HNUE JOURNAL OF SCIENCE
Natural Sciences 2024, Volume 69, Issue 2, pp. 125-132
This paper is available online at http://hnuejs.edu.vn/ns
DOI: 10.18173/2354-1059.2024-0027

NEW RECORDS OF ECHOLOCATION CALLS OF *Hipposideros khaokhouayensis*

Pham Van Nha[1,*], Nguyen Thanh Luong[2] and Vu Dinh Thong[2,3]
[1]*Faculty of Natural Sciences and Technology, Tay Bac University, Son La city, Vietnam*
[2]*Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology (VAST), Hanoi city, Viet Nam*
[3]*Graduate University of Science and Technology, Hanoi city, Vietnam*
*Corresponding author: Pham Van Nha, e-mail: phamvannha@utb.edu.vn

Received May 31, 2024. Revised June 20, 2024. Accepted June 26, 2024.

Abstract. Phou Khao Khouay leaf-nosed bat (*Hipposideros khaokhouayensis*) is a globally rare and vulnerable species of the family Hipposideridae. It has been known only from the type locality in Laos and two localities in Vietnam. Its echolocation calls were included in several publications but still poorly understood. Between November 2023 and April 2024, the authors conducted three field surveys in Lang Son province and Hai Phong city, northeastern Vietnam, in order to seek further understanding of its potential distribution and echolocation behavior. Twelve adult individuals of the species were captured using harp traps and mist nets for identification in the field based on their morphological diagnoses to confirm the target species' identity. The PCTape system was employed to record echolocation calls while bats were emerging from caves. Seven sound parameters (CF frequency, CF duration, tFM frequency, tFM bandwidth, tFM duration, pulse duration, and inter-pulse interval) of the recorded calls were analyzed and measured using the Selena software. Their values are in a range of 90.2 - 91.1 kHz, 3.1 - 8.9 ms, 75.6 - 84.5 kHz, 6.1 - 15.0 kHz, 0.9 - 1.7 ms, 4.5 - 10.1 ms and 15.1 - 31.4 ms, respectively. The present study results are considerably different from the descriptions in previous publications. This paper also provides the first data on the inter-pulse interval of the species echolocation calls and a methodological note on this sound parameter.

Keywords: bat, echolocation, Hipposideridae, Laos, Vietnam.

1. Introduction

Phou Khao Khouay leaf-nosed bat (*Hipposideros khaokhouayensis*) is a globally vulnerable and rare species of the family Hipposideridae [1]. The species was first described in the Phou Khao Khouay National Park in Lao People's Democratic Republic (Laos) and

has been listed as a "Vulnerable" (VU) in the IUCN Red List of Threatened Species [1], [2]. To date, it has been known from the type locality, which was formerly named Phou Khao Khouay National Biodiversity Conservation Area, in central Laos and two localities within northern Vietnam (Cat Ba National Park, Hai Phong city, and Son La province). However, the species is still poorly studied throughout its distributional range [3]-[12]. Its taxonomy has been clear with confirmation based on both morphological and genetic data [2]-[5]. On the other hand, its echolocation calls were only included or described in a few publications [2]-[5], [7], [8], [11], [12]. Remarkably, almost all previous publications only included the CF value or several sound parameters [3]-[5], [7], [8] and very few ones included detailed descriptions of the species calls [11], [12]. Between November 2023 and April 2024, the authors conducted a series of bat surveys in northeastern Vietnam with a focus on echolocation calls of Phou Khao Khouay leaf-nosed bat species. This paper provides new findings in the echolocation call parameters of the species from the Cat Ba National Park.

2. Content

2.1. Materials and methods

2.1.1. Bat capture and identification

Between November 2023 and April 2024, the authors conducted three bat surveys at Cat Ba National Park, Hai Phong city, and Van Lang district, Lang Son province, northeastern Vietnam. Bat capture and identification during the present study were implemented following Thong (2021) [4] and Thong (2023) [12]. Three mist nets of 6.0 m (height) × 9.0-12.0 m (length), mesh size 16 × 16 mm, and one four-bank harp trap [13] were used to capture bats near the studied caves and under forest canopies. All captured bats were studied following the guidelines of the American Society of Mammalogists [14], [15]. Each captured bat individual was carefully removed from the net or trap and then examined in reproductive status and age following Racey (2009) [16] and Brunet-Rossinni & Wilkinson (2009), respectively [17]. External characteristics and body size of every captured adult were compared with respective descriptions of the species in previous publications for morphological identification [2]-[12]). Photographs of representative adult individuals were taken before being released at the capture sites.

