

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

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**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

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### 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, 1983). 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.

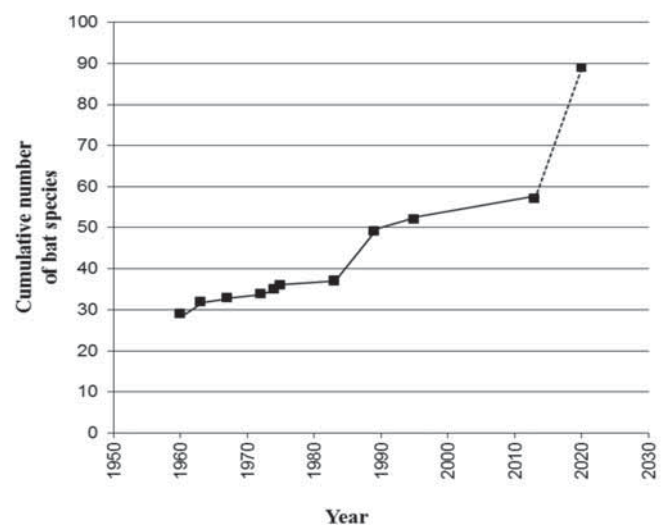


Fig. 2. Cumulative number of bat species recorded at Ulu Gombak Forest Reserve and the projected number (dashed line) of bat species after intensive DNA barcoding.

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)
<b>PTEEROPODIDAE</b>	
<i>Balionycteris maculata</i>	1,10,11,12,13
<i>Chironax melanocephalus</i> <sup>A</sup>	1,10,11
<i>Chironax melanocephalus</i> GOM01 <sup>a</sup>	14
<i>Cynopterus brachyotis</i>	1,10,11,12,13,14
<i>Cynopterus horsfieldi</i>	1,3,10,11,12,13
<i>Cynopterus</i> JLE sp. A	14
<i>Dyacopterus spadiceus</i>	13
<i>Eonycteris spelaea</i>	1,10,11,13
<i>Macroglossus lagochilus</i> <sup>b</sup>	1
<i>Macroglossus minimus</i> <sup>b</sup>	1
<i>Macroglossus sobrinus</i> <sup>B</sup>	10,11
<i>Megaerops ecaudatus</i>	9,11,13,14
<i>Penthetor lucasi</i>	1,10,11
<i>Pteropus vampyrus</i>	1,11
<i>Rousethus amplexicaudatus</i>	10,11,12
<b>EMBALLONURIDAE</b>	
<i>Emballonura monticola</i>	1,3,10,11
<i>Taphozous melanopogon</i>	1,11
<i>Taphozous saccolainus</i>	10,11
<b>NYCTERIDAE</b>	
<i>Nycteris javanica</i> <sup>C</sup>	10,11
<i>Nycteris tragata</i> <sup>c</sup>	13
<b>MEGADERMATIDAE</b>	
<i>Megaderma lyra</i>	2
<i>Megaderma spasma</i>	1,10,11
<b>RHINOLOPHIDAE</b>	
<i>Rhinolophus affinis</i>	3,13
<i>Rhinolophus luctus</i>	1,10,11,13
<i>Rhinolophus refulgens</i>	11
<i>Rhinolophus sedulus</i>	1,3,10,11,13
<i>Rhinolophus stheno</i>	10,11,13
<i>Rhinolophus trifolius</i>	3,10,11,13
<b>HIPPOSIDERIDAE</b>	
<i>Coelops frithii</i>	5,11
<i>Hipposideros bicolor</i> <sup>D</sup>	1,3,10,11,13
<i>Hipposideros bicolor</i> 131 <sup>d</sup>	14
<i>Hipposideros bicolor</i> 142 <sup>d</sup>	14
<i>Hipposideros cervinus</i> <sup>E</sup>	8,10,11,13
<i>Hipposidero cervinus</i> CMF02 <sup>e</sup>	14
<i>Hipposideros cineraceus</i>	1,3,11
<i>Hipposideros diadema</i>	1,3,10,11,13
<i>Hipposideros galeritus</i> <sup>e</sup>	1
<i>Hipposideros larvatus</i>	1,11,13
<i>Hipposideros sabanus</i>	10,11
<b>VESPETILIONIDAE</b>	
<i>Eptesicus circumdatus</i>	10,11
<i>Glischropus tylopus</i>	10,11,13
<i>Hesperoptenus blanfordi</i>	10,11
<i>Hesperoptenus doriae</i>	4,10,11
<i>Hesperoptenus tomesi</i>	10,11
<i>Kerivoula papillosa</i> <sup>F</sup>	2,11,13
<i>Kerivoula</i> sp. <sup>f</sup>	1
<i>Miniopterus schreibersii</i>	10,11
<i>Murina aenea</i>	7,11

Table 1.Cont'd.

