

Characterization of Echolocation Calls from Insectivorous Bats in Puting Bato Cave 5, Burdeos, Polillo Island, Philippines

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Acoustic recording is rarely applied in studying Philippine bats, which is reflected by the scarcity of related publications in the country. As a result, the echolocation calls of many local bat species are still unknown, and a reliable bat call library does not exist. Here, we described the echolocation call of some insectivorous bats in Polillo Island and used the call characteristics to define each species. We captured and recorded the echolocation calls of cave-dwelling bats in Puting Bato Cave 5 in October and November 2019. Using BatSound, we measured the spectral and temporal call characteristics and determined the call structure and duty cycle of all recorded individuals. Then, we applied multivariate discriminant function analysis (DFA) to classify the calls into species and identify the call parameter with the highest discriminating power. In total, we recorded 104 individual bats from five species belonging to four families. All species from families *Miniopteridae* and *Vespertilionidae* produced steep frequency-modulated (FM) sweeps, while the species belonging to families *Hipposideridae* and *Rhinolophidae* emitted calls with constant frequency and FM components. Among the recorded species, *Hipposideros coronatus* had the highest peak frequency record (> 150 kHz), while *Myotis macrotarsus* had the lowest (< 40 kHz). Meanwhile, the longest call duration was recorded from *Rhinolophus arcuatus* (> 40 ms), while *Miniopterus paululus* produced the shortest call (< 3 ms). The DFA perfectly classified the calls into the correct species and peak frequency was the most important predictor among the call parameters measured. Our results provide strong evidence that insectivorous bats in Puting Bato Cave 5 produce species-specific echolocation calls, and acoustic recording can be a reliable method to enhance research on these cave-dwelling bats.

Keywords: bat echolocation, bioacoustics, call characterization, cave, DFA, Polillo Island

INTRODUCTION

Mist nets and harp traps are the standard live capture methods used in bat sampling (Sikes *et al.* 2011). Mist nets are light and can be set in different environments, whereas harp traps have an easier retrieval mechanism. Despite the convenience of using nets, these traditional

methods were found to be insufficient in recording all species when used alone, or even combined, in bat surveys (Berry *et al.* 2004; Flaquer *et al.* 2007; Larsen *et al.* 2007; Pech-Canche *et al.* 2011). Bats appear to learn the position of the nets particularly when placed at the same location for consecutive days, and echolocating bats – mainly the high-frequency call producers – effectively evade the nets. Net avoidance results in a decrease in the number of

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captures, which often leads to underestimations of species diversity, richness, and activity.

Aside from the conventional netting methods, acoustic recording is also used in studying bats (Frick 2013). Echolocation call recording provides more non-invasive and passive monitoring of bats and their activity. Currently, bat detectors and ultrasonic recorders are becoming more sophisticated and reliable in documenting bat presence and activity (Frick 2013; Hogue and McGowan 2018; Banner *et al.* 2018). The use of echolocation calls in identifying species has shown promising results, as demonstrated in studies with high rates of correct classification of calls to species (Russo and Jones 2002; Carter *et al.* 2012; Rodriguez–San Pedro and Simonetti 2013; Mifsud and Vella 2019). In addition, certain call characters were found to be species-specific and contribute largely to the accurate classification of species through call analysis (Vaughan *et al.* 1997; Murray *et al.* 2001; Wordley *et al.* 2014; Zamora-Gutierrez *et al.* 2016). Furthermore, acoustic recordings were also effective in documenting bat activities – including roost emergence (Reiter *et al.* 2008), feeding (Griffin 2001), and migration (Ijas *et al.* 2017). Given the advantages of acoustic recording, certain information cannot be derived from call recordings – including actual counts, physical condition, and biological data such as sex, age, and morphometrics – which are essential in studying population estimates.

Neither of the given methods provides complete information on bats; therefore, the use of both netting and acoustic recording is recommended to maximize species documentation (Flaquer *et al.* 2007; Francl *et al.* 2011; Wordley *et al.* 2018). These methods are simultaneously used in other countries, but only netting is still being largely utilized in the Philippines. Bat surveys with acoustic recording are very limited (Sedlock 2001, 2002; Mould 2012; Sedlock *et al.* 2014) and acoustic monitoring of bat activities are seldom performed in the country (Dimaculangan *et al.* 2019; Sedlock *et al.* 2019). Moreover, studies that focused on the echolocation calls of Philippine bats were not available until the recent publication of Amberong *et al.* (2021).

The lack of comprehensive call libraries hampers the use of bioacoustics in studying Philippine bats. The country hosts 79 bat species, and around 54 species potentially use laryngeal echolocation (Heaney *et al.* 2010). Several of these species are also very cryptic (Heaney *et al.* 2010), and netting alone might not be sufficient to document their presence and activity. Acoustic recording can account for these bats that are difficult to capture, especially the echolocating species, which rely mostly on sound to move and forage. The establishment of local bat call libraries will support the use of acoustic recording in bat studies and promote bat acoustics research in the Philippines.

However, extensive acoustic records, call description, and locality-based information are needed to build a call library for Philippine bats.

Thus, here we provide an elaborate description of the echolocation calls of five insectivorous bat species occurring in Puting Bato Cave 5, Polillo Island, Quezon, Philippines with information on the structure and the spectral and temporal characteristics of their call. We performed a DFA to test the ability of the call characters to separate the species and determined the discriminating power of each call parameter measured. This information contributes to our current understanding of the acoustic diversity of laryngeal echolocating bats in the Philippines and highlights the need for bat acoustics research in the country. Our results would be helpful in the development of a local bat call library and may serve as a helpful guide to future researchers interested in studying Philippine bats.

MATERIALS AND METHODS

Study Area

We collected reference echolocation calls from insectivorous bats inhabiting Puting Bato Cave 5. It is one of the four identified caves in the Puting Bato cave system located near the beach shores of Brgy. Aluyon, Burdeos, Polillo Island (14° 54' 56.5" N, 121° 59' 59" E). Puting Bato Cave 5 is the smallest among the surveyed caves in Sitio Puting Bato, Polillo Island in terms of cave length (51.2 m) and entrance size (2.66 m²). There is only one entrance with two openings where the larger opening is mainly utilized by bats and swiftlets. Five out of 16 insectivorous bat species occurring in Polillo Island inhabit Puting Bato Cave 5 (Alviola 2000; Heaney *et al.* 2010) with an estimated population size of < 500 individuals inside the cave. These five species include *Hipposideros coronatus*, *Miniopterus paululus*, *M. eschscholtzii*, *Myotis macrotarsus*, and *Rhinolophus arcuatus*.