2.1.2. Echolocation recordings and analyses

Methods for recording and analyses of echolocation followed Thong (2023) [12] and Thong (2023) [18]. The PCTape system (480 kHz, 16 bit) was employed to record echolocation calls while bats were emerging from caves or approaching the mist net or trap. The Batman software, which displays colour sonograms of the detected echolocation signals in real time, was used to obtain high-quality sound sequences. The Selena software was used to analyze selected sound sequences. All PCTape, Batman, and Selena are custom-made programs developed by the University of Tübingen, Germany. The entire recorded calls of the Phou Khao Khouay leaf-nosed bat comprise different harmonics with the highest energy in the second one. The selected calls were analyzed and described following Thong (2023) [12], Thong (2023) [18], and Tian and Schnitzler (1997) [19]. Each entire harmonic of the analyzed calls comprises two components: constant-frequency (CF) followed by a downward frequency-modulated terminal sweep (tFM). The selected

sound sequences were displayed as sonograms with an FFT (Fast Fourier Transformation) of 256, Hann-window, and zero-padding. Seven sound parameters of the second harmonic of the calls [CF frequency, CF duration, tFM frequency, tFM bandwidth, tFM duration, pulse duration (PD), and inter-pulse interval (IPI)] were measured. The IPI was measured from the beginning of a call to the beginning of the following call. The CF frequency, tFM frequency, and tFM bandwidth were measured in kilohertz (kHz) while CF duration, tFM duration, PD, and IPI were measured in milliseconds (ms).

2.2. Results

2.2.1. Morphological identification

Over the three surveys, three males and nine females of Phou Khao Khouay leaf-nosed bats were captured in April 2024 near the exit of the Trung Trang Cave (20°47'13.56"N, 106°59'57.78"E, 22.0 meters above sea level), Cat Ba National Park, Cat Hai district, Hai Phong city, northeastern Vietnam. All captured individuals were adults and six of the nine captured females were pregnant. They clearly demonstrated the diagnostic morphological features of *H. khaokhouayensis*. The noseleaf of each individual is narrow with an expanded internarial septum forming an oval disc-like shape (Figure 1). The lateral margins of the posterior and anterior leaves are connected at their bases. The fur on the forehead is soft and bright yellow. The anterior leaf has a narrow median emargination on the frontal margin. Each ear is very broad in comparison with the body size and has a blunt tip. The pelage colour is in a range of light grey-brown and dark brown.

Figure 1. Frontal view of H. khaokhouayensis from Cat Ba National Park

2.2.2. New acoustic records

Every captured and uncaptured but detected individual of *H. khaokhouayensis* emitted multiharmonic echolocation calls with a typical "CF-FM" signal structure (Figure 2). The values of CF frequency, tFM frequency, and tFM bandwidth of the recorded calls are in ranges of 90.2 - 91.1 kHz, 75.6 - 84.5 kHz, and 6.1 - 15.0 kHz, whereas CF duration, tFM duration, pulse duration, and inter-pulse interval are in ranges of 3.1 - 8.9 ms, 0.9 - 1.7 ms, 4.5 - 10.1 ms, and 15.1 - 31.4 ms, respectively. The species emitted some short calls with a pulse duration of 4.5 - 4.6 ms among normal calls with a pulse duration longer than 7.0 ms (Figures 2, 3). The tFM bandwidth of each call was also adjusted even with a call group (Figure 4A). Remarkably, unusual calls (each of which comprises a pure CF component) were also emitted by the species among the short and normal calls (Figure 4B).

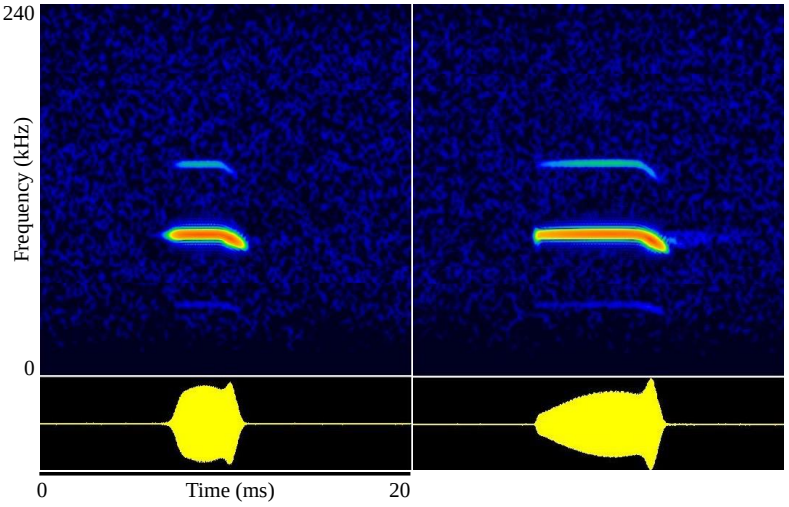

Figure 2. Sonograms and oscillograms of typical short (left) and normal (right) multiharmonic CF-FM calls of H. khaokhouayensis recorded in Cat Ba National Park

