

Article

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

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Abstract: This study is the first step towards more systematic monitoring of urban bat fauna in Vietnam and other Southeast Asian countries by collecting bat echolocation call parameters in Ho Chi Minh and Tra Vinh cities. We captured urban bats and then recorded echolocation calls after releasing in a tent. Additional bat's echolocation calls from the free-flying bats were recorded at the site where we captured bat. We used the obtained echolocation call parameters for a discriminant function analysis to test the accuracy of classifying these species based on their echolocation call parameters. Data from this pilot work revealed a low level of diversity for the studied bat assemblages. Additionally, the discriminant function analysis successfully classified bats to four bat species with an accuracy of >87.4%. On average, species assignments were correct for all calls from *Taphozous melanopogon* (100% success rate), for 70% of calls from *Pipistrellus javanicus*, for 80.8% of calls from *Myotis hasseltii* and 67.3% of calls from *Scotophilus kuhlii*. Our study comprises the first quantitative description of echolocation call parameters for urban bats of Vietnam. The success in classifying urban bats based on their echolocation call parameters provides a promising baseline for monitoring the effect of urbanization on bat assemblages in Vietnam and potentially also other Southeast Asian countries.

Keywords: acoustic parameters; discriminant function analysis; echolocation call; *Myotis hasseltii*; *Pipistrellus javanicus*; *Scotophilus kuhlii*; *Taphozous melanopogon*; urban bat



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1. Introduction

Vietnam is a country with a large diversity of bat species, counting about 120 species in the last comprehensive review [1]. Owing to the taxonomic description of new species previously unknown to the scientific community, the number of species observed for Vietnam has continuously increased over the past years (e.g., [2–6]). Thus far, the Vietnamese bat fauna has been mostly studied in natural habitats and protected areas [7–9]. Hence, we lack information on bat assemblages in habitats with a significant anthropogenic impact, for example, those in urban environments. In many developing countries, urban areas are expanding at unprecedented rates, exposing wildlife species to novel stressors such as high impervious surfaces, light pollution, and traffic. Some bat species appear to be particularly tolerant towards these stressors, whereas others seem sensitive [10]. This phenomenon turns bats into suitable indicator species and thus model taxa to study the impact of urbanization on wildlife species [10,11]. Most studies on urban bats have been conducted in so-called developed countries where urbanization is already high (e.g., [12]). However, the urban sprawl, the spatial spread of urbanization is usually highest in developing countries, from which we lack insights into how bats respond to urban stressors (but see [13]). Earlier studies showed a dramatic decline in species richness for cities in

Southeast Asia [14]. In Vietnam, the urbanization rate has increased in recent years [15,16] and, therefore, the bat fauna of recently urbanized areas will likely be heavily affected by human actions. The lack of empirical data on species richness of urban bats limits our ability to predict how the urban sprawl may impact bat assemblages in Vietnam in the future, i.e., which species will get extirpated from urbanized areas and which species will withstand anthropogenic changes. Here, we conducted a pilot study in two typical urban environments, a medium and a large-sized city, to shed light on whether or not classifying algorithms such as discriminant function analysis can be used for acoustic monitoring and automated identification of typical urban bats in Vietnam. Automatic classification of echolocation calls could support a more systematic monitoring activity of bats to understand how Southeast Asian bats respond to urban stressors.

Monitoring bat assemblages by conventional methods such as mist netting can be challenging because some species are difficult to capture. Since all bat species, except for pteropodids, use laryngeal echolocation to orient in darkness, acoustic surveys are increasingly used to survey bats in natural and urban environments [13,17]. Recent studies on urban bats looked explicitly at what urban factors explained species abundance and richness in urban and suburban areas [10,18–20] and what traits of bat species constituted a pre-adaptation for a city life [21,22]. This information can then formulate appropriate conservation measures to maintain or even restore bat species richness in urban environments [23]. Acoustic identification of bat species has also helped reveal cryptic species diversity, i.e., echolocation call parameters helped differentiate between two morphologically similar species [24]. For example, differences in call structures of *Miniopterus magnater* and *M. fuliginosus* helped to identify the species correctly, even though their morphology is almost identical [25]. In Vietnam, bat echolocation calls also helped identify cryptic species of the genera *Rhinolophus* and *Hipposideros* [26]. Many community studies have also used echolocation call parameters to classify bat species into functional groups. The most often used statistical approach for classifying bats based on call structure has been discriminant function analyses (DFA). For example, DFA helped assign Thai bats correctly to species in 96.7% of cases [27]. Similarly, bats with frequency modulating calls (FM calls) from India were assigned correctly in about 90% of cases using DFA [28]. Lastly, DFA may also support the differentiation of morphologically similar species [25] and the description of poorly studied bat assemblages [29].