Puting Bato Cave 5 is situated in a secondary lowland forest over limestone where the tallest vegetation consists of *Dendrocnide meyeniana*, *Ficus pseudopalma*, *Caryota* sp., and *Macaranga* sp. The understorey is a mixture of saplings, bamboos (*Bambusa* sp.), pandan (*Pandanus* sp.), aroids (*Alocasia* sp., *Homalonema* sp., *Rhaphidophora* sp.), ferns and allies (*Asplenium subnormale*, *Lygodium auriculatum*, *Selaginella fenixii*), and several shrubs (*Clerodendrum* sp., *Leea* sp.).

Voucher Call Recording

We captured cave-dwelling bats in Puting Bato Cave 5 to assess species identification and to record reference echolocation calls. A mist net was set 2 m from the cave

entrance at dawn between 04:00–05:30 AM during our fieldwork in October and November 2019. Identification of captured individuals was facilitated by a field guide (Ingle and Heaney 1992) and the updated identification following the Mammal Diversity Database (MDD 2021). We adapted the designation of *Miniopterus eschscholtzii* as a full species from being a subspecies of *Miniopterus schreibersii* found in the Philippines (MDD 2021). We also followed the designation of *Miniopterus paululus* as a full species distributed in the Philippines, Indonesia, and Malaysia after being separated from *Miniopterus australis* (MDD 2021). Important biological information – including sex, age, and basic morphometrics – were also recorded.

After the individual assessment, we recorded a reference echolocation call for each captured bat. Recordings were made using a Pettersson M500 USB Ultrasonic Microphone (Pettersson Elektronik AB, Uppsala, Sweden) connected to a computer with BatSound Touch Lite 1.2 (Pettersson Elektronik AB, Uppsala, Sweden). The majority of the bats were temporarily released inside an enclosure (2.13 m x 1.80 m x 1.20 m) during call recording. Meanwhile, horseshoe bats were hand-held around 30 cm from the ultrasonic microphone to record their calls. Since the quality of calls emitted and the consistency of call production varied greatly between individual bats, the resulting recordings had different durations. After each recording, the voucher call was saved as a waveform audio file (.wav) for analysis. Each file represented one individual bat. Finally, we individually released the bats after recording.

Animal handling and sample collection procedures were patterned from the standards of the American Society of Mammalogists (Sikes and ACUC-ASM 2016) and were approved by the animal care and use committee of the University of the Philippines Los Baños (UPLB).

Call Characterization

We used BatSound v.4.03 (Pettersson Elektronik AB, Uppsala, Sweden) program to examine the call recordings with the following spectrogram setting: Hanning window, 512-point fast Fourier transform (FFT), and 95% FFT overlap. In each recording file, we randomly selected six consecutive call pulses with a high signal-to-noise ratio to be characterized. We measured seven call characteristics as parameters in each call pulse. In this study, we define each call characteristic measured as follows: start frequency (SF) is the frequency at the onset of the call, end frequency (EF) is the frequency at the end of the call, peak frequency (PF) is the frequency containing the most energy in the call, minimum frequency (FMIN) is the lowest frequency in a call, maximum frequency (FMAX) is the highest frequency in a call, duration (DUR) is the time between the SF and EF of the call, and inter-pulse interval (IPI) is the time

between the EF of a call and the SF of the succeeding call. Frequency parameters were measured in kilohertz (kHz), while time parameters were measured in milliseconds (ms). Both SF and EF were determined by measuring the point at which the power began to rise above the background noise consistently (Kingston *et al.* 2003) (*i.e.* on average, –30 dB from the PF for FM calls while –20 dB from the PF for calls with CF component). Meanwhile, PF was extracted from the power spectrum of the call. On the other hand, FMIN, FMAX, and DUR were derived from the pulse characteristic analysis of the program with a pulse detection threshold of –60 dB [modified from Fenton *et al.* (2002)]. Then, IPI was manually calculated from the time records of SFs and EFs in the call sequence being analyzed. After measuring the call parameters from each call pulse, we calculated the mean and standard deviation of all call parameters from the six pulses and used them to represent the individual bats in the statistical tests. We also noted the structure of the echolocation call and the duty cycle (*i.e.* the percent of time spent emitting calls given a certain time period; $DUR / IPI \times 100$) of all individuals. In this study, we considered calls emitted $\leq 20\%$ of the time to be low duty cycle (LDC), while those produced $> 20\%$ of the time were high duty cycle (HDC) calls [modified from Jones (1999)].

Statistical Analysis

For each species, we determined the mean, standard deviation, and range of all call parameters. Results of Shapiro-Wilks and Levene's tests revealed that the call parameters of all species were not normally distributed and have unequal variances. With the condition of the data, we used Kruskal-Wallis H with Bonferroni correction in pairwise comparison to species sharing a similar call structure to test for differences in the call parameters.

To test the species-specific characteristic of bat echolocation calls, we applied a multivariate DFA with cross-validation using a stepwise method to call parameters from all species. In addition to the non-normal distribution of all call parameters, their covariance matrices were also heterogeneous (Box's $M = 791.66$, $F_{112, 653.35} = 3.714$, $p < 0.0001$), which violates the assumptions of DFA. However, multivariate tests such as DFA are not very sensitive to deviations given the large sample size and the absence of outliers (McDonald 2014). Wilks' λ values obtained from MANOVA were used to test the significance of the DFA models and the discriminating power of each call parameter. A classification matrix was also produced to determine the grouping accuracy of calls to species. We also performed Spearman's rank correlation to assess the relationship between the call parameters. All tests were performed in IBM SPSS Statistics v.20.0 (IBM Corp., Armonk, NY, USA), and values of $p < 0.05$ were considered significant in all tests.

RESULTS

In total, we characterized 624 echolocation calls of 104 individuals from five insectivorous bat species belonging to four families (Miniopteridae, Vespertilionidae, Hipposideridae, and Rhinolophidae) inhabiting Puting Bato Cave 5 (Table 1). We observed two types of frequency components in the structure of their echolocation calls: FM and CF (constant frequency). FM components are frequency fluctuations that can begin from a lower frequency to a higher frequency, or *vice versa*, over a very short period. On the other hand, CF components have very narrow bandwidths or an almost fixed frequency value throughout the long duration of the call. Overall, there were three different call designs each associated with at least one bat family (Figure 1).