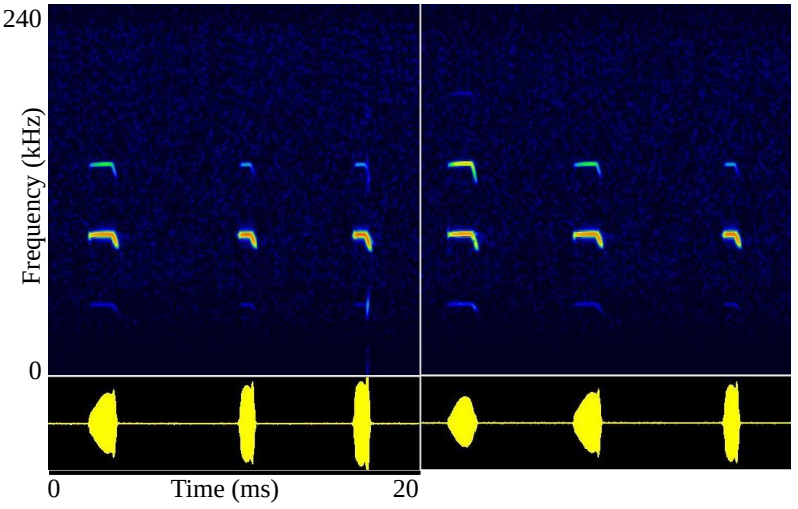

Figure 3. Sonograms and oscillograms of a group with one normal call and two short calls (left) and another group with two normal calls and one short call (right)

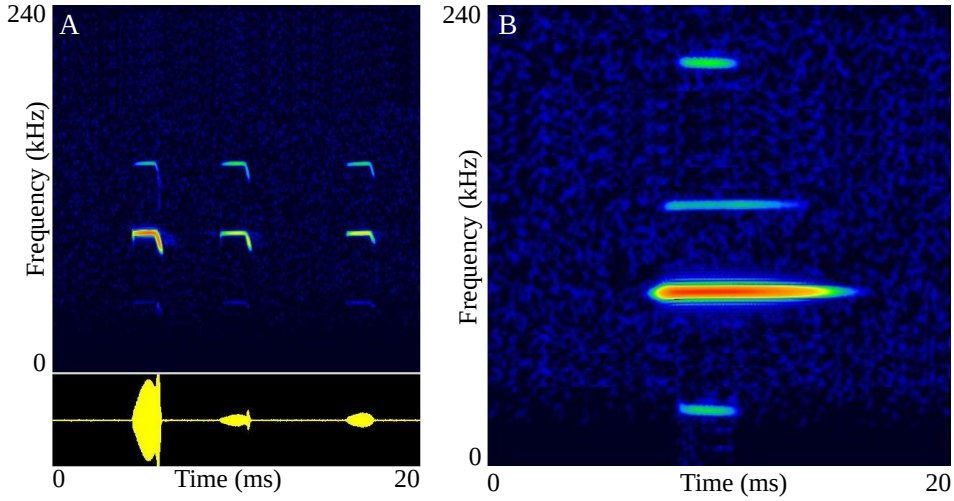

Figure 4. Sonograms and oscillograms of a normal call group (A) with the tFM bandwidth adjusted from 15.0 kHz down to 7.0 kHz and sonograms of an unusual call with only pure CF component (B)

2.3. Discussion

Table 1. Sound parameters of H. khaokhouayensis from the present study in comparison with previously published data

Data are presented as sample size (n), mean ± standard deviation, and range (minimum-maximum). Sample sizes from the present study are the numbers of signals measured within the three selected 1000-millisecond sound sequences. Abbreviations are given in the "Materials and Methods"