Given the relatively low number of bat species in most urban areas worldwide, we suggested that DFA could act as a powerful tool to identify bat species based on their acoustic parameters in urban environments. Here, we applied the DFA approach to describe bat assemblages in two urban ecosystems of Vietnam. Considering the low species richness in urban habitats, we expected that DFA could successfully identify bats to the species level in the majority of cases.

2. Materials and Methods

2.1. Bat Captured

We monitored bats in Ho Chi Minh City (10°48' N 106°39' E) and Tra Vinh City (9°56' N 106°21' E). While Ho Chi Minh is relatively large (about 9 million inhabitants in 2019) and has a long history of urban development, including historic and new buildings that provide roosting sites for many bats, Tra Vinh is relatively small (about 1.3 million inhabitants in 2019) and has expanded and developed in recent years. As a consequence, Tra Vinh hosts more green areas than Ho Chi Minh City. We conducted our study between 2019 and 2020. We captured bats between sunset and 2200 h using mist-nets (3 m height and 15 m long with 30 mm mesh). Nets were set up in parking areas, in front of entries to buildings or across water bodies in both cities. They were checked at 30 min intervals. In Tra Vinh City, bats were captured at five sites between August 2019 and May 2020 (Table 1), and in Ho Chi Minh City at six sites between May 2020 and August 2020 (Table 1).

Table 1. The description of sampling sites from two cities and survey effort.

#	City	Latitude	Longitude	Time	# Net	# Night	Note
1	Tra Vinh	9.921870008	106.346854	May 2020	1	2	Indoor parking area
2	Tra Vinh	10.82854802	106.7282675	May 2020	2	1	A small pond
3	Tra Vinh	10.87740471	106.6799775	August 2019 May 2020	2	4	Grassland with some trees
4	Tra Vinh	9.922454478	106.3476558	May 2020	1	1	Under the bridge
5	Tra Vinh	9.919190397	106.349743	May 2020	1	1	Abundance house
6	Ho Chi Minh	9.923449242	106.3434506	May 2020	1	1	Old building
7	Ho Chi Minh	9.938479504	106.337781	June 2020	2	1	Near Nhieu Loc canal
8	Ho Chi Minh	10.78259474	106.7016182	July 2020	2	1	Small canal
9	Ho Chi Minh	10.79325811	106.6957258	July 2020	2	1	Small canal
10	Ho Chi Minh	10.76487179	106.6356857	July 2020	2	1	Small canal
11	Ho Chi Minh	10.76132323	106.618494	June 2020	1	2	Small canal

Specimen collection protocols and animal operations followed the Institutional Ethical Committee of Southern Institute of Ecology, Vietnamese Academy of Science and Technology (certificate number 135/QĐ-STHMN of 09 December 2016 by Southern Institute of Ecology). Specimens were preserved in 70% ethanol and deposited in the zoological collection of the Southern Institute of Ecology as an initial specimen collection to foster future studies on bat taxonomy in this region.

2.2. Species Identification

Currently, we lack data on bat assemblage composition for urban ecosystems in Vietnam, yet we expected to encounter mostly generalist species. Before applying the DFA analysis based on acoustic parameters, it is necessary to know the echolocation call parameters of species. Accordingly, we first identified each captured bat based on morphological traits following a published key [1]. Measured traits included forearm length (FA; measured on a folded wing from the elbow to the outermost extremity of the wrist), head and body length (HB; from the tip of the nose to the anal opening), tail length (T; from the anal opening to the tip of the tail), ear length (E; from the inferior emargination to the tip of the pinna), tragus length (Tr; from the inferior emargination to the tip of the tragus), hindfoot (HF; from the tarsal joint to the outermost part of the claw of the longest finger).