FM Calls: Miniopteridae and Vespertilionidae

We characterized 294 FM calls from 25 *Miniopterus paululus*, four *Miniopterus eschscholtzii*, and 20 *Myotis macrotarsus* individuals. All three species emit calls at LDC (*M. paululus*: 6.28%; *M. eschscholtzii*: 6.01%; *M. macrotarsus*: 3.31%). Among the three species, the echolocation calls of *M. paululus* have the highest average measurement values for all frequency-related parameters, while those of *M. macrotarsus* have the lowest (Table 1). In contrast, the longest average call DUR and IPI were recorded from *M. macrotarsus* (DUR = 3.56 ± 0.48 ms; IPI = 107.65 ± 53.77 ms), while the lowest ones were from *M. paululus* (DUR = 2.85 ± 0.35

ms; IPI = 45.34 ± 15.03 ms). All call characteristics of *M. eschscholtzii* were in between the other two species (Table 1).

A Kruskal-Wallis test showed that all call parameters of the three species were significantly different (all $p < 0.001$). However, the pairwise comparison with Bonferroni correction indicated that differences in the call characteristics were only significant between *M. paululus* and *M. macrotarsus* (all adjusted $p < 0.001$). The difference in the call characteristics of *M. eschscholtzii* paired to *M. paululus* (SF, EF, PF, FMIN: adjusted $p = 0.178$; FMAX: adjusted $p = 0.179$; DUR, IPI: adjusted $p = 1.00$) and *M. macrotarsus* (SF, EF, FMIN: adjusted $p = 0.375$; PF, FMAX: adjusted $p = 0.376$; DUR: adjusted $p = 0.471$; IPI: adjusted $p = 0.178$) were not significant.

CF-FM Calls: Hipposideridae

A total of 156 calls from 26 *Hipposideros coronatus* individuals were characterized. The call of the species was distinct with each pulse beginning in a short CF component that terminates into a long FM drop (Figure 1). On average, *H. coronatus* produced calls at HDC (23.78%). The average bandwidth of the FM component for this species was 66.0 kHz. Moreover, the echolocation calls of the *H. coronatus* registered the highest frequency among the five species acoustically recorded (Table 1). We also noted that the call of this species is usually produced in a sequence containing three pulses.

Table 1. Descriptive statistics (mean \pm standard deviation, range) of each echolocation call characteristic from insectivorous bats in Puting Bato Cave 5, Polillo Island, Philippines. *n* is the number of individuals recorded for each species.

Species (<i>n</i>)	SF (kHz)	EF (kHz)	PF (kHz)	FMIN (kHz)	FMAX (kHz)	DUR (ms)	IPI (ms)
Hipposideridae							
<i>Hipposideros coronatus</i> (26)	158.25 \pm 0.90 (154.33–160.17)	92.69 \pm 0.95 (90.83–94.67)	150.79 \pm 2.94 (146.65–160.32)	96.08 \pm 1.42 (93.10–97.98)	145.57 \pm 6.00 (128.26–161.95)	3.22 \pm 0.13 (2.76–3.58)	13.54 \pm 1.53 (8.76–15.98)
Miniopteridae							
<i>Miniopterus paululus</i> (25)	122.45 \pm 5.19 (108.33–129.50)	59.45 \pm 1.61 (56.33–62.50)	69.68 \pm 2.14 (66.24–74.06)	58.29 \pm 1.86 (53.71–60.71)	125.35 \pm 5.72 (112.14–136.39)	2.85 \pm 0.35 (2.27–3.40)	45.34 \pm 15.03 (24.57–81.88)
<i>Miniopterus eschscholtzii</i> (4)	96.45 \pm 1.07 (95.67–98)	43.04 \pm 1.13 (41.83–44.17)	51.68 \pm 1.08 (50.62–52.90)	41.99 \pm 1.47 (39.88–43.29)	100.42 \pm 3.63 (97.49–105.31)	3.09 \pm 0.25 (2.79–3.34)	51.41 \pm 23.84 (30.29–76.89)
Vespertilionidae							
<i>Myotis macrotarsus</i> (20)	69.90 \pm 4.42 (62.17–78)	25.63 \pm 1.52 (22.83–29.17)	39.21 \pm 2.10 (36.46–44.11)	24.78 \pm 1.50 (22.14–28.16)	73.65 \pm 6.50 (64.45–90.66)	3.56 \pm 0.48 (2.65–4.25)	107.65 \pm 53.77 (22.66–245.23)
Rhinolophidae							
<i>Rhinolophus arcuatus</i> (29)	56.73 \pm 1.68 (53.50–60.33)	55.43 \pm 1.51 (52.50–58.83)	66.81 \pm 2.04 (62.17–69.34)	53.62 \pm 1.63 (49.64–56.64)	71.31 \pm 1.91 (67.22–74.54)	44.10 \pm 4.61 (37–57.54)	67.78 \pm 14.69 (40.43–110.68)

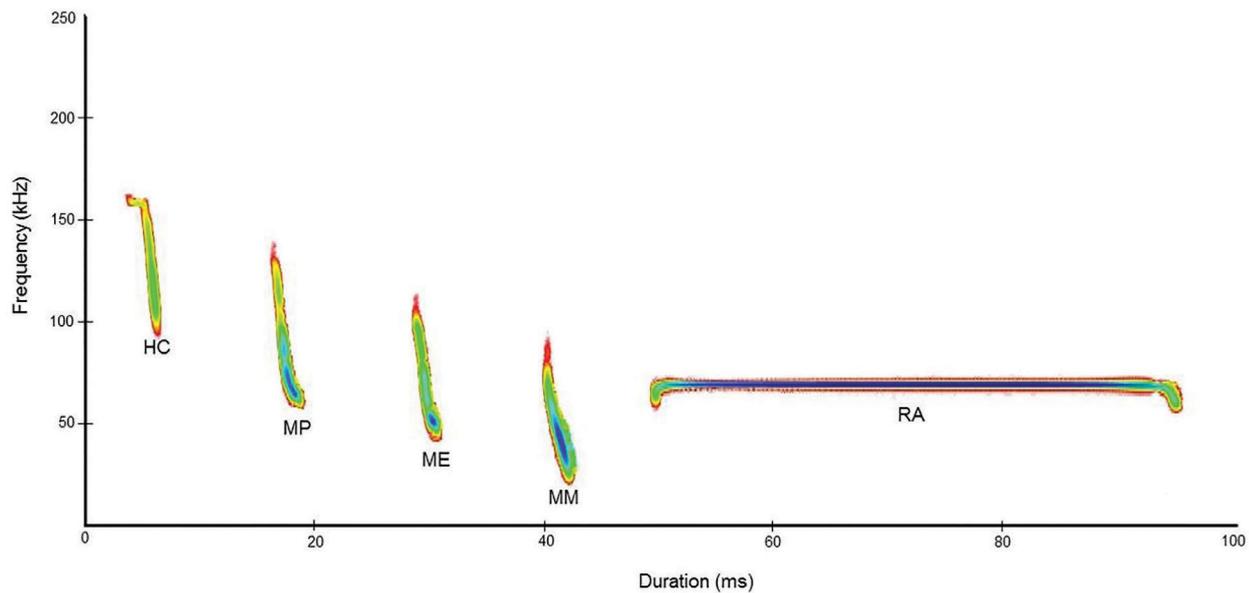


Figure 1. Echolocation calls of cave-dwelling insectivorous bats in Puting Bato Cave 5: HC – *Hipposideros coronatus*; MP – *Miniopterus paululus*; ME – *Miniopterus eschscholtzii*; MM – *Myotis macrotarsus*; RA – *Rhinolophus arcuatus*. Color spectrum indicates relative sound pressure levels. Calls were visualized in the same plot size and their shape and size are relative to each other. Gaps between calls do not represent IPIs.