Data sources	n	CF frequency	CF duration	tFM frequency	tFM bandwidth	tFM duration	Pulse duration	Inter-pulse interval
This study	26	90.7 ± 0.2 90.2 - 91.1	5.9 ± 1.5 3.1 - 8.9	80.1 ± 2.5 75.6 - 84.5	10.6 ± 2.6 6.1 - 15.0	1.3 ± 0.2 0.9 - 1.7	7.2 ± 1.3 4.5 - 10.1	23 ± 5.6 15.1 - 31.4
Thong (2023) [12]	81	92.8 ± 0.9 91.8 - 94.4	6.4 ± 0.9 4.5 - 8.3	79.8 ± 1.8 76.6 - 83.6	13.0 ± 2.0 8.5 - 16.9	1.1 ± 0.2 0.8 - 1.7	7.6 ± 0.9 5.3 - 9.7	-
Thong et al. (2021) [11]	2	91.2 - 92.1	-	-	-	-	7.7 - 8.8	-
Tuneu-Corral (2019) [3]	N/A	87.0 - 91.0	-	-	-	-	-	-
Kruskop (2013) [8]	N/A	87.0 - 94.3	-	-	-	-	-	-
Abramov &	N/A	94.3	-	-	-	-	-	-

Kruskop (2012)								
Thong et al. (2008) [5]	1	94.2	-	-	-	-	-	-
Guillen-Servent & Francis (2006) [2]	6	87.2 - 91.1	-	-	-	-	3.0 - 5.0	-

To our knowledge, data on echolocation calls of *H. khaokhouayensis* was included in several previous publications [2]-[5], [7], [8], [11], [12]. However, almost all of those publications just included the CF frequency and CF duration of the species calls [2]-[5], [7], [8], [11]. Considerably, at least two of those publications, Kruskop (2013) and Tuneu-Corral (2019), only cited data from the literature [3], [8]. Before the present study, the report of Thong (2023) was the only publication containing descriptions of the species calls [12]. Remarkably, the inter-pulse interval (IPI) of the species calls was not included in any previous publication although it is one of the commonly described sound parameters in bat echolocation research. To provide initial data for a broader view and understanding of the species calls, this paper provides the first data on the IPI of the species calls (Table 1). In fact, IPI values are normally in a very wide variation, even within a group of calls, because every bat often adjusts its calls to adapt to the surrounding environments and space (Figures 3, 4; Table 1; [22]). Therefore, the author here recommends that IPI should not be measured or included in the descriptions of echolocation calls which were recorded while the bat was under control by humans (e.g. inside a flight tent, zipline, etc.). Otherwise, the described IPI from those recordings may contain biased values.

The present study results not only include the first information on IPI but also provide new data on sound parameters which are considerably different from respective ones in previous publications (Table 1). Guillen-Servent & Francis (2006) provided the first echolocation data of *H. khaokhouayensis* with CF frequency and pulse duration in the range of 87.2-91.1 kHz and 3.0-5.0 ms, respectively [2]. These values were cited in subsequent publications [3], [8]. The CF frequency from the present study overlaps with that in the publication of Guillen-Servent & Francis (2006) but is distinctly lower than that in other previous publications (Table 1).

3. Conclusions

The present study provides new data on the echolocation calls of the Phou Khao Khouay leaf-nosed bat (*Hipposideros khaokhouayensis*). The IPI sound parameter of the species is first described in this paper. However, it is normally in a wide variation because bats often adjust their calls with different IPI values to adapt to the surrounding environments. The new data on echolocation characteristics from the present study would be essential for the monitoring and conservation of the species in the future.

Acknowledgments: The authors would like to thank the Vietnam Academy of Science and Technology for their support under grant number QTBG01.03/23-24; and to the following people and institutions for their various support: Hans-Ulrich Schnitzler, Annette Denzinger, Christian Dietz of the University of Tuebingen, Germany; Doctorates of the Institute of Ecology and Biological Resources, VAST; Nguyen Van Thiu, Vu Hong Van and Nguyen Xuan Khu of Cat Ba National Park.

REFERENCES

[1] Douangboubpha B, (2020). *Hipposideros khaokhouayensis* (amended version of 2019 assessment). The IUCN Red List of Threatened Species 2020: e.T136819A166602959. https://dx.doi.org/10.2305/IUCN.UK.2020-1.RLTS.T136819A166602959.en. Accessed on 04 June 2024.

[2] Guillen-Servent A & Francis CM, (2006). A new species of bat of the *Hipposideros bicolor* group (Chiroptera: Hipposideridae) from Central Laos, with evidence of convergent evolution with Sundaic taxa. *Acta Chiropterologica*, 8(1), 39-61.

[3] Tuneu-Corral C, (2019). Phou Khao Khouay leaf-nosed bat (Hipposideros khaokhouayensis). In: Wilson DE, Mittermeier RA, (1st ed.). *Handbook of the mammals of the world - Volume 9: Bats*. Lynx Edicions, Barcelona, p. 255.

