99.5	BOLD:AAA9800
BGH-4	*Hipposideros* cervinusCMF02	100.0	BOLD:AAB6249
BGM-5	*Cynopterus brachyotis*	99.7	BOLD:AAA9800
BGM-7	*Megaerops ecaudatus*	99.2	BOLD:ABA9836
BGM-6	*Hipposideros* cervinusCMF02	99.6	BOLD:AAB6249
BGM-8	*Hipposideros* cervinusCMF02	99.5	BOLD:AAB6249
BGM-9	*Cynopterus* JLE sp. A	99.0	BOLD:AAA9308
TF-5	*Cynopterus brachyotis*	99.1	BOLD:AAA9800
TF-6	*Cynopterus* JLE sp. A	100.0	BOLD:AAA9308
TF-8	*Cynopterus brachyotis*	98.2	BOLD:AAA9800
TF-9	*Hipposideros* cervinusCMF02	100.0	BOLD:AAB6249
TF-15	*Hipposideros* cervinusCMF02	100.0	BOLD:AAB6249
TF-20	*Hipposideros* cervinusCMF02	100.0	BOLD:AAB6249
TI-10	*Hipposideros* cervinusCMF02	97.5	BOLD:AAB6249
TI-13	*Hipposideros* bicolor131	99.7	BOLD:AAD3329
TI-14	*Hipposideros* cervinusCMF02	99.8	BOLD:AAB6249
TI-16	*Hipposideros* cervinusCMF02	99.5	BOLD:AAB6249
TI-18	*Hipposideros* cervinusCMF02	100.0	BOLD:AAB6249
TI-21	*Hipposideros* cf. bicolor (*H.* bicolor142)100.0	BOLD:AAC0445	
TI-22	*Hipposideros* cervinusCMF02	99.8	BOLD:AAB6249
TI-23	*Hipposideros* cervinusCMF02	100.0	BOLD:AAB6249
TI-24	*Hipposideros* cervinusCMF02	100.0	BOLD:AAB6249
TI-7	*Hipposideros* cervinusCMF02	100.0	BOLD:AAB6249
TI-8	*Hipposideros* cervinusCMF02	100.0	BOLD:AAB6249
TF-7	*Cynopterus brachyotis*	98.5	BOLD:AAA9800
TI-12	*Hipposideros* cf. bicolor (*H.* bicolor142)100.0	BOLD:AAC0445	

divergence (4.2%) from conspecifics from Indonesia strongly suggests this is a cryptic species newly uncovered by our survey. Which is the valid *C. melanocephala* and whether the species are allopatric or both present at Ulu Gombak remains to be seen. The high proportion of cryptic species sampled during relatively small-scale surveys suggests that bat diversity at Ulu Gombak is not yet completely known and is significantly underestimated.

The DNA barcodes from our survey were assigned a species identification with high probability using the BOLD identification engine. This was also the case for the dark taxa due to the extensive DNA barcode reference library for Southeast Asian bats in BOLD (largely from Francis et al., 2010). DNA barcodes for *H. bicolor* fell into two distinct clusters (see Francis et al., 2010; Wilson et al., 2013). Similarly, the deep DNA barcode variation within morphological species in *Cynopterus* had been encountered in prior DNA barcode surveys conducted at other locations. *C.* JLE sp. A is also known as "*C.* cf. *brachyotis* Forest" (Francis et al., 2010) and has recently been subject to morphometric cluster analysis (Jayaraj et al., 2012). These results support the view that DNA barcoding provides an accurate, rapid and cost-effective approach for identification of bats at Ulu Gombak. The high number of cryptic complexes in our surveys supports the suggestion of Francis et al. (2010) that the number of bat species in Southeast Asia is significantly underestimated. The projected number of 89 bat species for Ulu Gombak (Fig. 2) provides a benchmark for future, more intensive, surveys using multiple trapping methods and covering a larger area of the reserve, but critically, incorporating DNA barcoding for species recognition.

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APPENDIX B: Abstract for seminar presented.

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Are butterflies, bats and beetles good biodiversity indicators in tropical Southeast Asia? An assessment using four key criteria and DNA barcodes

[1,2]**Syaripuddin, K.**, [1]**Sing, K.W.**, [1,2]**Wilson, J.J.**
[1]Museum of Zoology, Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia
[2]Ecology & Biodiversity Program, Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia
Email: knisa.syar12@gmail.com

Urbanization and rapid human population growth are threatening the habitats available for wildlife in Southeast Asia. Informed conservation decision-making requires meaningful biodiversity assessment yet performing an inventory of all the species present at a site is impossible. A small group of species is frequently used as a proxy for "total" biodiversity, and various attributes required by bioindicator groups have been suggested. We propose four key criteria: i) tractable taxonomy, ii) easily surveyed, iii) broadly distributed higher taxa but specialized species, iv) diversity patterns reflected in other groups; and assessed three groups - butterflies, bats and beetles - against these criteria to determine their potential as bioindicators in Southeast Asia. DNA barcodes from butterflies, bats and beetles sampled during standardized surveys at Rimba Ilmu Botanic Garden and Ulu Gombak Forest Reserve were sorted into molecular operational taxonomic units (MOTU) and assigned species and family names. Beetle and butterfly sampling required a similar number of person-hours per species, which was an order of magnitude lower than that required for bat sampling. It was easier to generate DNA barcodes for butterflies and bats than for beetles. Most butterfly and bat families were found at both sites but the species of all three groups showed little overlap between sites (<15%). The species richnesses of all three groups were correlated with each other, but only bat and butterfly species richness was strongly correlated and statistically significant. Based on our four key criteria butterflies showed the most potential as bioindicators and should be given more prominence in the evaluation of biodiversity of sites in Southeast Asia.