TROPICAL LIFE SCIENCES RESEARCH

Acoustic Characterisation of Bats on Penang Island, Malaysia: First Call Descriptions for Biodiversity Monitoring

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Submitted: 21 November 2025; Accepted: 26 August 2026; Early view: 5 November 2026
To cite this article: Nur-Izzati Abdullah, Hao-Chih Kuo and Lee-Sim Lim (in press). Acoustic characterisation of bats on Penang Island, Malaysia: First call descriptions for biodiversity monitoring. *Tropical Life Sciences Research.*

Highlights:

- The first bat echolocation documentation and acoustic database specifically focused on the bat community on Penang Island.
- The echolocating calls from a total of 12 bat species from families Vespertilionidae, Megadermatidae, Emballonuridae, Rhinolophidae and Hipposideridae were characterised and compared in this study.
- All these characterised echolocating calls were uploaded to ChiroVox – The Bat Call Library (https://www.chirovox.org/). The call IDs for the deposited calls from this study were reported here for future references in research and monitoring works.

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EARLY VIEW

Acoustic Characterisation of Bats on Penang Island, Malaysia: First Call Descriptions for Biodiversity Monitoring

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Abstract: The bat diversity on Penang Island is increasingly threatened by deforestation, agricultural expansion and urbanisation. Although acoustic monitoring is widely used in bat research and conservation, the acoustic diversity of the island's bat fauna remains poorly understood. This study characterises and compares echolocation calls of 12 bat species on Penang Island. Species from families Vespertilionidae, Megadermatidae, Emballonuridae and *Coelops frithii* from Hipposideridae produce frequency-modulated calls, and the remaining Hipposideridae species alongside all species from Rhinolophidae produce constant-frequency calls. Principal component analysis based on frequency parameters well cluster samples by species, with bandwidth and lowest frequency particularly informative for species discrimination.

Comparisons with published mainland data reveals variation in call frequencies among certain species, although these differences should be interpreted cautiously due to limited sample sizes and the absence of molecular validation. This study provides baseline acoustic information to support future bat monitoring and conservation efforts on Penang Island.

Keywords: Bat Acoustics, Call Variation, Island Fauna, Peninsular Malaysia

INTRODUCTION

As of May 2026, 1,511 bat species have been recorded all over the world (Simmons & Cirranello, 2026). Malaysia currently records 143 bat species which represent nearly 10% from the global bat fauna and 40% of Malaysian mammal species (Medway, 1983; Kingston *et al*., 2012; Senawi & Norhayati, 2021; Abdullah *et al*., 2022). There are 11 species of bats that are endemic in Malaysia (Hutson *et al*., 2001). Peninsular Malaysia alone hosts 143 bat species (Lim *et al*., 2017, Senawi & Norhayati, 2021) and therefore Peninsular Malaysia is one of the bat diversity hotspots in the world (Kingston *et al.*, 2003; Kingston *et al.*, 2006). In fact, bat is one of the largest mammalian orders within Peninsular Malaysia (Kingston *et al.*, 2003).

Bats play crucial roles in ecosystems as pollinators and seed dispersers for a vast variety of plants (Aziz *et al*., 2017) as well as natural insect pest suppressors (Srilopan *et al*., 2018). However, habitat loss due to deforestation and urbanisation poses a significant threat to bat populations (Kingston, 2013; Struebig *et al*., 2015). The degradation and fragmentation of their natural habitats reduce roosting and foraging opportunities, ultimately leading to population declines. These threats significantly increase the risk of bat species facing extinction (Cardillo *et al*., 2006). Conservation efforts, such as habitat restoration and protected areas, are essential to mitigate these risks and ensure the survival of bat populations.

Insectivorous bats are known for their ability to echolocate (Griffin, 1958; Fujioka *et al*., 2016). Echolocation is the process by which an individual “visualises” its surroundings using the echoes of their own emitted sounds (Griffin *et al.*, 1960; Jones, 2005). Many bat species exhibit acoustic plasticity, whereby echolocation call parameters such as frequency, duration and bandwidth can shift in response to environmental conditions, particularly habitat structure and foraging space (Ulanovsky & Moss, 2008; Jones & Holderied, 2007; Schnitzler & Kalko, 2001; Obrist, 1995). Bats

rely on echolocation for spatial orientation (Thomas *et al*., 2004), foraging (Thomas *et al*., 2004; Wright *et al*., 2011), and obstacle avoidance (Orbach & Fenton, 2010; Corcoran & Weller, 2018).

Because echolocation calls are species-specific and adapted to their ecological needs, variations in call parameters allow for species-level identification through acoustic analysis (Fenton & Bell, 1981). Distinct call structures reflect differences in foraging strategies, habitat use and social behaviors, making echolocation a valuable tool for studying bat ecology. Acoustic monitoring provides a non-invasive method for tracking bat populations, assessing biodiversity, and detecting shifts in bat activity over time, which is crucial for conservation efforts.

Penang Island has been highly urbanised and industrialised over the past century (Tan *et al*., 2010). However, in this island, there are areas that still serve as agriculture lands (Elhadary *et al*., 2013; Mohammed *et al*., 2015) and reserves for nature, particularly tropical rainforests (Kanniah & Ho, 2017; Masum *et al*., 2017) and mangrove forests (Beh *et al*., 2010). This variety of vegetation provides different habitats to accommodate diverse bat fauna that are ideal to explore the bat echolocation calls and acoustic evolution within the island. However, knowledge about the call diversity of bat species on Penang Island mainly rely on studies on the geographic populations outside the island. To date, echolocation calls have been reported for only one species from Penang Island (Huang *et al*., 2019), indicating that acoustic reference data for this island remain limited.

The aim of this study is to describe the echolocation calls emitted by selected insectivorous bat species on Penang Island recorded from our past surveys. We also discuss the importance of acoustic information and database towards bat conservation and monitoring among Penangites.

MATERIALS AND METHODS

Bat Trapping and Bat Call Recording

Bats were trapped using harp traps and mist nets (9 m × 4 m with a mesh size of 2.5 mm) throughout Penang Island during dry seasons (February to October) from 2014 to 2016 (see details in Nur-Izzati *et al*., 2022). The harp traps were opened before 19:00 and checked at 20:00 and 22:00 as well as 07:00 the following morning. Mist nets were operated between 19:00 to 22:00 and checked every 15 to 20 minutes.

Captured bats were identified based on their external morphological features following established regional identification keys (Kingston *et al.*, 2006; Francis, 2008), which were adapted to incorporate subsequent taxonomic revisions (Soisook *et al.* 2016; Murray *et al.* 2018).

In Peninsular Malaysia, two morphologically overlapped while acoustically well distinguishable Hipposideros species co-occur: *H. kunzi* and *H. bicolor*, emitting calls with peak frequencies at around 142 and 131 kHz, respectively (Murray *et al.*, 2018). With both skull measurements and DNA evidence lacked for our samples and thus the species identity was unable to be unambiguously determined, we assigned our acoustic samples to *H. cf. kunzi* based on their call peak frequencies (see Results).