FM-CF-FM Calls: Rhinolophidae

Among the five species, only *Rhinolophus arcuatus* produced calls with an FM-CF-FM structure, and 174 echolocation calls from 29 individuals of the species were characterized. Generally, the call of *R. arcuatus* starts in a short FM rise, followed by the CF component, and ends in an equally short FM drop (Figure 1). The long CF component in the call of *R. arcuatus* easily distinguishes the species from the rest of the bats inhabiting Puting Bato Cave 5. Moreover, the long CF component granted the species to have the longest call DUR recorded among the species examined (Table 1). In addition, the species also emits calls at HDC (65.06%) similar to *H. coronatus*.

DFA

All five species were included in the DFA. The small sample size of *M. eschscholtzii* did not have a significant effect on the overall classification. Out of the seven call parameters measured, only SF, EF, PF, and DUR were included in the final model. Call parameters FMAX, FMIN, and IPI failed the tolerance test of DFA (partial $F < 3.84$), which measures the significance of predictors (*i.e.* the call parameters) in the classification model.

The best model produced 100% correct classification in both original and cross-validated group membership (Table 2). A MANOVA indicated that the discrimination of the data was significantly different from random (Wilks' $\lambda = 8.116 \times 10^{-7}$, $F_{16, 293.9} = 1, 792, p < 0.0001$), and the

first two discriminant functions (df) explained most of the variation in the DFA (df 1 = 75.1%, df 2 = 20.6%). All functions combined showed a high discriminating ability; however, df 4 alone was weak (Wilks' $\lambda = 0.997$, $\chi^2 = 0.342, p = 0.559$). In all function except df 4, the call parameters also have nearly perfect correlation with each other (canonical correlation coefficients: df 1 = 0.999, df 2 = 0.996, and df 3 = 0.980). PF and call DUR have the highest variable effect in df 1 (PF = 0.872) and df 2 (DUR = 0.663), respectively. The group centroid (*i.e.* mean discriminant function score) of each species was unique and spread distantly from each other, which suggests the complete separation of species and the distinctness of their calls (Figure 2).

The increasing Wilks' λ value of the call parameters was illustrated in the following order: IPI > FMAX > DUR > SF > FMIN = EF > PF. In this case, PF has the strongest discriminating power (Wilks' $\lambda = 0.003, p < 0.001$), while the weakest predictor was IPI (Wilks' $\lambda = 0.389, p < 0.001$). Correlation analysis indicated that most of the call parameters were strongly correlated with each other (Table 3). Frequency-related parameters were positively correlated to other frequency-related parameters, while they were negatively correlated to time-related parameters, and *vice versa*.

Table 2. Classification matrix for all species. Overall classification rate was 100% ($n = 104$) for both the original and cross-validated group memberships.

Model	True groups	Predicted group membership				
		<i>H. coronatus</i>	<i>M. paululus</i>	<i>M. eschscholtzii</i>	<i>M. macrotarsus</i>	<i>R. arcuatus</i>
Original	<i>H. coronatus</i>	26	0	0	0	0
	<i>M. paululus</i>	0	25	0	0	0
	<i>M. eschscholtzii</i>	0	0	4	0	0
	<i>M. macrotarsus</i>	0	0	0	20	0
	<i>R. arcuatus</i>	0	0	0	0	29
	Total n	26	25	4	20	29
	n correct	26	25	4	20	29
	% correct	100	100	100	100	100
Cross-validated	<i>H. coronatus</i>	26	0	0	0	0
	<i>M. paululus</i>	0	25	0	0	0
	<i>M. eschscholtzii</i>	0	0	4	0	0
	<i>M. macrotarsus</i>	0	0	0	20	0
	<i>R. arcuatus</i>	0	0	0	0	29
	Total n	26	25	4	20	29
	n correct	26	25	4	20	29
	% correct	100	100	100	100	100

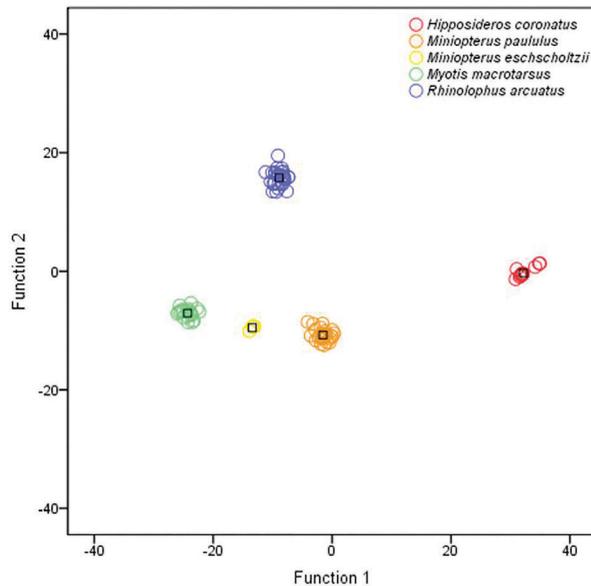


Figure 2. Canonical discriminant function biplot showing group centroid of each cave-dwelling insectivorous bat species sampled in Puting Bato Cave 5, Polillo Island, Philippines.

DISCUSSION

Effect of Recording Method on Echolocation Calls

Bats vary their echolocation calls depending on the amount of clutter in their environment (Schnitzler and

Kalko 2001). However, the extent of variation between calls recorded in different clutter conditions still depends on the type of echolocation calls produced by the bat species. Calls dominated by an FM component, similar to the signals of species from family Miniopteridae and Vespertilionidae in this study, are produced at LDC. To avoid forward masking, LDC bats do not emit calls and listen to returning echoes simultaneously. Instead, they produce short duration calls at long intervals and wait for the returning echo before emitting another call thus separating the pulse and target echo in time (Fenton *et al.* 2012). The LDC bats in this study are associated with edge spaces (Sedlock *et al.* 2019) where clutter is minimal; however, the conditions of the enclosure where their calls are recorded simulate a narrow or cluttered environment (Siemers 2004). To adapt to increasing clutter, bats modify their call by increasing the frequency ranges and shortening the call duration and inter-pulse interval (Schnitzler and Kalko 2001; Jones and Holderied 2007). This is to compensate for the rapid return of target echo and avoid self-deafening. Thus, only the calls used by these bats in cluttered environments were recorded, and differences in the echolocation call features and characteristics may be observed when compared to bat individuals recorded in free flight.