Bat Species	Reference Source(s)
<i>Murina cyclotis</i>	11,13
<i>Murina suilla</i>	10,11,13
<i>Myotis horsefieldii</i>	11
<i>Myotis montivagus</i>	3,10,11
<i>Myotis muricola</i> <sup>G</sup>	3,10,11
<i>Myotis mystacinus</i> <sup>g</sup>	1
<i>Myotis ridleyi</i>	10,11
<i>Philetor brachypterus</i>	6,10,11,13
<i>Phoniscus atrox</i>	1,3,4,10,11
<i>Pipistrellus</i> sp. <sup>h</sup>	1
<i>Pipistrellus stenopterus</i> <sup>h</sup>	11
<i>Scotophilus kuhlii</i> <sup>i</sup>	10,11
<i>Scotophilus temminckii</i> <sup>i</sup>	1
<i>Tylonycteris pachypus</i>	1,3,10,11
<i>Tylonycteris robustula</i>	1,10,11,13
<b>MOLOSSIDAE</b>	
<i>Chaerephon</i> sp.	1,11
<i>Cheiromeles torquatus</i>	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. melanocephalus*GOM01.

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. bicolor*<sup>131</sup> and *H. bicolor*<sup>142</sup>) (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 melanocephala*<sup>GOM01</sup> had not been reported in prior studies, but the deep DNA barcode