Calls were recorded individually in the field using a Pettersson M500 ultrasonic microphone (Pettersson Elektronik AB, Sweden) with BatSound® Touch Lite (https://batsound.com/product/batsound-touch-lite/). Calls were recorded either by hand release or a flight tent constructed from mosquito netting (2 m x 2 m x 3 m). For hand release recordings, bats were initially held approximately 30 cm from the microphone for initial recording before being allowed to fly, with the microphone to obtain reference calls before release, with the microphone directed toward the flying individual. Recordings were obtained under a range of field conditions, including cluttered forest understorey, forest edge, and semi-open habitats depending on trapping location and release environment. However, habitat-specific comparisons of echolocation parameters were not conducted due to limited sample sizes within each habitat category. In total, 74 individual bats representing 12 species were recorded and included in this study. Selected high-quality recordings were deposited in ChiroVox - Library of Bat Calls (https://www.chirovox.org/) with ChiroVox UID A001051-A001141 to support transparency, future validation and comparative acoustic studies.

Data Analysis

Echolocation calls recorded during the search phase with high signal-to-noise ratio and minimal strong echoes were selected as reference calls, as they are generally more stable and easily detectable (Simmons *et al.*, 1979; Fenton & Bell, 1981; O'Farrell & Gannon, 1999; Schnitzler *et al*., 2003). For each usable recording sequence, up to five high-quality search-phase pulses were selected for acoustic measurement to minimise the influence of background noise, recording

artefacts and distorted calls. Consequently, the total number of analysed pulses varied among species depending on recording quality and the number of individuals successfully recorded.

Calls were analysed using BatSound version 4.01 (Pettersson Elektronik AB) with the fast Fourier transform (FFT) size of 512 points as defaults. Call structure was determined by visual inspection. Measurements were taken from the harmonic with a maximum energy, following standard acoustic analysis procedures for insectivorous bats. Six acoustic parameters were calculated, namely pulse duration (D), interpulse interval (IPI), peak frequency (FmaxE), start frequency (SF), highest frequency (HF) and lowest frequency (LF). A seventh parameter, bandwidth (BW), was obtained by subtracting LF from HF.

Recordings obtained from both hand-release and flight-tent conditions were analyse together. Because echolocation calls may vary depending on recording context, flight behaviour and environmental conditions, interspecific comparisons were interpreted cautiously, particularly for species represented by small sample sizes or single individuals. Juvenile individuals were excluded from acoustic analyses to minimise ontogenetic variation in echolocation parameters. Sex-specific differences were not assessed due to limited sample sizes across several taxa. Recordings obtained from hand-release and flight-tent conditions were analysed together; however, only search-phase calls with high signal-to-noise ratio and minimal distortion were selected to reduce potential behavioural and recording-context effects.

To aid in visualizing patterns and simplifying the dataset, we applied Principal Component Analysis (PCA) to reduce dimensionality while preserving as much variability as possible. PCA was used as an exploratory tool only and not for species classification. By transforming correlated variables into a smaller set of uncorrelated principal components (PCs), PCA makes it easier to interpret relationships within the data. It also allows multidimensional data to be plotted in two or three dimensions, helping to detect clusters, trends and outliers. Ultimately, PCA makes datasets more manageable for further analysis.

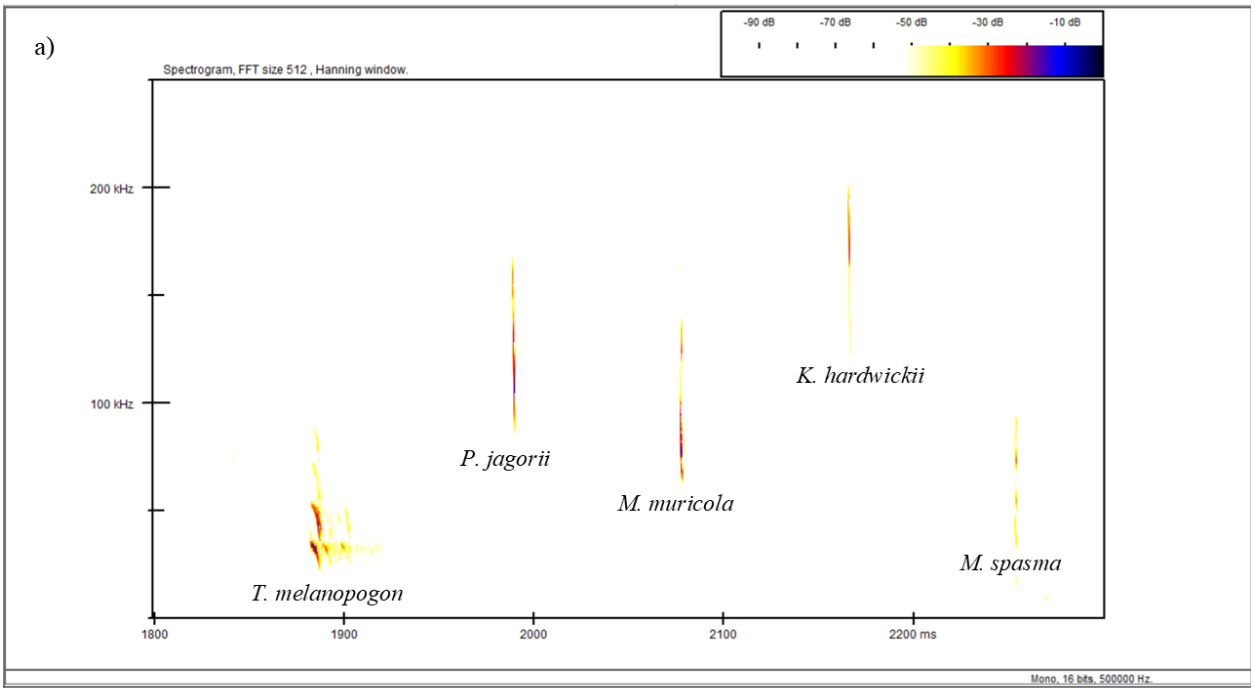
PCA was performed using the prcomp() function in R (version 4.6.0). We included only the spectral variables, namely FmaxE, SF, HF, LF and BW in PCA, aiming to evaluate whether and how these correlated variables differentially contributed to species discrimination. As the PCA entries, all five parameters were standardised to z-scores to account for differences in measurement scales (scale.=TRUE).