2.3. Acoustics Recording and Data Analysis

We then recorded echolocation calls of bats that flew singly in a tent made out of mosquito net (dimensions: 2 m height \times 4 m length \times 2 m width). We acknowledge that the use of a tent could affect call parameters of flying bats. However, we considered this is the only plausible way to describe the echolocation call parameters, given the paucity of data on the echolocation call behavior of urban bats in Vietnam. Additionally, we recorded bat echolocation calls at each mist netting sites to obtain echolocation calls of free-flying animals. To avoid misidentification, we compared the spectrogram of echolocation calls from free-flying animal to those of animals flying in the confined space of the tent. Echolocation calls were recorded by a U2 microphone attached to an ultrasonic recorder (SM4-Bat, Wildlife Acoustics). The sampling frequency was set at 256 kHz and the resolution at 16bit. We placed the device in front of the tent, about three meters away, enabling us to record calls while bats were flying in the tent. We recorded at least ten files for each animal to obtain acoustic recordings of sufficient quality for further analysis.

We only used acoustic recordings with a high signal-to-noise ratio to generate spectrograms and to measure acoustic parameters. In total, we analyzed 233 echolocation calls (Table S3) of four studied species, of which 49 of *Scotophilus kuhlii*, 43 of *Taphozous melano-*

pogon, 50 of *Pipistrellus javanicus*, and 91 of *Myotis hasseltii*. We used Avisoft SasLab Pro (Avisoft Bioacoustics, Berlin, Germany) to generate the spectrogram of animal echolocation calls and quantify the call parameters. The spectrogram was generated by the following settings: FFT size at 1024, frame size at 100%, Hann window [27]. The duration of calls was measured based on the oscillogram. To avoid a potential bias by the analyzing person, we used the function “Automatic Parameter Measurements”. Additionally, by using the automatic parameter measurements function, we aimed to provide information that was automatically generated as a basis for further studies that involve, e.g., machine learning for bat identification. The acoustics parameters were chosen based on information from a previous study [27]: the frequency of maximum energy (kHz) (FMAXE), start frequency (sf), terminal frequency (tf) maximum frequency (hf), minimum frequency (lf). The frequency range or bandwidth (fr) was calculated by subtracting the maximum frequency by minimum frequency. The automated analyses by the software AVISOFT determined the max, min, and peak frequency at the onset point of the call, at the maximum energy point of the spectrogram, and the endpoint of the call (Figure S2). The maximum frequency (hf) variable is the highest frequency across the call, in this case, was the highest value out of three max frequency values that the software measures at three points. The minimum frequency (lf) is the lowest frequency of the call, and it was the lowest value of three min values calculated by the software. The start frequency (sf) and terminal frequency (tf) were selected as the peak frequency at the onset and end of each call, respectively.

As one of our goals, we tested if urban bat species could be identified correctly by echolocation call parameters only. To this end, we applied a discriminant function analysis (DFA). Before conducting the DFA, we first tested the co-linearity between acoustic parameters using the variance inflation vector (VIF). We first ran the regression model with all studied variables by using the function “lm” and then calculated the VIF value by using the function “vif” of package faraway [30]. We removed those parameters with VIF values > 10 [31]. We used the function “lda” from the package MASS to conduct the discriminant function analysis for our data (See Table S3). We used the function “LinearDA” of package “PredPsych” with a leave-one-out cross-validation method to test the classification model due to the small sample size [27,28]. All the analysis was done by using RStudio 4.3 [32].

3. Results

3.1. The Species Diversity and Their Acoustic Parameters

In our study, we confirmed the presence of six bat species for Ho Chi Minh City and Tra Vinh City, belonging to the families Vespertilionidae (4 species), Emballonuridae (1 species) and Pteropodidae (1 species) (Table S1, Figure S1, morphological parameters in Table S2). The Jackknife estimator for species richness revealed the total number of bat species to equal 3.88 (± 0.88 ; mean \pm one standard deviation) in Tra Vinh City and 5.71 (± 1.21) in Ho Chi Minh City. The species accumulation curve (Figure S2) showed the number of captured species was below the total estimated number of species at both study sites. Species recorded for Ho Chi Minh City included *Pipistrellus javanicus*, *Scotophilus kuhlii* and *Myotis hasseltii*. In Tra Vinh City, we recorded *Taphozous melanopogon*, *S. kuhlii* and *Cynopterus sphinx* by using the mist-net during the sampling; and *S. heathii* from dried specimens collected by our colleagues in Tra Vinh University.