[4] Vu DT, (2021). *Taxonomy and echolocation of Vietnamese bats*, (1st ed.). Publishing House for Science and Technology, Hanoi, Vietnam, p. 258.

[5] Vu DT, Dietz C, Schinitzler H-U, Denzinger A, Furey NM, Borisenko AV & Bates PJJ, (2008). First record of *Hipposideros khaokhouayensis* (Chiroptera: Hipposideridae) from Vietnam. *HNUE Journal of Science*, 53(5), 138-143.

[6] Vu DT & Furey NM, (2008). The bat fauna of Cat Ba Biosphere Reserve. *Academia Journal of Biology* (formerly *Journal of Biology*), 30(3), 73-77 (in Vietnamese).

[7] Abramov AV & Kruskop SV, (2012). The mammal fauna of Cat Ba Island, Northern Vietnam. *Russian Journal of Theriology*, 11(1), 57-72.

[8] Kruskop SV, (2013). *Bats of Vietnam: Checklist and an identification manual* (2nd ed.). Moscow, Russia, p. 299.

[9] Vu DT, Do TD & Nguyen VT, (2016). An overview of bat research in Cat Ba biosphere reserve with remarks on previous records. *Proceedings of the 2nd national scientific conference on biological research and teaching in Vietnam*. Publishing House of the Vietnam National University, 737-744.

[10] Vu DT, Sichanh S & Nha PV, (2020). The conservation status of bats (Mammalia: Chiroptera) in Cat Ba National Park, northeastern Vietnam. *HNUE Journal of Science*, 65(10), 92-98.

[11] Vu DT, Denzinger A, Nguyen VS, Nguyen TTH, Hoang TT, Dao NL, Pham VN, Nguyen VV, Pham DT, Tuanmu M-N, Huang JC-C, Ladthavong T, Nguyen TL. & Schnitzler H-U, (2021). Bat Diversity in Cat Ba Biosphere Reserve, Northeastern Vietnam: A Review with New Records from Mangrove Ecosystem. *Diversity*, 13(376), 1-29.

[12] Vu DT, (2023). New Data on Distribution, Morphology and Echolocation of *Hipposideros khaokhouayensis* Guillén-Servent & Francis, 2006 (Chiroptera: Hipposideridae). *Acta Zoologica Bulgarica*, 75(4), 469-476.

[13] Francis CM, (1989). A comparison of mist nets and two designs of harp traps for capturing bats. *Journal of Mammalogy*, 70(4), 865-870.

[14] Sikes RS & Animal Care and Use Committee of the American Society of Mammalogists, (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.

[15] Sikes RS & Gannon WL, (2011). Guidelines of the American Society of Mammalogists for the use of wild mammals in research. *Journal of Mammalogy*, 92(1), 235-253.

[16] Racey PA, (2009). Reproductive assessment of bats. In: Kunz TH, Parsons S (eds.). *Ecological and Behavioural Methods for the Study of Bats*, (2nd ed.), p. 249-264. The John Hopkins University Press.

[17] Brunet-Rossinni AK & Wilkinson GS, (2009). Methods for age estimation and the study of senescence in bats. In: Kunz TH, Parsons S (eds.). *Ecological and Behavioural Methods for the Study of Bats*, (2nd ed.), p. 315-325. The John Hopkins University Press.

[18] Vu DT, (2023). Remarks on the diversity and echolocation calls of hipposiderid bats (Chiroptera: Hipposideridae) in Cuc Phuong National Park, Northeastern Vietnam. *Academia Journal of Biology*, 45(4), 1-9.

[19] Tian B & Schnitzler H-U, (1997). Echolocation signals of the greater horseshoe bat (Rhinolophus ferrumequinum) in transfer flight and during landing. *The Journal of the Acoustical Society of America*, 101(4), 2347-2364.

[20] Neuweiler G, Metzner W, Heilmann U, Riibsamen R, Eckrich M & Costa HH, (1987). Foraging behaviour and echolocation in the rufous horseshoe bat (Rhinolophus rouxi) of Sri Lanka. *Behavioral Ecology and Sociobiology*, 20, 53-67.

[21] Schnitzler H-U & Denzinger A, (2011). Auditory fovea and Doppler shift compensation: adaptations for flutter detection in echolocating bats using CF-FM signals. *Journal of Comparative Physiology A*, 197, 541-559.

[22] Starik N & Göttert T, (2022). Bats adjust echolocation and social call design as a response to urban environments. *Frontiers in Ecology and Evolution*, 10: 939408. doi: 10.3389/fevo.2022.939408.