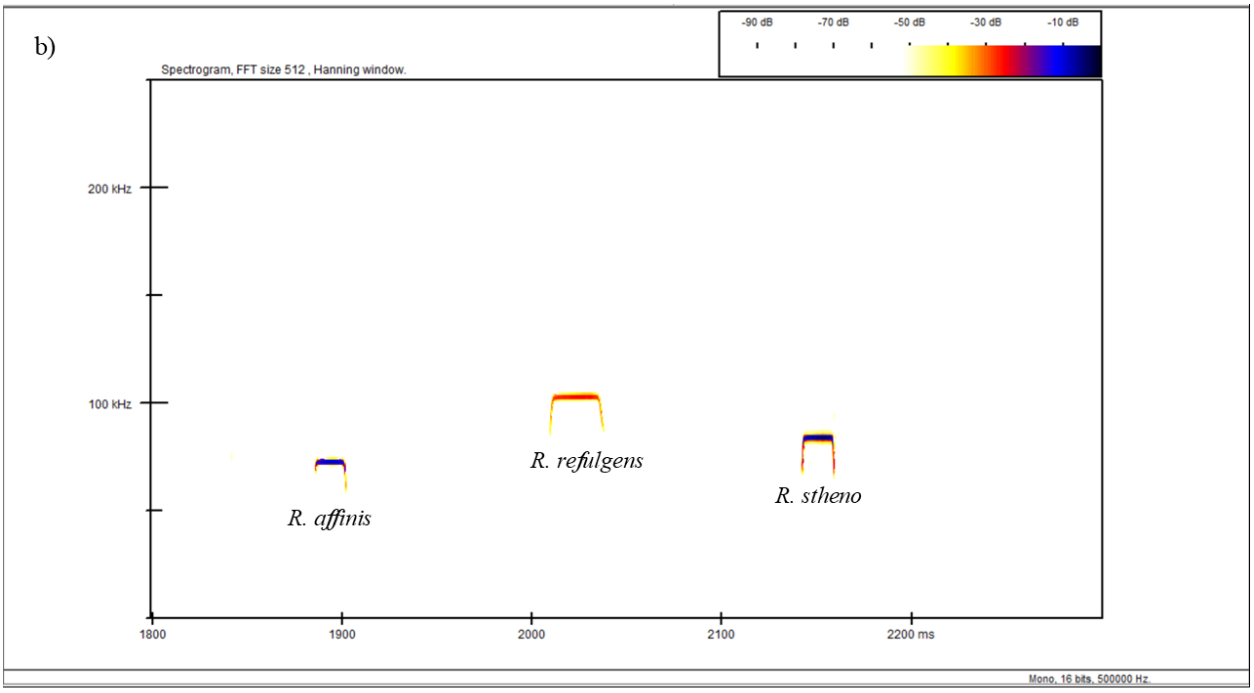
RESULTS

Echolocation calls of 11 bats species of five families were reported in this survey. These included *Hipposideros* cf. *kunzi*, *Hipposideros galeritus* and *Hipposideros armiger* of Hipposideridae, *Rhinolophus affinis*, *Rhinolophus refulgens* and *Rhinolophus stheno* of Rhinolophidae, *Megaderma spasma* of Megadermatidae, *Taphozous melanopogon* of Emballonuridae, and *Phoniscus jagorii, Kerivoula hardwickii*, and *Myotis muricola* of Vespertilionidae. Calls of an additional Hipposideridae species—*Coelops frithii* from literature were also included in comparison (Huang *et al*., 2019). In total, 74 bat individuals representing 12 species were analysed (Table 1).

Murray *et al.* (2018) reported the CF-component frequencies of the calls of *H. kunzi* and *H. bicolor* in the ranges of 135.8–147.5 kHz (n = 842) and 124.9–135.8 kHz (n = 136), respectively. The peak frequencies (FmaxE; quasi-equal to the CF-component frequency) of our acoustic samples from this species complex ranged in 137.2–141.2 kHz, well consistent with those of *H. kunzi*. Therefore, we assigned all these samples to *H.* cf. *kunzi*.

Different call structures were recorded for the 12 bat species. Bats from family Vespertilionidae, Emballonuridae and Megadermatidae, along with *C. frithii* from the family Hipposideridae produce frequency-modulated (FM) calls. Rhinolophid and Hipposiderid bats produce calls dominated by a constant-frequency (CF) component (Figure 1). Reflecting such a distinction, Rhinolophid and Hipposiderid species emitted calls with longer durations (D) than those of the other species although the most pronounced, non-overlapping differences existed between Rhinolophidae and all other species: Rhinolophidae and the other species had D in ranges of > 10 and < 10 ms, respectively (Table 1). Contrastingly, the interpulse interval (IPI) did not show patterns specific to taxonomic groups; in fact, IPI showed extreme variations within species (Table 1), limiting its usage in species discrimination.


a)
-90 dB
-70 dB
-50 dB
-30 dB
-10 dB
Spectrogram, FFT size 512 , Hanning window.
200 kHz
100 kHz
T. melanopogon
P. jagorii
M. muricola
K. hardwickii
M. spasma
1800
1900
2000
2100
2200 ms
Mono, 16 bits, 500000 Hz


b)
-90 dB
-70 dB
-50 dB
-30 dB
-10 dB
Spectrogram, FFT size 512 , Hanning window.
200 kHz
100 kHz
R. affinis
R. refulgens
R. stheno
1800
1900
2000
2100
2200 ms
Mono, 16 bits, 500000 Hz

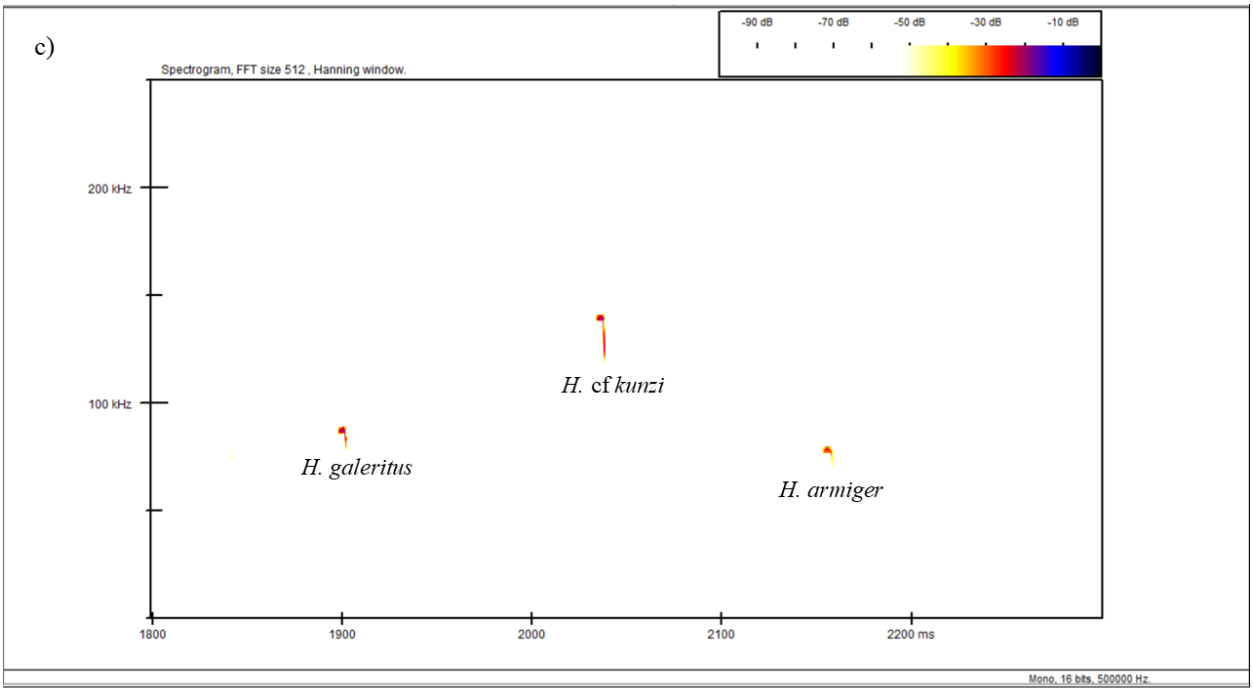


Figure 1. The sonograms of bat calls of a) FM call structures, b) FM-CF-FM call structures and c) CF-FM call structures.

Table 1. Call parameters of 12 bats species from Penang Island.