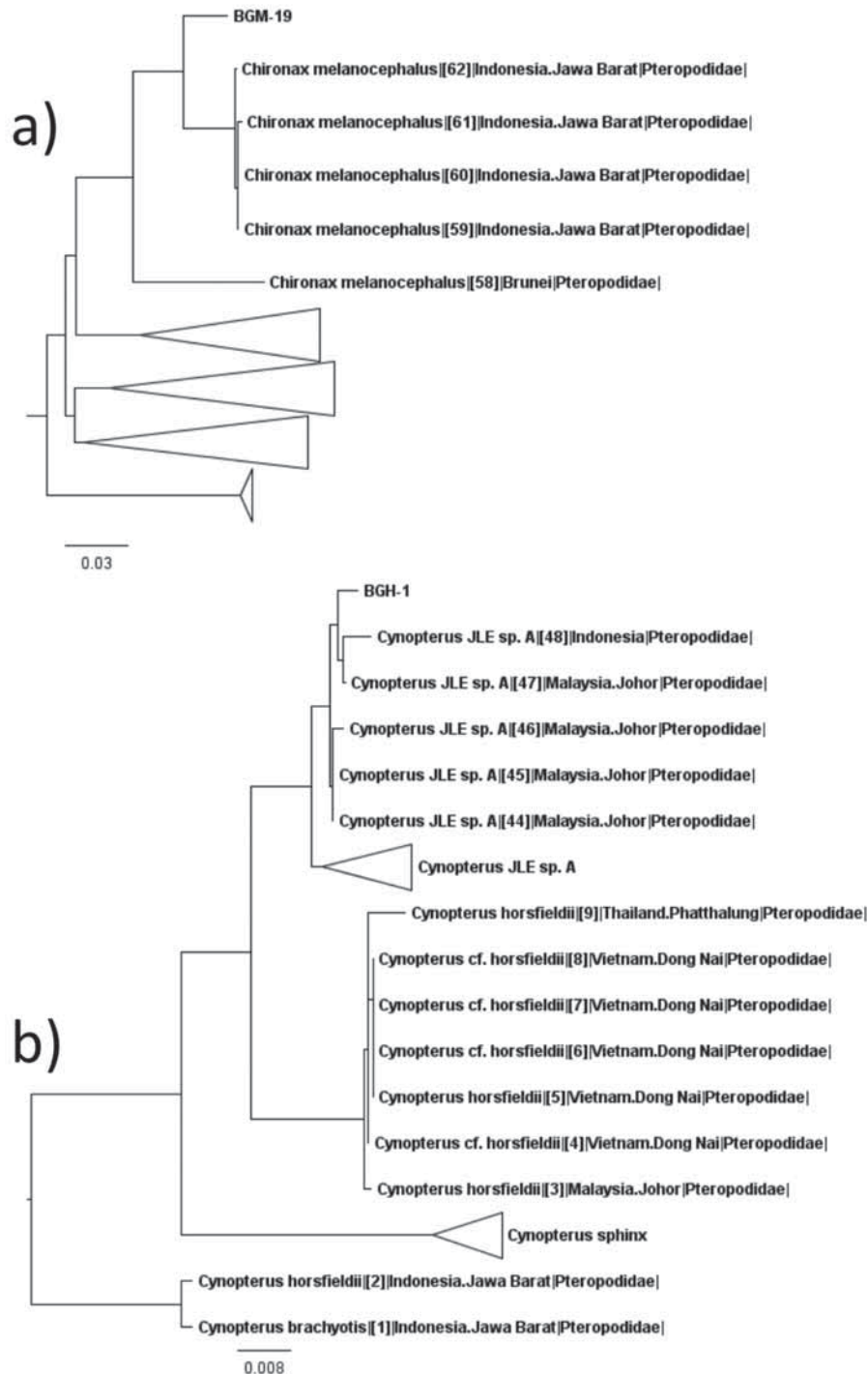


Fig. 3. Neighbour-joining trees produced by BOLD identification engine for the identification of DNA barcodes (a) BGM-19 and (b) BGH-1 from bats sampled at Ulu Gombak. Triangles represent clusters of multiple barcodes; height being proportional to the number of barcodes and width proportional to the 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	<i>Cynopterus</i> JLE sp. A	99.7	BOLD:AAA9308
BGM-10	<i>Cynopterus brachyotis</i>	99.3	BOLD:AAA9800
BGM-11	<i>Cynopterus brachyotis</i>	99.5	BOLD:AAA9800
BGH-12	<i>Hipposideros cervinus</i> CMF02	100.0	BOLD:AAB6249
BGM-14	<i>Megaerops ecaudatus</i>	99.4	BOLD:ABA9836
BGM-15	<i>Cynopterus brachyotis</i>	99.7	BOLD:AAA9800
BGM-16	<i>Megaerops ecaudatus</i>	98.7	BOLD:ABA9836
BGM-17	<i>Cynopterus brachyotis</i>	99.8	BOLD:AAA9800
BGM-18	<i>Megaerops ecaudatus</i>	99.3	BOLD:ABA9836
BGM-19	<i>Chironax melanocephalus</i> ( <i>C. melanocephalus</i> GOM01)	95.8	BOLD:AAE9045
BGM-20	<i>Cynopterus</i> JLE sp. A	99.3	BOLD:AAA9308
BGM-21	<i>Cynopterus brachyotis</i>	98.7	BOLD:AAA9800
BGM-22	<i>Cynopterus brachyotis</i>	99.5	BOLD:AAA9800
BGM-23	<i>Megaerops ecaudatus</i>	98.7	BOLD:ABA9836
BGM-24	<i>Megaerops ecaudatus</i>	99.7	BOLD:ABA9836
BGM-25	<i>Cynopterus brachyotis</i>	99.7	BOLD:AAA9800
BGM-26	<i>Megaerops ecaudatus</i>	98.4	BOLD:ABA9836
BGM-27	<i>Cynopterus brachyotis</i>	99.5	BOLD:AAA9800
BGM-2	<i>Hipposideros cervinus</i> CMF02	99.8	BOLD:AAB6249
BGM-3	<i>Cynopterus brachyotis</i>	99.5	BOLD:AAA9800
BGH-4	<i>Hipposideros cervinus</i> CMF02	100.0	BOLD:AAB6249
BGM-5	<i>Cynopterus brachyotis</i>	99.7	BOLD:AAA9800
BGM-7	<i>Megaerops ecaudatus</i>	99.2	BOLD:ABA9836
BGM-6	<i>Hipposideros cervinus</i> CMF02	99.6	BOLD:AAB6249
BGM-8	<i>Hipposideros cervinus</i> CMF02	99.5	BOLD:AAB6249
BGM-9	<i>Cynopterus</i> JLE sp. A	99.0	BOLD:AAA9308
TF-5	<i>Cynopterus brachyotis</i>	99.1	BOLD:AAA9800
TF-6	<i>Cynopterus</i> JLE sp. A	100.0	BOLD:AAA9308
TF-8	<i>Cynopterus brachyotis</i>	98.2	BOLD:AAA9800
TF-9	<i>Hipposideros cervinus</i> CMF02	100.0	BOLD:AAB6249
TF-15	<i>Hipposideros cervinus</i> CMF02	100.0	BOLD:AAB6249
TF-20	<i>Hipposideros cervinus</i> CMF02	100.0	BOLD:AAB6249
TI-10	<i>Hipposideros cervinus</i> CMF02	97.5	BOLD:AAB6249
TI-13	<i>Hipposideros bicolor</i> 131	99.7	BOLD:AAD3329
TI-14	<i>Hipposideros cervinus</i> CMF02	99.8	BOLD:AAB6249
TI-16	<i>Hipposideros cervinus</i> CMF02	99.5	BOLD:AAB6249
TI-18	<i>Hipposideros cervinus</i> CMF02	100.0	BOLD:AAB6249
TI-21	<i>Hipposideros</i> cf. <i>bicolor</i> ( <i>H. bicolor</i> 142)	100.0	BOLD:AAC0445
TI-22	<i>Hipposideros cervinus</i> CMF02	99.8	BOLD:AAB6249
TI-23	<i>Hipposideros cervinus</i> CMF02	100.0	BOLD:AAB6249
TI-24	<i>Hipposideros cervinus</i> CMF02	100.0	BOLD:AAB6249
TI-7	<i>Hipposideros cervinus</i> CMF02	100.0	BOLD:AAB6249
TI-8	<i>Hipposideros cervinus</i> CMF02	100.0	BOLD:AAB6249
TF-7	<i>Cynopterus brachyotis</i>	98.5	BOLD:AAA9800
TI-12	<i>Hipposideros</i> cf. <i>bicolor</i> ( <i>H. bicolor</i> 142)	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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