We recorded the echolocation calls from captured animals of *T. melanopogon* (three individuals), *Myotis hasseltii* (six individuals), *Pipistrellus javanicus* (three individuals) and *S. kuhlii* (three individuals). Acoustic parameters of echolocation calls are listed in Table 2 for each captured species. Acoustic parameters of *S. heathii* were not available because this species was not captured during our field campaign.

Table 2. Acoustic parameters of recorded species, d = duration, FMAXE = frequency at maximum energy, hf = the highest frequency entire the spectrogram, lf = the lowest frequency entire the spectrogram, sf = started frequency, tf = terminal (end) frequency, fr = the bandwidth of frequency of the call, n = number of call in the analysis.

Species	Location	d (ms)	FMAXE (kHz)	hf (kHz)	lf (kHz)	sf (kHz)	tf (kHz)	fr (kHz)	N	Reference
<i>Scotophilus kuhlii</i>	Vietnam	5.48 ± 23.8	45.64 ± 3.32	76.27 ± 6.56	38.94 ± 2.40	60.41 ± 7.21	42.66 ± 3.18	37.32 ± 7.06	49	This study
<i>Scotophilus kuhlii</i>	India	2.96 ± 0.38	45.26 ± 0.77	N/a	N/a	56.67 ± 2.06	43.53 ± 0.76	13.1 ± 2.25	2	[1]
<i>Taphozous melanopogon</i>	Vietnam	6.45 ± 1.13	33.07 ± 5.90	59.10 ± 5.11	23.19 ± 1.25	32.70 ± 3.27	31.09 ± 5.12	35.91 ± 5.22	43	This study
<i>Taphozous melanopogon</i>	Thailand	6.02 ± 3.4	29.71 ± 2.67	76.15 ± 20.18	20.37 ± 6.2	36.6 ± 10.44	22.58 ± 5.58	55.78 ± 20.32	33	[2]
<i>Taphozous melanopogon</i>	Malaysia	6–14	N/a	N/a	N/a	26–30	24–26	N/a	N/a	[3]
<i>Pipistrellus javanicus</i>	Vietnam	4.78 ± 1.06	50.30 ± 5.09	88.27 ± 3.50	42.27 ± 2.66	68.23 ± 4.05	48.03 ± 3.61	46.00 ± 3.48	50	This study
<i>Pipistrellus javanicus</i>	Nepal	3.84	36.85	42	36.19		52.81			[4]
<i>Myotis hasseltii</i>	Vietnam	3.86 ± 0.50	50.06 ± 3.09	84.63 ± 5.42	38.82 ± 3.12	67.55 ± 3.75	47.80 ± 2.31	45.81 ± 6.31	91	This study
<i>Myotis hasseltii</i>	Malaysia	2.5–5.5	N/a	N/a	N/a	82–104	N/a	N/a	N/a	[3]

Out of four studied species that we analyzed concerning their acoustic parameters *Scotophilus kuhlii* demonstrated large differences between echolocation call properties emitted by animals flying in the tent and free-flying conspecifics (Table 3). We observed significant differences in six out of seven acoustic parameters to this species, except for the lowest frequency (Table 3). In all studied species, we observed the effect of the flight environment on the duration of the call (Table 3). We did not observe a significant difference for FMAXE between calls from bats flying in the tent and free-flying conspecifics for *Myotis hasseltii* ($t = -0.4311$, $p = 0.67$) and *Taphozous melanopogon* ($t = 0.55174$, $p = 0.58$). For other parameters, we observed various differences depending on the species.

Table 3. Statistical parameters for testing differences in acoustic parameters between bats that flying in a tent and free-flying conspecifics.