Species	ChiroVox ID (UID)	n	No. of pulses	Call structure	Call parameters						
					D (ms)	IPI (ms)	FMaxE (kHz)	SF (kHz)	HF (kHz)	LF (kHz)	BW (kHz)
Phoniscus jagorii†	A001083-A001100	1	90	FM	1.4 (0.5-2.7)	29.9 (8.9-237.8)	116.8 ± 12.8	128.0 ± 23.5	149.8 ± 23.2	86.2 ± 5.0	63.5 ± 22.9
Myotis muricola†	A001079-A001081	1	18	FM	2.6 (1.7-3.8)	87.8 (41.3-231.4)	79.6 ± 10.7	102.7 ± 12.6	111.0 ± 7.9	52.0 ± 2.8	59.0 ± 7.4
Kerivoula hardwickii†‡	A001075-A001078	4	20	FM	1.7 (1.0-2.3)	15.6 (7.6-83.5)	158.3 ± 18.7	173.0 ± 21.0	198.4 ± 12.4	97.2 ± 6.0	101.3 ± 14.6
Megaderma spasma†	A001082	1	5	FM	1.1 (1.0-1.3)	393.6 (139.1-1000.0)	75.4 ± 21.4	75.5 ± 21.4	80.2 ± 20.4	66.6 ± 21.5	13.6 ± 3.6

Taphozous melanopogon‡	A001101-A001103	3	15	FM	3.2 (1.2-5.0)	20.8 (3.9-52.8)	28.5 ± 5.4	30.1 ± 5.9	31.3 ± 5.6	19.8 ± 4.0	11.5 ± 2.7
Coelops frithii‡*	A001051-A001052	1	10	FM	0.8 (0.5-1.2)	14.1 (11.0-22.0)	140.9 ± 24.2	141.3 ± 24.5	183.0 ± 2.9	117.5 ± 6.4	65.5 ± 6.3
Hipposideros cf. *kunzi*†‡	A001056-A001067	12	60	CF-FM	5.8 (4.2-8.0)	12.8 (3.4-136.4)	139.8 ± 1.1	139.6 ± 1.2	140.5 ± 1.2	115.2 ± 4.6	25.3 ± 4.4
Hipposideros galeritus‡	A001068-A001074	7	35	CF-FM	5.6 (2.4-9.5)	27.6 (7.8-87.5)	92.1 ± 0.9	91.8 ± 1.0	92.6 ± 1.1	81.4 ± 2.6	11.2 ± 2.1
Hipposideros armiger‡	A001053-A001055	3	15	CF-FM	6.9 (6.1-8.4)	34.3 (13.9-192.1)	71.7 ± 0.2	71.5 ± 0.3	72.0 ± 0	62.0 ± 1.7	10.0 ± 1.7
Rhinolophus affinis‡	A001104-A001120	17	85	CF-FM	29.9 (17.5-48.3)	42.2 (3.5-381.3)	73.7 ± 0.3	72.8 ± 0.8	74.4 ± 0.5	63.0 ± 3.4	11.4 ± 3.4
Rhinolophus refulgens†‡	A001121-A001138	18	90	CF-FM/ FM-CF-FM	24.4 (12.2-46.5)	25.6 (2.8-113.3)	103.9 ± 1.3	101.3 ± 3.6	104.4 ± 1.4	81.2 ± 5.2	23.2 ± 5.6
Rhinolophus stheno†‡	A001139-A001144	6	30	CF-FM/ FM-CF-FM	27.5 (14.9-52.6)	27.1 (4.3-117.8)	86.8 ± 1.0	86.0 ± 1.2	87.1 ± 0.7	73.4 ± 5.2	14.0 ± 5.2

Notes: n = number of individuals, D—pulse duration, IPI—interpulse interval, FMaxE—peak frequency, SF—start frequency, HF—highest frequency, LF—lowest frequency, BW—bandwidth. Values are presented in mean (range) for D and IPI and in mean ± standard deviation for the other parameters. †flight tent: calls recorded during free flight within a flight tent; ‡hand released: calls recorded immediately after released by hand into open space; *published by Huang *et al.* (2019)

We used PCA to summarise call variation in frequency parameters. The identified first two principal components, PC1 and PC2, collectively accounted for 98.3% of the total variance (PC1 and PC2 accounted for 82.6% and 15.7, respectively; Figure 2). On PC1, the loadings of the five frequency parameters were all negative and similar in magnitude (Table 2). This indicated that PC1 represented the general frequency of a call, with large PC1 scores corresponding to calls at

low frequencies. On PC2, the bandwidth (BW) and the lowest frequency (LF), displaying positive and negative loadings there, respectively, had loading magnitudes much greater than those of the other parameters (Table 2). Accordingly, large PC2 scores corresponded to calls with broad BW and/or low LF.

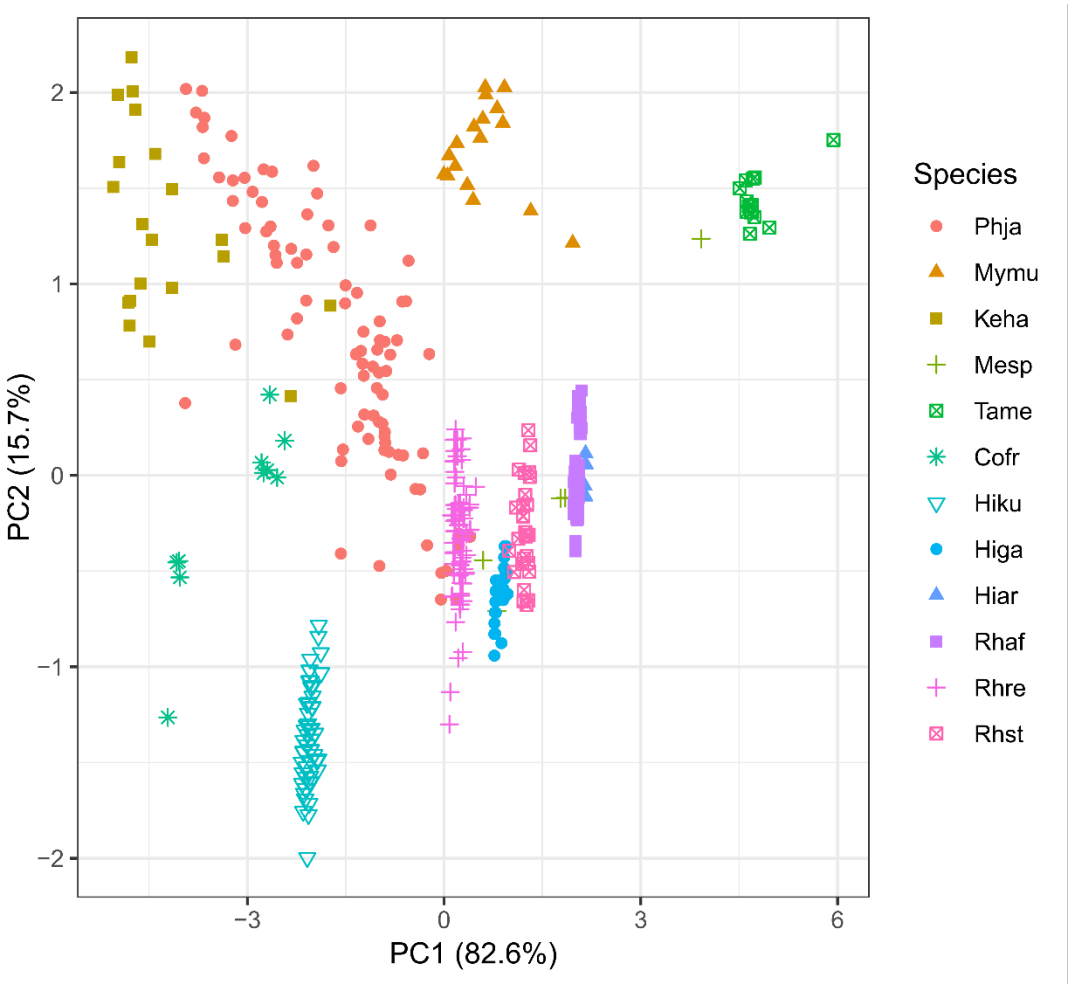


Figure 2. Principal Component Analysis (PCA) based on the five spectral call parameters listed in Table 1.