On the other hand, echolocation calls with a CF component like those produced by *Hipposideros coronatus* and *Rhinolophus arcuatus* in this study are emitted at HDC. HDC

Table 3. Spearman’s rank correlation coefficient for parameters used in DFA models. Values indicate the magnitude of correlation between call parameters (approaching 1 – strong, approaching 0 – weak). Sign determines directionality of correlation.

	SF	EF	PF	FMIN	FMAX	DUR
EF	0.7395					
PF	0.6955	0.9533				
FMIN	0.7474	0.9804	0.9459			
FMAX	0.9177	0.8270	0.8177	0.8157		
DUR	-0.6597	-0.3705	-0.3543	-0.3795	-0.5600	
IPI	-0.7587	-0.8018	-0.8116	-0.8071	-0.7924	0.4776

bats have evolved anatomical modifications, especially the cochlea of their ear and the neuronal system responsible for processing acoustic information to detect sounds within a specific and narrow frequency range (Fenton *et al.* 2012). In addition, HDC bats can listen to returning echoes while emitting acoustic signals since self-generated call pulses and target echoes are separated in frequency. By lowering the frequency of the emitted calls, HDC bats compensate for the Doppler-induced increase in the frequency of the returning echo to match the frequency range that the species can detect (Schnitzler and Kalko 2001; Fenton *et al.* 2012). The ability of HDC bats to compensate for the Doppler effect also allows them to be unaffected by masking problems, especially for species with long CF components in their call (Schnitzler and Kalko 2001). Recording calls from these species inside an enclosure will generate relatively shorter call durations due to the short travel distance of the signal but their CF components, which contain the frequency mainly used to acoustically identify species in this group, will not be affected or significantly change (Siemers 2004). Therefore, echolocation calls recorded from HDC bat individuals released in an enclosure or hand-held can still be used as a reliable reference in acoustic identification of these species in the field.

FM Calls: *Miniopteridae* and *Vespertilionidae*

Species producing FM calls in our study exhibited acoustic distinctness between each other; however, statistical analysis showed no significant difference between the call characteristics of *Miniopterus eschscholtzii* and the other two species. Particularly, the call characters of *M. eschscholtzii* overlapped with the other two FM-producing bats. Studies of Vaughan *et al.* (1997), Russo and Jones (2002), and Wordley *et al.* (2014) also observed overlaps in the call characters of other FM-producing bats. Though our study identified peak frequency as the strongest call predictor, we also found end frequency as one of the strongest call characters in differentiating the species. This was similar to the reports of previous studies along with other call characters such as start and peak frequencies, and call duration (Vaughan *et al.* 1997; Russo and Jones 2002; Wordley *et al.* 2014).

Most of the measured call characteristics in these species were similar to the records of previous studies in the Philippines (Sedlock 2001; Sedlock *et al.* 2019; Amberong *et al.* 2021). The call peak frequency of *Miniopterus paululus* in our study (66.24–74.06 kHz) was comparable to the peak frequency of the same species recorded by Sedlock *et al.* (2019) in Laguna (62.0–73.0 kHz) (identified as *Miniopterus australis*) and by Amberong *et al.* (2021) in Bulacan with a slightly wider range (63.7–90.0 kHz). Moreover, the call duration of the species in Laguna had a wider range (2.5–13.0 ms) compared to the records in Bulacan (2.0–3.7 ms) and in our study (2.27–3.4 ms), which might be attributed to the difference in the recording methods employed. Echolocation calls of *M. paululus* in Laguna were recorded from free-flying bats (Sedlock *et al.* 2019), while the calls of the species from Bulacan (Amberong *et al.* 2021) and in our study were recorded from individuals released in enclosures. Recording FM-dominated calls inside enclosures or flight cages usually result in calls that are produced by bats in cluttered environments having shorter duration and higher frequency range (Siemers 2004). Meanwhile, the minimum frequency of *M. eschscholtzii* in our study (41.99 ± 1.47 kHz) was almost similar to the records of the same species in Laguna (45.6 ± 0.7 kHz) (identified as *Miniopterus schreibersii*) and Bulacan (44.3 ± 0.6 kHz) (Sedlock 2001; Sedlock *et al.* 2019; Amberong *et al.* 2021). On the other hand, the echolocation calls of *Myotis macrotarsus* is not mentioned in any literature, and – to our knowledge – our study is the first to document and characterize the echolocation call of this species. *Myotis macrotarsus* is distributed across Malaysia and the Philippines (Duya *et al.* 2019), and intraspecific call variation in this species can be potentially high.

CF-FM Calls: *Hipposideridae*

The CF in the call of roundleaf bats is distinguishable from other families and their CF-FM call design separates them from other bats that also produce calls with CF component. Members of the family *Hipposideridae* typically produce calls with a prominent CF component (Hughes *et al.* 2010;

Webala *et al.* 2019; Amberong *et al.* 2021); however, the echolocation call of *Hipposideros coronatus* does not follow this form. Comparing spectrograms and call duration records, the CF component in the echolocation call of *H. coronatus* is the shortest in duration among other roundleaf bat species recorded in the Philippines (Sedlock 2001; Mould 2012; Sedlock *et al.* 2019; Amberong *et al.* 2021). The call duration of *H. coronatus* was even shorter than our records of *Myotis macrotarsus*, which produces only pure FM calls. In addition, the call peak frequency of *H. coronatus* recorded by Sedlock *et al.* (2014) in Bohol (156 kHz) is within the range of our records (146.65–160.32 kHz) of this species. Thus, *H. coronatus* currently has the shortest call duration among Philippine *Hipposideros* species and the highest frequency call record among all bat species with echolocation call record in the country.