Acoustic Parameter	<i>Pipistrellus javanicus</i>	<i>Scotophilus kuhlii</i>	<i>Myotis hasseltii</i>	<i>Taphozous melanopogon</i>
duration	−7.1731 (0) *	−7.4182 (0) *	−9.8947 (0) *	−3.8096 (0.0) *
FMAXE(kHz)	2.691 (0.01) *	3.6796 (0.001) *	−0.4311 (0.67)	0.5517 (0.58)
hf (kHz)	−1.4085 (0.17)	5.9849 (0) *	1.7652 (0.08)	0.8711 (0.39)
lf (kHz)	−1.3701 (0.19)	−1.9089 (0.07)	−7.4933 (0) *	−8.9108 (0.0) *
sf (kHz)	−0.0027 (0.99)	3.2563 (0.002) *	2.2077 (0) *	−2.6367 (0.01) *
tf (kHz)	−0.0937 (0.92)	3.7237 (0.001) *	0.7845 (0.43)	0.8740 (0.38)
fr (kHz)	−0.4044 (0.69)	7.4684 (0) *	4.692 (0) *	2.3334 (0.02) *

* denoted the statistical significance of difference.

3.2. The Echolocation Call Classification by Discriminant Function Analysis

For our final DFA, the frequency range was excluded as a factor since the VIF value was 38.15. Parameters used in the final model included duration (VIF = 2.06), the frequency at maximum energy (VIF = 5.14), the highest frequency (VIF = 6.79), the lowest frequency (VIF = 6.09), started frequency (VIF = 8.57), and terminal (end) frequency (VIF = 6.53). The accuracy of classification of all calls equaled 79.0%, and the accuracy of the classification model equaled 80% based on the leave-one-out cross-validation method. Specifically, DFA correctly assigned 100% of calls to *T. melanopogon* (Table 3 and Figure 1). The DFA identified bat calls with high accuracy to *M. hasseltii* and *P. javanicus* at 80.1% and 70.0%, respectively, but at lower accuracy to *S. kuhlii* (67.3%, Table 4). We observed some level of misidentification for calls of these species (Table 3). According to the DFA, these species also lumped as a group within the bat assemblage (Figure 1).

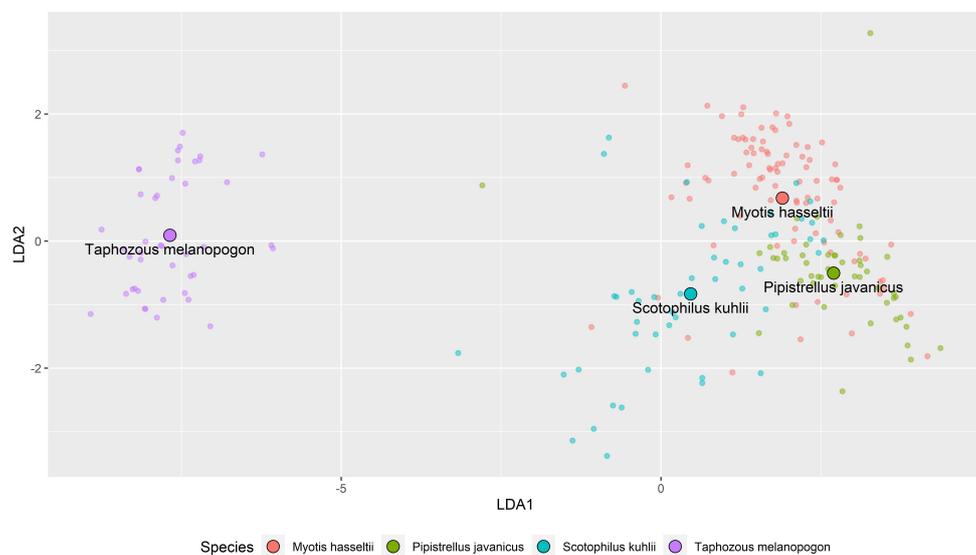


Figure 1. Plot of the linear discriminant functions LDA1 and LDA2 that separate echolocation calls of *Taphozous melanopogon* from those of the vespertilionid species.

Table 4. Cross-validation classification matrix for studied species.