Notes: Principal component 1 (PC1) and principal component 2 (PC2) together account for 98% of the total variance. The analyzed species include *Phoniscus jagorii* (Phja), *Myotis muricola* (Mymu), *Kerivoula hardwickii* (Keha), *Megaderma spasma* (Mesp), *Taphozous melanopogon* (Tame), *Coelops frithii* (Cofr), *Hipposideros* cf. *kunzi* (Hiku), *Hipposideros galeritus* (Higa), *Hipposideros armiger* (Hiar), *Rhinolophus affinis* (Rhaf), *Rhinolophus refulgens* (Rhre) and *Rhinolophus stheno* (Rhst).

T**able 2.** Principal Component Analysis (PCA) variable loadings for the first two principal components (PC1 and PC2).

Variable	PC1	PC2
FmaxE	-0.476	-0.230
SF	-0.485	0.012
HF	-0.484	0.173
LF	-0.405	-0.624
BW	-0.376	0.726

Note: FMaxE = peak frequency, SF = start frequency, HF = high frequency, LF = low frequency, BW = bandwidth

Based on aforementioned variable loadings, PC1 indicated that *K. hardwickii*, *C. frithii* and *H.* cf. *kunzi* emitted high-frequency calls (small PC1 scores), *T. melanopogon* emitted low-frequency calls (large PC1 scores), and *M. muricola*, *H. galeritus*, *H. armiger* alongside all Rhinolophidae species emitted calls at intermediate frequencies (Figure 2). *P. jagorii* and *M. spasma* had call frequencies varied widely to the point that each overlapped with several other species (Figure 2). Based on PC2, calls of *K. hardwickii*, *M. muricola* and *T. melanopogon* were characterised by broad BW and/or low LF (large PC2 scores), while those of Rhinolophid and Hipposiderid species (including *C. frithii*) showed the opposite patterns (small PC2 scores); calls of *P. jagorii* and *M. spasma* had BW and/or LF in between and overlapping with the former two groups (Figure 2).

DISCUSSION

In this study, *Phoniscus jagorii*, *Coelops frithii*, *Myotis muricola* and *Megaderma spasma* were each represented by a single individual. All high-quality recordings, including those from these taxa, were retained to maximise dataset completeness; however, the findings for these species should be interpreted cautiously because of the limited sample size.

A key limitation is the low number of recordings available for several species, with some represented by only a few individuals. Although some taxa are considered widespread, obtaining high-quality echolocation recordings under standardised field conditions was constrained by low capture success, environmental variability and strict call-quality selection criteria. In addition, echolocation call characteristics may vary with biological factors such as sex and age, as well as behavioural and recording context (Russo & Jones, 2002). Although juveniles were excluded to minimize ontogenetic effects, variation associated with sex and recording context could not be

fully assessed because of uneven sampling. Therefore, this dataset should be regarded as a preliminary regional acoustic reference rather than a comprehensive call library, and future studies with larger sample sizes and standardised sampling protocols are needed to better characterise intraspecific variation.

Global repositories such as ChiroVox may help overcome these limitations by providing standardised, curated echolocation reference calls for cross-regional comparison (Gorfol *et al*., 2022). Integration of such databases would improve species-level identification confidence and strengthen regional call libraries, particularly in under-sampled yet bat diversity rich tropical regions. Nevertheless, this study provides baseline acoustic information for Penang Island and contributes to bat monitoring and conservation. Future studies incorporating larger sample sizes, longer sampling term and broader habitat coverage are needed to refine species-level acoustic characterisation.

Echolocation frequency is widely used to distinguish cryptic bat species when morphological traits are insufficient (Jones & Barlow, 2004). CF calls are specialised for detecting fluttering prey and typically have narrow bandwidths and long durations, whereas FM calls provide greater spatial resolution in cluttered habitats (Schnitzler & Kalko, 2001). These differences explain the distinct clustering of Rhinolophidae and Hipposideridae relative to Vespertilionidae, Emballonuridae and Megadermatidae observed in the present study. Similar convergence in echolocation structure has been associated with comparable flight behaviour and foraging strategies in similar environments (Jung *et al*., 2014). Echolocation is also a flexible trait influenced by behavioural and environmental conditions (Jakobsen *et al*., 2013; Surlykke & Kalko, 2008). However, because habitat use was not directly assessed, interpretation is limited to acoustic variation.

Dejtaradol (2009) previously reported echolocation as the main distinguishing feature within *Rhinolophus refulgens*. However, subsequent taxonomic studies demonstrated that *R. refulgens* can be reliably distinguished from closely related taxa based on a combination of DNA barcodes and subtle morphological characters, including differences in forearm and skull measurements and the morphology of the supraorbital region (Soisook *et al*., 2016; Francis, 2019).

Hipposideridae bats exhibit high cryptic diversity, with narrowband echolocation calls often showing clear interspecific divergence (Jones & Barlow, 2004; Kingston *et al.*, 2001). Francis *et al.* (1999) reported a 19 kHz difference between *Hipposideros ridleyi* and *H. orbiculus*, while

Kingston *et al.* (2001) identified two cryptic *H. bicolor* lineages in Peninsular Malaysia with distinct peak frequencies (131 and 142 kHz), later recognised as *H. bicolor* and *H. kunzi* (Murray *et al.,* 2018). Such differences may function in prey detection, intraspecific communication (Bradbury & Vehrencamp, 1998) or habitat adaptation.

Spectral parameters are generally considered more robust descriptors than temporal parameters because they are less influenced by flight behaviour and recording geometry, making them particularly suitable for comparative acoustic studies (Russo & Jones, 2002; Surlykke & Kalko, 2008). Nevertheless, the substantial acoustic similarity observed here suggests that spectral parameters alone have limited discriminatory power for separating these cryptic taxa on Penang Island. The discrepancy from mainland records may reflect geographic or population-level variation; however, this interpretation remains tentative without complementary genetic evidence. Species identification was therefore based on morphological confirmation using updated regional taxonomic references. Future molecular studies are required to determine whether island isolation has contributed to the observed acoustic similarity.

Hipposideros galeritus is another species that showed potential geographical variation in their echolocating calls. Our call data based on a total of seven individuals included in this study emitted calls at peak frequency between 91kHz to 94kHz, which is the lowest among the reported to date. Existing records of peak frequency for *H. galeritus* were between 112kHz to 116kHz from the population from at central peninsular India (Srinivasulu *et al.*, 2015), between 99kHz-116kHz from the Vietnamese population (Győrössy *et al.* 2024), between 98.5kHz–102.5kHz from the Cambodian population (Phauk *et al.*, 2013), and 97.66kHz ± 0.7 from the Sri Lanka island population (Kusuminda *et al.*, 2019).