FM-CF-FM Calls: Rhinolophidae

The horseshoe bat *Rhinolophus arcuatus* is the only species we recorded with FM-CF-FM call design. Typical for horseshoe bats, the long CF in their call mainly contributes to their long call duration (Vaughan *et al.* 1997; Russo and Jones 2002; Hughes *et al.* 2010; Hackett *et al.* 2017). The call of *R. arcuatus* is the longest among the echolocation calls characterized in our study and was comparable to the records of the species in Bulacan (34.7–56.2 ms) (Amberong *et al.* 2021). In terms of call frequencies, the peak frequency of *R. arcuatus* recorded in our study in Polillo Island (66.81 ± 2.04 kHz) is close to the values reported in Bohol (68.7 ± 1.4 kHz) and Bulacan (65.0 ± 1.8 kHz) (Sedlock *et al.* 2014; Amberong *et al.* 2021). The peak frequency of the individuals recorded in Mt. Makiling (71.2 ± 0.4 kHz) is slightly higher than the other existing records (Sedlock 2001).

Call Frequency and Morphometric Correlation

Interspecific and intraspecific call variations are observed in many bat species worldwide. Apart from geographic location, the differences in call characteristics and design among and within species might also be affected by environmental features (Fawcett *et al.* 2015; Chaverri and Quiros 2017) and morphometric differences (Huihua *et al.* 2003; Wu *et al.* 2015). Thiagavel and colleagues (2017) determined that forearm length is negatively correlated with call PF in evening bats (Vespertilionidae). Similarly, Hughes *et al.* (2010) also observed this inverse relationship on species from families Rhinolophidae and Hipposideridae in Thailand. Both studies noticed the decreasing trend in call frequencies across species with increasing forearm length. We observe the same pattern in the FM-producing species in our study if the application of Thiagavel *et al.*'s observations extends to long-fingered bats (Miniopteridae) as being closely related

to evening bats (Miller-Butterworth *et al.* 2007). Among the three species, *Myotis macrotarsus* with the longest forearm length had the lowest peak frequency while *Miniopterus paululus* with the shortest forearm length had the highest peak frequency. We also observe this in *Rhinolophus arcuatus*, a medium-sized horseshoe bat that produces call frequency values lower than smaller species but higher than larger species when compared to other Philippine horseshoe bats (Sedlock 2001, 2002; Sedlock *et al.* 2014; Dimaculangan *et al.* 2019; Amberong *et al.* 2021). However, this pattern is not observed in Philippine roundleaf bats. *Hipposideros coronatus* currently has the highest call peak frequency record among the roundleaf bat species with recorded calls in the Philippines (Sedlock 2001, 2002; Mould 2012; Sedlock *et al.* 2014; Amberong *et al.* 2021), but its forearm length is longer than *Hipposideros pygmaeus* – the smallest roundleaf bat in the Philippines (Heaney *et al.* 2010), which only produce calls with peak frequencies at 90–95 kHz (Sedlock *et al.* 2014) to 105–115 kHz (Amberong *et al.* 2021). Although several species have already been acoustically recorded in the Philippines, their echolocation calls were not completely described and only one frequency parameter in their calls was measured. Our study only included the five species, and more information is required to establish call frequency and morphometric correlation in Philippine bats.

DFA Classification Success and Predictors

Numerous studies have demonstrated the species-specific characteristic of bat echolocation calls and our study also demonstrates this. In our study, the DFA provided a perfect classification rate for the five species. Classification success was higher than the reports of Amberong *et al.* (2021) on several Philippine bats and the studies in other southeast Asian countries (Hughes *et al.* 2010, 2011; Chou and Cheng 2012; Phauk *et al.* 2013; Pham *et al.* 2021). However, most of these studies categorized more species compared to our study, which might affect the accuracy of discrimination by the models. In addition, the final model only included the parameters SF, EF, PF, and DUR, but the removal of FMIN, FMAX, and IPI did not increase the overall classification rate of the model. Disregarding the stepwise method in the DFA, all calls in our study would still be classified to the correct species even if the model contained all seven parameters. The three parameters were only removed in the final model since their partial F values were below the minimum value requirement. Moreover, IPI may have also been removed since it is the weakest predictor. Conversely, peak frequency had the most discriminating power among the call parameters used. Numerous studies have noted the importance and high significance of peak frequency in classifying calls to correct species (Vaughan *et al.* 1997; Russo and Jones

2002; Carter *et al.* 2012; Wordley *et al.* 2014; Lloyd 2017; Mifsud and Vella 2019). Peak frequency is the frequency with the highest sound pressure level (Denzinger *et al.* 2016), which is related to the relative loudness of sound. By listening to a particular range of loudness, the chance of detection increases. Therefore, it may be possible that producing calls with distinct peak frequencies enables the bats to recognize only the echoes of their own echolocation signal.

CONCLUSION

Our findings reveal that insectivorous bats of Puting Bato Cave 5 are as acoustically diverse as they are taxonomically. Interspecific call variation exists between the five cave-dwelling species in Puting Bato Cave 5 and most of these species exhibit intraspecific call variation as well. Several species were already found to remain morphologically cryptic but are acoustically and genetically different across populations (Kingston *et al.* 2001; Thabah *et al.* 2006). Numerous bat species in the Philippines form species complexes (Heaney *et al.* 2010), and describing their echolocation calls may potentially contribute to resolving their taxonomy along with morphological, morphometric, and genetic evidence.

Our study also supports the need for the acoustic recording of bats in the Philippines. Our method is limited to hand-held and enclosure-release recordings, and we recommend recording calls from free-flying bats to collect a wider variety of call repertoire and compare the signals produced by species in different clutter conditions. We also recommend recording echolocation calls of other species from various localities to have a more inclusive and comprehensive call library for Philippine bats. We believe that it will advance acoustic recording as a tool for studying and monitoring bats and promote the study of bat acoustics in the country. Many of our species remain understudied and combining the traditional capture methods with acoustic recording will augment bat research in the Philippines.

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REFERENCES

- ALVIOLA PA. 2000. The distribution and ecology of bats in Polillo Islands, Philippines. In: Wildlife of Polillo Island, Philippines. Glossop, UK: Viper Press. p. 105–124.
- AMBERONG AGT, FIDELINO JS, DUCO RAJ, LEDESMA MM, DUYA MV, ONG PS, DUYAMRM. 2021. Toward a Philippine bat call library: acoustic characterization of insectivorous bats in Bulacan, Luzon Island, Philippines. *Philippine Journal of Science* 150(S1): 473–485.
- BANNER KM, IRVINE KM, RODHOUSE TJ, WRIGHT WJ, RODRIGUEZ RM, LITT AR. 2018. Improving geographically extensive acoustic survey designs for modelling species occurrence with imperfect detection and misidentification. *Ecology & Evolution* 8(12): 6144–6156.
- BERRY N, O'CONNOR W, HOLDERIED MW, JONES G. 2004. Detection and avoidance of harp traps by echolocating bats. *Acta Chiropterologica* 6(2): 335–346.
- CARTER GG, LOGSDON R, ARNOLD BD, MENCHACA A, MEDELLIN RA. 2012. Adult vampire bats produce contact calls when isolated: acoustic variation by species, population, colony and individual. *PLoS ONE* 7(6): e38791.
- CHAVERRI G, QUIROS OE. 2017. Variation in echolocation call frequencies in two species of free-tailed bats according to temperature and humidity. *The Journal of the Acoustical Society of America* 142(1): 146–150.
- CHOU C, CHENG H. 2012. Echolocation calls of the eleven insectivorous bats of Taiwan. *Taiwan Journal of Biodiversity* 14(3–4): 33–62.
- DENZINGER A, KALKO EKV, TSCHAPKA M, GRINNEL AD, SCHNITZLER HU. 2016. Guild structure and niche differentiation in echolocating bats. In: *Bat Bioacoustics: Springer's Handbook of Auditory Research*, Vol. 54. New York: ASA Press. p. 141–166.