As predicted group	True Group				Sum
	<i>Myotis hasseltii</i>	<i>Pipistrellus javanicus</i>	<i>Scotophilus kuhlii</i>	<i>Taphozous melanopogon</i>	
<i>Myotis hasseltii</i>	73	14	15		102
<i>Pipistrellus javanicus</i>	13	35	1		49
<i>Scotophilus kuhlii</i>	5	1	33		39
<i>Taphozous melanopogon</i>	0	0		43	43
Total	91	50	49	43	233
n correct	73	35	33	43	184
% correct	80.80	70.00	67.34	100.0	78.96

4. Discussion

Our capture effort revealed a low species diversity of insectivorous bat species in the urban environments of Ho Chi Minh City and Tra Vinh City. All observed species are considered generalist species since they are common in Southeast Asian cities, including those in Vietnam [1,33]. The first study in Vietnam on urban bats was conducted in Hanoi, where five bat species were reported [34]. For the larger metropolitan area of Hanoi, previous

studies reported 13 species [1,34,35]. In Ho Chi Minh City, the largest city in Vietnam, the number of bat species was 13 species for the whole metropolitan area [1]. Three out of these 13 species were recorded for the most heavily urbanized areas in the centre, namely *Taphozous melanopogon*, *T. theobaldi*, and *Scotophilus heathii* [1]. This study added three more species to this list: *S. kuhlii*, *Pipistrellus javanicus* and *Myotis hasseltii*, making it six species for the urban ecosystem of Ho Chi Minh City. This study captured nearly 80% of all bat species previously recognized for Vietnam cities based on mist-netting effort. Therefore, future bat fauna surveys should expand to capturing bats close to roosting sites in addition to foraging grounds.

To our knowledge, the echolocation call parameters documented in our study are the first reported for the studied species in Vietnam. Some notes on the frequency ranges and peak frequencies at maximum energy for our study species can be found in work by Kruskop [1], but this source lacks many details of the echolocation calls. *Myotis hasseltii* was reported to produce FM calls with high intensity, particularly for frequencies around 45–50 kHz [1]. Our data is consistent with those from previous reports for the species in Vietnam. The species was recently recorded for Ha Noi City [35], but without a detailed description of the specific echolocation call parameters. Individuals reported here showed echolocation calls with a duration of about 3.86 ms (sd = 0.92 ms), and a frequency with maximum energy at about 50.0 ± 2.8 kHz. The pulse duration of calls emitted by this species is close to the lowest value observed before in conspecifics from Malaysia, ranging between 2.5 and 5.5 ms [36]. The start frequency was lower in our study than in Malaysia, with a mean frequency of 67.6 kHz (± 3.7 kHz) compared to a range of 82 to 104 kHz for conspecifics from Malaysia [36]. Although *Pipistrellus javanicus* is a common species in Vietnam and recorded in various habitats [1,9,37], we are the first to describe the species' echolocation call parameters. Previous studies noted that this species has a primary echolocation call frequency with maximum energy at around 50 to 55 kHz [1]. Our data fall within this range. The frequency parameters of echolocation reported for Vietnamese *P. javanicus* is relatively high compared to those reported for the same species in Nepal [38], except for the lowest frequency (Table 2).

We report the first echolocation call parameters for *Scotophilus kuhlii* in Vietnam. These call parameters are consistent with those reported before by other studies from India [28,39] and China [40]. However, the spectrogram pattern of echolocation calls from our collection is relatively different from conspecifics recorded in China, where the echolocation call consists of four harmonics [40]. The variation in echolocation call of this species was reported to vary due to differences in habitat and geographic environments [28,41,42], but the difference in shape and pattern of call spectrograms produced by Chinese specimens and those from Indian and Vietnam are documented for the first time. Spectrograms of species-specific echolocation calls were similar in two studies from India even though the recording method differed [28,39]. It is noteworthy that even when applying the same recording method, i.e., recording bat's acoustic after release, led to dissimilar spectrograms for the same species [28,40], highlighting that echolocation call parameters of this species are variable and highly context-dependent. Since the species has a wide distribution range, further studies on species acoustic and molecular identity are needed to explain this large intra-specific variation of echolocation call parameters.

Even though we confirmed *Scotophilus heathii* in Tra Vinh City, we did not capture live animals with our mist-nets. Therefore, the acoustic's parameter of echolocation calls could not be analyzed for this species. Previous studies noted the FMAXE for *S. heathii* equalled 30 kHz [1], which is much lower than that of *S. kuhlii* (45.6 kHz in this study). In future, it is necessary to capture live animals this species to document the species' echolocation calls and to test for difference between these two species.