Echolocation structure is influenced by habitat complexity and foraging behaviour (McKenzie *et al*., 1995; Gao *et al*., 2011), and geographic variation in bat calls is well documented, with frequency shifts of 5–10 kHz often observed alongside high individual variability (Jiang *et al.*, 2015). Environmental conditions, habitat type, and foraging mode further shape call structure (Barclay *et al.*, 1999; Obrist, 1995; Jones & Teeling, 2006). However, disentangling these effects is challenging due to interacting influences of genetic drift, ecological selection and social factors may interact to influence call design (Jiang *et al.*, 2015).

Penang Island's isolation may contribute to local acoustic variation. The island was connected to the Sunda Shelf during the Late Pleistocene, with separation occurring after Holocene sea-level rise approximately 8,000–10,000 years ago (Solihuddin, 2014). As insular populations likely originated from mainland conspecifics (Kruskop, 2011), broadly similar species composition and acoustic traits are expected. However, ecological differences in habitat structure may still drive subtle divergence in call design (Shuyi *et al*., 1999). In the absence of genetic or population-level data, interpretations of insular divergence remain tentative.

Phoniscus jagorii exhibited differences in peak frequency compared to mainland records. However, this comparison is based on recordings from a single individual, as reported by Nur-Izzati *et al*. (2022). Although only a single individual was captured, multiple high quality search phase echolocation calls were recorded from this individual within a flight tent allowing 90 pulses to be analysed and providing a robust description of its call characteristics despite the limited number of individuals. Nevertheless, analysing multiple pulses from a single individual does not account for potential intraspecific acoustic variation. Consequently, the observed differences may reflect recording context, harmonic selection or intraspecific variation rather than true geographic divergence. With only eleven known voucher specimens of *Phoniscus jagorii* (three from Penang Island) kept in the museums (personal database of Gabor Csorba, Hungarian Natural History Museum, Budapest, Hungary) and the scarcity in the literature, it reflects the rarity of this species in Peninsular Malaysia and throughout Southeast Asia (Oo *et al.*, 2019; Thong *et al.*, 2021). Therefore, we proceed to describe the calls of the single *Phoniscus jagorii* individual for future conservation and monitoring importance of this species. In addition, our description here is the first detailed description of its echolocation calls from Penang Island. However, the absence of genetic data limits evolutionary interpretation, and further integrative studies incorporating larger sample sizes and molecular analyses are needed to confirm whether these differences represent geographic variation.

Islands are important reservoirs of bat diversity but are often subject to conservation pressures (Jones *et al*., 2009). Given limited knowledge of bat assemblages in Penang, further ecological and taxonomic studies are needed to support conservation planning. While this study provides baseline acoustic data, results should be interpreted cautiously due to limited sample sizes. Nevertheless, regional acoustic reference developed here provides an important foundation for passive acoustic monitoring on Penang Island, enabling future biodiversity assessments and long-term conservation programs. Continued monitoring, together with public education

highlighting the ecological roles of bats as pollinators, seed dispersers and insect pest regulators, will further strengthen bat conservation efforts in the region.

CONCLUSION

This study provides the first preliminary acoustic reference dataset for bat species on Penang Island, contributing new regional information on echolocation call characteristics for multiple insectivorous bat taxa. The results demonstrate that spectral parameters are useful for discriminating most species, especially the band width and the lowest frequency. On the other hand, species identification based solely on echolocation calls is insufficient, and integrating acoustic monitoring with other lines of evidence such as skull measurement and DNA barcodes is warranted. This combined approach improves taxonomic reliability and strengthens the development of regional acoustic reference libraries.

The study also demonstrates that island bat assemblages may exhibit acoustic variation relative to mainland populations; however, these differences should be interpreted cautiously due to limited sample sizes and potential behavioural and recording-context effects. Nevertheless, this study provides an important baseline for future bat research and acoustic monitoring on Penang Island.

For bat conservation and monitoring in Penang, the findings support the continued use of integrated survey methods combining mist-netting, harp trapping and acoustic recording to improve detection coverage and species identification accuracy. The establishment of comprehensive local reference libraries, supported by larger sample sizes and future genetic validation, will further enhance biodiversity assessment and conservation planning in the region.

ACKNOWLEDGEMENTS

Lee-Sim Lim would like to thank Idea Wild (https://ideawild.org/) for sponsoring a Pettersson M500 ultrasonic microphone (Pettersson Elektronik AB, Sweden) and a tablet for her acoustic research (LIMMALA0614-00). We would like to express our highest appreciation to Dr Joe Chun-Chia Huang for training the first author in acoustic research methods and skills, nevertheless to Dr. Nurul Ain Elias for sharing bat trapping skills in hilly habitat. Our heartfelt thanks to all field assistants especially Nur Nabila Shuib, Mohamad Fikri Rasib, Nur Fazrina Jainulabadeen, Choi

Kha Yee, Jimmy Wong Cheng Min and Nur Hafizah binti Ibrahim and UNIMAS team (Nurul Farah Diyana Ahmad Tahir, Sultana Parvin Habeebur Rahman, Nurshilawati Abdul Latip, Mohd Isham Mohd Azhar, Mohd Amsyari Morni, Julius Williams, Qhairil Shyamri Rosli and Rafik Murni). Besides, we would like to thank the Forestry Department of Peninsular Malaysia and Department of Wildlife and National Parks, Peninsular Malaysia for giving the permit for us to trap bats in Penang National Park and selected forest reserves within Penang Island. I also would like to thank Tropical Spice Garden, Freedom Eco Farm, Comfort Organic Farm, Penang Bat Cave Temple and Penang Peranakan Mansion for giving us the permission and opportunity to trap within their property. This project was funded by USM short term grant (304/PJJAUH/6313060) and Malaysian Ministry of Higher Education, Fundamental Research Grant (R503-KR-FRG001-0000002460-K134) given to Lee-Sim Lim.

AUTHORS' CONTRIBUTIONS

Lee-Sim Lim was the principal investigator of this project who had conceptualised the central research idea. Lee-Sim Lim was also the main supervisor to the Nur Izzati Abdullah who guided her and supervised the research direction and progress of this project. Nur Izzati Abbdullah and Lee-Sim Lim collected and compiled echolocation data. Nur Izzati Abdullah obtained echolocation call parameters. Hao-Chih Kuo conducted statistical analyses, final checked and validated all data and analyses. Nur Izzati Abdullah drafted the manuscript. Finally, Lee-Sim Lim and Hao-Chih Kuo edited it.

REFERENCES

Abdullah, M. T., Hall, L., Anwarali Khan, F., & Azhar, I. (2022). Bats of Malaysia. Cover 22 July 2021: Penerbit Universiti Malaysia Terengganu.

Aziz, S. A., Clements, G. R., McConkey, K. R., Sritongchuay, T., Pathil, S., Abu Yazid, M. N. H., Campos-Arceiz, A., Forget, P.M., & Bumrungsri, S. (2017). Pollination by the locally endangered island flying fox (*Pteropus hypomelanus*) enhances fruit production of the economically important durian (*Durio zibethinus*). *Ecology and Evolution*, *7*(21), 8670-8684.

Barclay, R. M., Fullard, J. H., & Jacobs, D. S. (1999). Variation in the echolocation calls of the hoary bat (*Lasiurus cinereus*): influence of body size, habitat structure, and geographic location. *Canadian Journal of Zoology,* 77(4), 530-534.