- DIMACULANGAN FD, JACOBSON AC, ALVIOLA PA, ALVAREZ JD, SEDLOCK JL. 2019. Seasonal emergence counts from a multispecies horseshoe bat (Chiroptera: Rhinolophidae) roost in the Philippines. *Journal of Bat Research & Conservation* 12(1): 46–51.
- DUYA MR, ALVAREZ JDV, FIDELINO J, GATANBALBAS M, PEDREGOSA M, VELEZ MJ, JAKOSALEM PG, TANALGO K, GARCIA JLL, CACHO MA. 2019. *Myotis macrotarsus*. The IUCN Red List of Threatened Species 2019: e.T14178A22065997.
- FAWCETT K, JACOBS DS, SURLYKKE A, RATCLIFFE JM. 2015. Echolocation call in bat, *Rhinolophus capensis*: the influence of clutter, conspecifics and prey on call design and intensity. *Biology Open* 4: 693–701.
- FENTON MB, FAURE PA, RATCLIFFE JM. 2012. Evolution of high duty cycle in bats. *The Journal of Experimental Biology* 215: 2935–2944.
- FENTON MB, TAYLOR PJ, JACOBS DS, RICHARDSON EJ, BERNARD E, BOUCHARD S, DEBAEREMAEKER KR, TER HOFSTEDE H, HOLLIS L, LAUSEN CL, LISTER JS, RAMBALDINI D, RATCLIFFE JM, REDDY E. 2002. Researching little-known species: the African bat *Otomops martiensenni* (Chiroptera: Molossidae). *Biodiversity and Conservation* 11: 1583–1606.
- FLAQUER C, TORRE I, ARRIZABALAGA A. 2007. Comparison of sampling methods for inventory of bat communities. *Journal of Mammalogy* 88(2): 526–533.
- FRANCL K, BLAND RC, LUCAS JS, BRACK V. 2011. Comparison of survey techniques for documenting summer bat communities in Pennsylvania and New Jersey. *Journal of the Pennsylvania Academy of Science* 85(2/3): 52–56.
- FRICK WF. 2013. Acoustic monitoring of bats, considerations of options for long-term monitoring. *Therya* 4(1): 69–78.
- GRIFFIN DR. 2001. Return to the magic well: echolocation behavior of bats and responses of insect prey. *Bioscience* 51(7): 555–556.
- HACKETT TD, HOLDERIED MW, KOINE C. 2017. Echolocation call description of 15 species of Middle Eastern desert dwelling insectivorous bats. *Bioacoustics* 26(3): 217–235.
- HEANEY LR, DOLAR ML, BALETE DS, ESSELSTYN JA, RICKART EA, SEDLOCK JL. 2010. Synopsis of Philippine mammals. Field Museum of Natural History. http://archive.fieldmuseum.org/philippine_mammals/
- HOGUE AS, MCGOWAN AT. 2018. Comparison of driving transect methods for acoustic monitoring of bats. In: *Bats*. IntechOpen. Retrieved on 07 Jan 2021 from <https://www.intechopen.com/books/bats/comparison-of-driving-transect-methods-for-acoustic-monitoring-of-bats>
- HUGHES AC, SATASOOK C, BATES PJJ, SOISOOK P, SRITONGCHUAY T, JONES G, BUMRUNGSRI S. 2010. Echolocation call analysis and presence-only modelling as conservation monitoring tools for rhinolophoid bats in Thailand. *Acta Chiropterologica* 12(2): 311–327.
- HUGHES AC, SATASOOK C, BATES PJJ, SOISOOK P, SRITONGCHUAY T, JONES G, BUMRUNGSRI S. 2011. Using echolocation calls to identify Thai bat species: Vespertilionidae, Emballonuridae, Nycteridae and Megadermatidae. *Acta Chiropterologica* 13(2): 447–455.
- HUIHUA Z, SHUYI Z, MINGXUE Z, JIANG Z. 2003. Correlation between call frequency and ear lengths in bats belonging to families Rhinolophidae and Hipposideridae. *Journal of Zoology* 259(2): 189–195.
- IJAS A, KAHILAINEN A, VASKO VV, LILLEY TM. 2017. Evidence of the migratory bat, *Pipistrellus nathusii*, aggregating to the coastlines of the northern Baltic Sea. *Acta Chiropterologica* 19(1): 127–139.
- INGLE NR, HEANEY LR. 1992. A key to the bats of the Philippine Islands. *Fieldiana Zoology, New Series* No. 69. Field Museum of Natural History: Chicago, IL. 44p.
- JONES G. 1999. Scaling of echolocation call parameters in bats. *Journal of Experimental Biology* 202: 3359–3367.
- JONES G, HOLDERIED MW. 2007. Bat echolocation calls: adaptation and convergent evolution. *Proceedings of the Royal Society B* 274: 905–912.
- KINGSTON T, JONES G, AKBAR Z, KUNZ TH. 2003. Alternation of echolocation calls in 5 species of aerial-feeding insectivorous bats from Malaysia. *Journal of Mammalogy* 84(1): 205–215.
- KINGSTON T, LARAMC, JONES G, AKBAR Z, KUNZ TH, SCHNEIDER CJ. 2001. Acoustic divergence of two cryptic *Hipposideros* species: a role for social selection? *Proceedings of the Royal Society B: Biological Sciences* 268(1474): 1381–1386.
- LARSEN RJ, BOELGER KA, GENOWAYS HH, MASEFIELD WP, KIRSCH RA, PEDERSEN SC. 2007. Mist netting bias, accumulation curves, and the rediscovery of two bats on Montserrat (Lesser Antilles). *Acta Chiropterologica* 9(2): 423–435.