Taphozous melanopogon from our study sites emitted echolocation calls with four harmonics, similar to those reported before for this taxon [27,43]. However, the fourth harmonic was often in lower intensity, compared to the first and second harmonic. The frequency at the maximum energy occurs for the second or first harmonic, at 33.1 ± 5.9 kHz, which was higher than those reported before at 29.7 kHz for conspecifics from Thailand [27] and at 30.1 kHz from conspecifics from China [43]. The start frequency of the species in

this study was lower than those recorded for Thai conspecifics but slightly higher than those recorded for Malaysian conspecifics. On the contrary, the call's terminal frequency was higher in specimens from Vietnam compared with those from Thailand or Malaysia (Table 1).

In previous studies, discriminant function analysis classified FM calling bat species at an accuracy of 70 to 90%, depending on the taxonomic group studied [27–29]. In our study, the classifying species' accuracy was 79.0% and deemed acceptable for identifying species based on their acoustic parameters. A misidentification was reported in some studies before due to the complex echolocation calls and importantly, the overlap in species echolocation call parameters in species of the same taxonomic group and the same functional guild [27–29]. Three out of four species studied produced broadband FM calls and only *Taphozous melanopogon* emitted multi-harmonics calls. Therefore, the discriminant function analysis successfully distinguished the echolocation calls of *Taphozous melanopogon* from those of the other species (Table 4 and Figure 1). On the other hand, our dataset demonstrated the similarity of echolocation call parameters for the three vespertilionid species (Table 2). Therefore, the accuracy of assigning to species based on their acoustic parameters was low. The species with the lowest correct identification level was *S. kuhlii* (67.3%), perhaps caused by the high diversity of call properties. Calls of this species were misidentified as *M. hasseltii* or *P. javanicus*. *M. hasseltii* and *S. kuhlii* were foraging at the same place and were captured simultaneously. The similarity in habitat used and the preference for similar insect prey may highlight the membership to the same foraging guild, which suggests convergent evolution of echolocation call parameters [44]. Another reason that might affect the classification accuracy was the use of flying tent to record species echolocation call. For two species with no effect of flying tent on FMAXE, the most crucial acoustic parameter in studying bat calls, species classification accuracy based on their acoustic parameters was high, 100% for *T. melanopogon* and 80.8 for *M. hasseltii*. On the other hand, the tent's confined space affects FMAXE, of the other two species. Hence the percentage of accuracy in classifying at 67.3% to *S. kuhlii* and 70.0% to *P. javanicus*.

5. Conclusions

Our study confirmed the occurrence of more than three bat species for the urban area of Ho Chi Minh City (six species) and for that of Tra Vinh city (four species). Overall, we consider the species richness to be low compared to the bat fauna in more forested sites or protected areas. We first described echolocation calls of four urban bats species in Vietnam. However, using a flying tent was perhaps not an adequate method to record and describe some bat species' echolocation calls. Our study showed a high level of accuracy in using the acoustic parameters of bats to classify species based on DFA even when recording only a few echolocation calls. Therefore, the results provide a promising first step towards monitoring bat assemblages in urban areas in Vietnam in an automated and thus more comprehensive way. However, future studies are needed to shed light on the bat assemblages in suburban and rural areas. Further, we are in need of a comprehensive bat call library that will facilitate long-term monitoring programs to examine the effect of urbanization on bat assemblages.

Supplementary Materials: The following are available online at <https://www.mdpi.com/1424-2818/13/1/18/s1>. Table S1. List of recorded species and some notes for specimens. Table S2. Selected morphological parameters (mm; mean and range) and body mass (in g) of the species described in this study. Values are given at mean (Avg), minimum-maximum (Min-max), *n*. Min-max (*n*) indicates that the number of examined specimens for that measurement is different from other measurements—denotes data not available. Abbreviations and definitions for measurements are given in the text of method section. Table S3: The call parameters of studied species that used for the Discriminant Function Analysis. Figure S1. The lateral view of captured bat species: *Pipistrellus javanicus* (a); *Scotophilus kuhlii* (b); *Myotis hasseltii* (c); *Taphozous melanopogon* (d). Figure S2. The species accumulation curve based on sampling effort by night of sampling for survey in Tra Vinh City (a) and Ho Chi Minh City (b) shows the continuously growing of the species richness in two survey sites. Figure S3. The illustration of automatic parameter measurements by the software. The

spectrum-base parameters that were measured including max frequency (1), peak frequency (2) and min frequency (3) at three points of start (s), maximum energy (p), and end of the call (e).

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