Beh, B. C., MatJafri, M. Z., & Lim, H. S. (2010). Remotely based monitoring of the mangroves over Penang Island, Malaysia. In *Multispectral, Hyperspectral, and Ultraspectral Remote Sensing Technology, Techniques, and Applications III* (Vol. 7857, pp. 162-167). International Society for Optics and Photonics.

Bradbury, J. W., & Vehrencamp, S. L. (1998). Signal design rules. *Principles of animal communication* (Vol. 132, pp. 571-618). Sunderland, Massachusetts: Sinauer Associates.

Cardillo, M., Mace, G. M., Gittleman, J. L., & Purvis, A. (2006). Latent extinction risk and the future battlegrounds of mammal conservation. *Proceedings of the National Academy of Sciences of the United States of America,* 103(11), 4157-4161.

Corcoran, A. J., & Weller, T. J. (2018). Inconspicuous echolocation in hoary bats (*Lasiurus cinereus*). *Proceedings of the Royal Society B: Biological Sciences*, 285(1878), 20180441.

Dejtaradol, A. (2009). *A taxonomic review of Rhinolophus pusillus and Rhinolophus lepidus (Chiroptera: Rhinolophidae) in Thailand.* Doctoral dissertation. Prince of Songkla University, Thailand.

Elhadary, Y. A. E., Samat, N., & Obeng-Odoom, F. (2013). Development at the peri-urban area and its impact on agricultural activities: An example from the Seberang Perai Region, Penang State, Malaysia. *Agroecology and Sustainable Food Systems*, 37(7), 834-856.

Fenton, M. B., & Bell, G. P. (1981). Recognition of species of insectivorous bats by their echolocation calls. *Journal of Mammalogy,* 62(2), 233-243.

Francis, C. M. (2019). *Field guide to the mammals of South-east Asia*. Bloomsbury Publishing.

Francis, C. M. (2008). *A field guide to the mammals of South-East Asia*: New Holland Publishers.

Francis, C. M., D. Kock and J. Habersetzer. 1999. Sibling species of *Hipposideros ridleyi* (Mammalia, Chiroptera, Hipposideridae). *Senckenbergiana Biologica*, 79, 255–270.

Fujioka, E., Aihara, I., Sumiya, M., Aihara, K., & Hiryu, S. (2016). Echolocating bats use future-target information for optimal foraging. *Proceedings of the National Academy of Sciences*, 113(17), 4848-4852.

Gao, L., Balakrishnan, S., He, W., Yan, Z., & Müller, R. (2011). Ear deformations give bats a physical mechanism for fast adaptation of ultrasonic beam patterns. *Physical review letters*, 107(21), 214301.

Görföl, T., Huang, J. C. C., Csorba, G., Győrössy, D., Estók, P., Kingston, T., Szabadi, K. L., McArthur, E., Senawi, J., Furey, N. M., Tu, V. T., Thong, V. D., Khan, F. A. A., Jinggong, E. R., Donnelly, M., Kumaran, J. V., Liu, J. N, Chen, S. F., Tuanmu, M. N., Ying-Yi Ho, Y.

Y., Heng-Chia Chang, H. C., Nurul-Ain, E., Nur-Izzati, A., Lim, L. S., Squire, C. D., & Zsebők, S. (2022). ChiroVox: a public library of bat calls. *PeerJ*, 10, e12445.

Griffin, D.R., Webster, F.A., & Michael, C.R. (1960). The echolocation of flying insects by bats. *Animal behaviour*, 8(3), 141-154.

Griffin, D. R. (1958). *Listening in the dark: The acoustic orientation of bats and men.* Yale University Press.

Győrössy, D., Csorba, G., Szabadi, K. L., Estók, P., Tu, V. T., Thong, V. D., Furey, N. M., Huang, J. C. C., Tuanmu, M-N., F., Dai, Zsebők, S. & Görföl, T. (2024). The calls of Vietnamese bats: a major step toward the acoustic characterization of Asian bats. *Scientific reports*, 14(1), 23335.

Huang, J. C. C., Nur-Izzati, A., Nurul-Ain, E., Sah, S. A. M., & Lim, L. S. (2019). The first record of *Coelops frithii* (Blyth, 1848) to Penang, Malaysia, with a note of the potential acoustic plasticity of the species. *Journal of Bat Research & Conservation,* 12(1), 21-26.

Jakobsen, L., Ratcliffe, J. M., & Surlykke, A. (2013). Convergent acoustic field of view in echolocating bats. *Nature*, 493(7430), 93-96.

Jiang, T., Wu, H., & Feng, J. (2015). Patterns and causes of geographic variation in bat echolocation pulses. *Integrative zoology,* 10(3), 241-256.

Jones, K. E, Mickleburgh, S. P., Sechrest, W., & Walsh, A. L. (2009). Global overview of the conservation of island bats: importance, challenges and opportunities. *Island Bats: Evolution, Ecology, and Conservation.*, 496-530.

Jones, G., & Holderied, M. W. (2007). Bat echolocation calls: adaptation and convergent evolution. *Proceedings of the Royal Society B: Biological Sciences*, 274(1612), 905-912.

Jones, G., & Teeling, E. C. (2006). The evolution of echolocation in bats. *Trends in Ecology & Evolution,* 21(3), 149-156.

Jones, G. (2005). Echolocation. *Current Biology*, 15(13), R484-R488.

Jones, G., & Barlow, K. E. (2004). Cryptic species of echolocating bats. In J. T., C. M., & M. Vater (Eds.), *Echolocation in Bats and Dolphins* (pp. 345 - 349). Chicago University Press.

Jung, K., Molinari, J., & Kalko, E. K. (2014). Driving factors for the evolution of species-specific echolocation call design in new world free-tailed bats (Molossidae). *PloS one*, 9(1), 1-9.

Kanniah, K. D., & Ho, C. S. (2017). Urban forest cover change and sustainability of Malaysian cities. *Chemical Engineering Transactions*, *56*, 673-678.

Kingston, T. (2013). Response of bat diversity to forest disturbance in Southeast Asia: insights from long-term research in Malaysia. In *Bat evolution, ecology, and conservation* (pp. 169-185). Springer, New York, United States.

Kingston, T., Juliana, S., Nurul-Ain, E., Hashim, R., & Zubaid, A. (2012). The Malaysian bat conservation research unit: from a national model to an international network. *Malaysian Applied Biology*, 41(2), 1-10.

Kingston, T., Lim, B. L., & Akbar, Z. (2006). *Bats of Krau wildlife reserve*: Penerbit Universiti Kebangsaan Malaysia.

Kingston, T., Jones, G., Akbar, Z., & Kunz, T. H. (2003). Alternation of echolocation calls in 5 species of aerial-feeding insectivorous bats from Malaysia. *Journal of Mammalogy, 84*(1), 205-215.

Kruskop, S. V. (2011). New data on the bat fauna of Con Dao Islands (Ba Ria-Vung Tau Province, Vietnam). *Russian Journal of Theriology*, 10(2), 37-46.

Kusuminda, T., Mannakkara, A., Amarasinghe, C. J., Karunarathna, M. K., Patterson, B. D., & Yapa, W. B. (2019). Echolocation call characteristics of Hipposiderid bats in Sri Lanka.