- LLOYD B. 2017. Bat call identification manual for Department of Conservation's spectral bat detectors. Retrieved on 16 Feb 2021 from https://ftp.doc.govt.nz/public/folder/CpR1cRv_cE_rqb9ua5WRTg/electronics/Acoustic%20Recorders/Bat_Call_ID_Spectral.pdf
- [MMD] Mammal Diversity Database. 2021. Retrieved on 07 Sep 2021 from www.mammaldiversity.org
- MCDONALD JH. 2014. Handbook of biological statistics, 3rd ed. Baltimore, MD: Sparky House Publishing.
- MIFSUD CM, VELLAA. 2019. Acoustic characterization of bats from Malta: setting baseline for monitoring and conservation of bat populations. *Bioacoustics* 28(5): 427–442.
- MILLER-BUTTERWORTH CM, MURPHY WJ, O'BRIEN SJ, JACOBS DS, SPRINGER MS, TEELING EC. 2007. A family matter: conclusive resolution of the taxonomic position of the long-fingered bats, *Miniopterus*. *Molecular Biology and Evolution* 24(7): 1553–1561.
- MOULD A. 2012. Cave bats of the central west coast and southern section of the northwest Panay peninsula, Panay Island, the Philippines. *Journal of Threatened Taxa* 4(11): 2993–3028.
- MURRAY KL, BRITZKE ER, ROBBINS LW. 2001. Variation in the search-phase calls of bats. *Journal of Mammalogy* 82(3): 728–737.
- PECH-CANCHE JM, ESTRELLAE, LOPEZ-CASTILLO DL, HERNANDEZ-BETANCOURT SF, MORENO CE. 2011. Complementarity and efficiency of bat capture methods in a lowland tropical dry forest of Yucatan, Mexico. *Revista Mexicana de Biodiversidad* 82: 896–903.
- PHAM LK, TRAN BV, LE QT, NGUYEN TT, VOIGT CC. 2021. Description of echolocation call parameters for urban bats in Vietnam as a step towards a more integrated acoustic monitoring of urban wildlife in southeast Asia. *Diversity* 13(1): 18.
- PHAUK S, PHEN S, FUREY NM. 2013. Cambodian bat echolocation: a first description of assemblage call parameters and assessment of their utility for species identification. *Cambodian Journal of Natural History* 2013(1): 16–26.
- REITER G, HUTTMEIR U, KRAINER K, SMOLE-WIENER K, JERABEK M. 2008. Emergence behavior of Lesser horseshoe bat (*Rhinolophus hipposideros*): intracolony variation in time and space (Carinthia and Salzburg, Austria). *Berichte des naturwissenschaftlichen-medizinischen Verein Innsbruck* 95: 81–93.
- RODRIGUEZ–SAN PEDRO A, SIMONETTI JA. 2013. Acoustic identification of four species of bats (Order Chiroptera) in central Chile. *Bioacoustics* 22(2): 165–172.
- 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.
- SCHNITZLER HU, KALKO EK. 2001. Echolocation by insect-eating bats. *Bioscience* 51(7): 557–569.
- SEDLOCK JL. 2001. Inventory of insectivorous bats in Mt. Makiling, Philippines using echolocation call signatures and a new tunnel trap. *Acta Chiropterologica* 3(2): 163–178.
- SEDLOCK JL. 2002. Autecology and the conservation of insectivorous bats in Mt. Makiling, Philippines. *Silliman Journal* 42(1): 163–201.
- SEDLOCK JL, JOSE RP, VOGT JM, PAGUNTALAN LMJ, CARIÑO AB. 2014. A survey of bats in a karst landscape in the central Philippines. *Acta Chiropterologica* 16(1): 197–211.
- SEDLOCK JL, STUART AM, HORGAN FG, HADI B, JACOBSON AC, ALVIOLA PA, ALVAREZ JDV. 2019. Local-scale bat guild activity differs with rice-growth stage at ground level in the Philippines. *Diversity* 11(9): 148.
- SIEMERS BM. 2004. Bats in the field and in a flight cage: recording and analysis of their echolocation calls and behavior. In: *Bat Echolocation Research: tools, techniques and analysis*. Bat Conservation International. Austin, Texas. p. 107–113.
- SIKES RS, [ACUC-AMS] Animal Care and Use Committee of the American Society of Mammalogists. 2016. 2016 Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. *Journal of Mammalogy* 97(3): 663–688.
- SIKES RS, GANNON WL, [ACUC-AMS] Animal Care and Use Committee of the American Society of Mammalogists. 2011. Guidelines of the American Society of Mammalogists for the use of wild animals in research. *Journal of Mammalogy* 92(1): 235–253.
- THABAH A, ROSSITER SJ, KINGSTON T, ZHANG S, PARSONS S, MYA MYA K, ZUBAID A, JONES G. 2006. Genetic divergence and echolocation call frequency in cryptic species of *Hipposideros larvatus s.l.* (Chiroptera: Hipposideridae) from the Indo-Malayan region. *Biological Journal of the Linnean Society* 88(1): 119–130.

- THIAGAVEL J, SANTANA SE, RATCLIFFE JM. 2017. Body size predicts echolocation call peak frequency better than gape height in vespertilionid bats. *Science Report* 7: 828.
- VAUGHAN N, JONES G, HARRIS S. 1997. Identification of British bat species by multivariate analysis of echolocation call parameters. *Bioacoustics* 7(3): 189–207.
- WEBALA PW, RYDELL J, DICK CW, MUSILA S, PATTERSON BD. 2019. Echolocation calls of high-duty cycle bats (Hipposideridae and Rhinonycteridae) from Kenya. *Journal of Bat Research and Conservation* 12(1): 10–20.
- WORDLEY CFR, FOUIEK, MUDAPPAD, SANKARAN M, ALTRINGHAM JD. 2014. Acoustic identification of bats in the southern western Ghats, India. *Acta Chiropterologica* 16(1): 213–222.
- WORDLEY CFR, SANKARAN M, MUDAPPA D, ALTRINGHAM JD. 2018. Heard but not seen: comparing bat assemblages and study methods in a mosaic landscape in western Ghats of India. *Ecology and Evolution* 8(8): 3883–3894.
- WU H, JIANG TL, MULLER R, FENG J. 2015. The allometry of echolocation call frequencies in horseshoe bats: nasal capsule and pinnae size are better predictors than forearm length. *Journal of Zoology* 297(3): 211–219.
- ZAMORA-GUTIERREZ V, LOPEZ-GONZALEZ C, MACSWINEY-GONZALEZ MC, FENTON B, JONES G, KALKO EKV, PUECHMAILLE SJ, STATHOPOULOS V, JONES K. 2016. Acoustic identification of Mexican bats based on taxonomic and ecological constraints on call designs. *Methods in Ecology and Evolution* 7(9): 1082–1091.