Lim, V. C., Ramli, R., Bhassu, S., & Wilson, J. J. (2017). A checklist of the bats of Peninsular Malaysia and progress towards a DNA barcode reference library. *PloS one*, *12*(7), e0179555.

Masum, K. M., Mansor, A., Sah, S. A. M., & San Lim, H. (2017). Effect of differential forest management on land-use change (LUC) in a tropical hill forest of Malaysia. *Journal of environmental management*, *200*, 468-474.

McKenzie, N. L., Gunnell, A. C., Yani, M., & Williams, M. R. (1995). Correspondence Between Plight Morphology and Foraging Ecology in Some Palaeotropical Bats. *Australian Journal of Zoology, 43*(3), 241-257.

Medway, L. (1983). *The Wild Mammals of Malaya (Peninsular Malaysia and Singapore)*. 2nd Edition. Oxford University Press, Kuala Lumpur. 130 pp.

Mohammed, K. S., Elhadary, Y., Samat, N., & Omar, N. Q. (2015). GIS and remote sensing techniques for measuring agriculture land loss in Balik Pulau region of Penang state, Malaysia. *Asian Journal of Agriculture and Rural Development*, 5(2), 30-41.

Murray, S. W., Khan, F. A., Kingston, T., Zubaid, A., & Campbell, P. (2018). A new species in the *Hipposideros bicolor* group (Chiroptera: Hipposideridae) from Peninsular Malaysia. *Acta Chiropterologica*, *20*(1), 1-29.

Nur-Izzati, A. Nurul-Ain, E., Huang, J. C. C., Khan, F. A. A., & Lim, L. S. (2022). Bat species in the forests reserves of Penang Island after 2010: with two more new records filed. *Journal of Sustainability and Science Management*, 17(5), 13-22.

Obrist, M. K. (1995). Flexible bat echolocation: the influence of individual, habitat and conspecifics on sonar signal design. *Behavioral ecology and sociobiology*, 36(3), 207-219.

O'Farrell, M. J., Miller, B. W., & Gannon, W. L. (1999). Qualitative identification of free-flying bats using the Anabat detector. *Journal of Mammalogy,* 80(1), 11-23.

Orbach, D. N., & Fenton, B. (2010). Vision impairs the abilities of bats to avoid colliding with stationary obstacles. *PLoS One*, 5(11), 1-7.

Oo, S.S.L., Kingston, T., Francis, C., Rosell-Ambal, R.G.B. & Tabaranza, B. (2019). *Phoniscus jagorii*. *The IUCN Red List of Threatened Species* 2019: e.T10976A21974660. https://dx.doi.org/10.2305/IUCN.UK.2019-3.RLTS.T10976A21974660.en. Accessed on 12 June 2026.

Phauk, S., Phen, S., & Furey, N. M. (2013). Cambodian bat echolocation: a first description of assemblage call param. Cambodian Journal of Natural History, 16.

Russo, D., & Jones, G. (2002). Identification of twenty-two bat species (Mammalia: Chiroptera) from Italy by analysis of time-expanded recordings of echolocation calls. *Journal of Zoology*, 258(1), 91-103.

Senawi, J. & Norhayati, A. (2021). *Kelawar Malaysia / Bats of Malaysia*. Kementerian Tenaga & Sumber Asli, Malaysia. pp. 80.

Simmons, N. B. & Cirranello, A. L. (2026). *Bat Species of the World: A taxonomic and geographic database*. *Version 1.10*. [https://batnames.org/] Accessed on 05/18/2026.

Simmons, J. A., Fenton, M. B., & O'Farrell, M. J. (1979). Echolocation and pursuit of prey by bats. *Science,* 203(4375), 16-21.

Schnitzler, H. U., Moss, C. F., & Denzinger, A. (2003). From spatial orientation to food acquisition in echolocating bats. *Trends in Ecology & Evolution*, 18(8), 386-394.

Schnitzler, H. U., & Kalko, E. K. (2001). Echolocation by insect-eating bats: we define four distinct functional groups of bats and find differences in signal structure that correlate with the typical echolocation tasks faced by each group. *Bioscience*, 51(7), 557-569.

Soisook, P., Karapan, S., Srikrachang, M., Dejtaradol, A., Nualcharoen, K., Bumrungsri, S., Lin Oo, S.S., Aung, M.M., Bates, P.J., Harutyunyan, M., Buś, M.M. & Bogdanowicz, W. (2016). Hill forest dweller: a new cryptic species of Rhinolophus in the 'pusillus group' (Chiroptera: Rhinolophidae) from Thailand and Lao PDR. *Acta Chiropterologica*, 18(1), 117-139.

Solihuddin, T. (2014). A drowning Sunda Shelf model during last glacial maximum (LGM) and Holocene: A review. *Indonesian Journal on Geoscience*, 1(2), 99-107.

Srilopan, S., Bumrungsri, S., & Jantarit, S. (2018). The wrinkle-lipped free-tailed bat (*Chaerephon plicatus* Buchannan, 1800) feeds mainly on brown planthoppers in rice fields of central Thailand. *Acta Chiropterologica*, 20(1), 207-219.

Srinivasulu, B., Srinivasulu, C., & Kaur, H. (2015). Echolocation calls of four species of leaf-nosed bats (genus Hipposideros) from central peninsular India. *Current Science*, 108(6), 1055-1057.

Struebig, M. J., Wilting, A., Gaveau, D. L., Meijaard, E., Smith, R. J., Abdullah, T., Abram, N., Alfred, R., Ancrenaz, M., Augeri, D.M. & Belant, J. L. (2015). Targeted conservation to safeguard a biodiversity hotspot from climate and land-cover change. *Current Biology*, 25(3), 372-378.

Surlykke, A., & Kalko, E. K. (2008). Echolocating bats cry out loud to detect their prey. *PLoS one*, 3(4), 1-10.

Shuyi, Z., Jiang, F., Zhenxin, L., Lianxi, S., & Lixin, W. (1999). Comparison of the echolocation signals in three species of bats at fly. *Dong wu xue bao. Acta Zoologica Sinica*, 45(4), 385-389.

Tan, K. C., San Lim, H., MatJafri, M. Z., & Abdullah, K. (2010). Landsat data to evaluate urban expansion and determine land use/land cover changes in Penang Island, Malaysia. *Environmental Earth Sciences*, 60(7), 1509-1521.

Thomas, J. A., Moss, C. F., & Vater, M. (Eds.). (2004). *Echolocation in bats and dolphins*. University of Chicago Press.

Thong, V. D., Denzinger, A., Sang, N. V., Huyen, N. T. T., Thanh, H. T., Loi, D. N., Nha, P.V., Viet, N.V., Tien, P.D., Tuanmu, M.N., Thongphachanh, L., Luong, N. T., Huang, J.C.C., & Schnitzler, H. U. (2021). Bat diversity in Cat Ba Biosphere Reserve, northeastern Vietnam: a review with new records from mangrove ecosystem. *Diversity*, 13(8), 376.

Ulanovsky, N., & Moss, C. F. (2008). What the bat's voice tells the bat's brain. *Proceedings of the National Academy of Sciences,* 105(25), 8491-8498.

Wright, G. S., Wilkinson, G. S., & Moss, C. F. (2011). Social learning of a novel foraging task by big brown bats, *Eptesicus fuscus*. *Animal Behaviour*, 82(5), 1075-1083.