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# Importance of Mangroves for Bat Research and Conservation: A Case Study from Vietnam with Notes on Echolocation of *Myotis hasselti*

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**Citation:** Thong, V.D.; Denzinger, A.; Long, V.; Sang, N.V.; Huyen, N.T.T.; Thien, N.H.; Luong, N.K.; Tuan, L.Q.; Ha, N.M.; Luong, N.T.; et al. Importance of Mangroves for Bat Research and Conservation: A Case Study from Vietnam with Notes on Echolocation of *Myotis hasselti*. *Diversity* **2022**, *14*, 258. https://doi.org/10.3390/d14040258

Academic Editor: Michael Wink

Received: 12 March 2022
Accepted: 24 March 2022
Published: 30 March 2022



**Abstract:** Mangrove ecosystems play important ecological roles, including the mitigation of global climate change and biodiversity conservation. However, they have received little attention from scientists for the research and conservation of bats and general biodiversity. In Vietnam, bat species inhabiting mangroves have been relatively unstudied, while this ecosystem is located along the country's coastal zones and has declined dramatically due to the development of agriculture, wind energy and other threats. To initially fill this gap, five bat surveys were conducted between September 2019 and November 2021 within Ha Long Bay and Ben Tre province, which contain representative mangrove areas of northern and southern regions of Vietnam, respectively. Bats were captured using mist nets, mobile nets and hand nets. Their echolocation calls were recorded and analyzed using the PCTape system and Selena software, respectively. Five species were captured and recorded: *Cynopterus brachyotis*, *Macroglossus minimus*, *Myotis hasselti*, *Myotis pilosus* and *Taphozous melanopogon*. They are all new to both Ha Long Bay and Ben Tre province. Four species (*C. brachyotis*, *M. minimus*, *M. hasselti* and *M. pilosus*) have been rarely documented from other ecosystems in Vietnam but have commonly been recorded and captured in mangrove areas. Of these species, *M. pilosus* is a globally "Vulnerable" species. While searching for prey, *Myotis hasselti* emitted high energy echolocation calls sweeping from about 96 to about 24 kHz with a signal duration of about 5 ms. This species sometimes uses social calls of a horseshoe-shaped structure, which last about 15 ms and are emitted about 26 ms in front of a search call. Results from our surveys indicated the importance and potential of mangroves for bat research and conservation.

**Keywords:** bat; Ben Tre; echolocation; Ha Long Bay; mangrove; Vietnam: wind energy

## 1. Introduction

Mangrove ecosystems are important in the prevention of erosion, protection of coastal communities from extreme weather events and mitigation of global climate change [1]. They are present throughout the coastal zones of Vietnam [2]. Unfortunately, over 38% of mangroves in the country were lost during the war and are still at high risk by expansion of shrimp aquaculture, infrastructure development and climate change [2]. Ha Long Bay in Quang Ninh province is one of the best-known sites for the rapid development of tourism. It contains a large portion of remaining mangroves in northern Vietnam [3]. In southern Vietnam, a large portion of remaining mangroves remains in Ben Tre province with approximately 6098 ha distributed in three coastal districts: Binh Dai, Ba Tri and Thanh Phu. Remarkably, Quang Ninh and Ben Tre have been identified as two of the most sensitive provinces in Vietnam to sea level rise [3,4]. In fact, mangrove areas of Binh Dai, Ba Tri and Thanh Phu decreased by 49.4%, 33.3% and 59.3% respectively between 1998 and 2015, and continue to be lost by the expansion of rice crops and other threats [5]. On the other hand, over 45% of the remaining mangrove areas in Ben Tre province will be lost when the sea level rises up to 1 m in the future [5]. The loss of mangroves in the two areas and other coastal zones of the country leads to biodiversity loss [6]. To our knowledge, before September 2019, bats of Ha Long Bay within Quang Ninh province and Ben Tre province were not included in any previous publication in every aspect. To initially fill in the gap and provide scientific data for research, monitoring and conservation actions of bats in these areas and other mangrove ecosystems, we conducted bat surveys in both Ha Long Bay within Quang Ninh province and Ben Tre province with an emphasis on mangroves. These two localities are representative of the remaining mangrove in northern and southern regions of Vietnam, but also show the highest loss of this ecosystem [3–5]. Results from the surveys provide the first records of bats from both Ha Long Bay and Ben Tre province as well as new data on echolocation calls of a *Myotis* species.

## 2. Materials and Methods

### *2.1. Bat Capture and Morphological Measurements*

Field surveys were conducted during one phase in Ha Long Bay, Quang Ninh province (7–11 September 2020) and four phases in Ben Tre province (24–26 September 2019 and 4–6 January 2021 in the Thanh Phu district; 10–13 November 2020 and 26–29 March 2021 in the Binh Dai district) (Figure 1 and Table 1). The mangrove flora of each study site consists of dense vegetation with at least 11 common plant species (*Acanthus ilicifolius*, *Aegiceras corniculatum*, *Avicennia marina*, *A. officinalis*, *Bruguiera gymnorrhiza*, *Kandelia candel*, *Lumnitzera racemosa*, *Rhizophora apiculate*, *R. stylosa*, *Sonneratia caseolaris* and *Thespesia populnea*), which were identified by botanists of IEBR and CBES (unpublished reports). Bats were captured and handled following guidelines recommended by the American Society of Mammalogists [7,8]. Mist nets of various sizes (3.0–6.0 m (height) × 6.0–20 m (length), mesh size 16 × 16 mm) were set up across the channels in mangrove areas between 17:30 h and 22:00 h. A mobile "V shaped net", which was made of two fishing rods and a short mist net, was also employed to capture bats when they emerged from their roosts and were foraging over water. Each bat was removed carefully from the nets and placed in a cotton bag. Selected external measurements were obtained in the field using an ABSOLUTE Coolant Proof Caliper Series 500, Mitutoyo America Corporation: FA, forearm length—from the extremity of the elbow to the extremity of the carpus with the wings folded; EH, ear height—length of ear conch; TIB, tibia length—from the knee joint to the ankle; HF, hind-foot length—from the extremity of the heel behind the os calcis to the extremity of the longest digit, excluding the hairs or claws; tail, tail length—from the anal opening to the tip of the tail. The measurement values were round up to the nearest 0.1 mm. The above measurements are described and illustrated in Bates and Harrison [9], Borissenko and Kruskop [10] and Kruskop [11]. Reproductive status and age were assessed following Racey [12] and Brunet-Rossinni and Wilkinson [13], respectively.

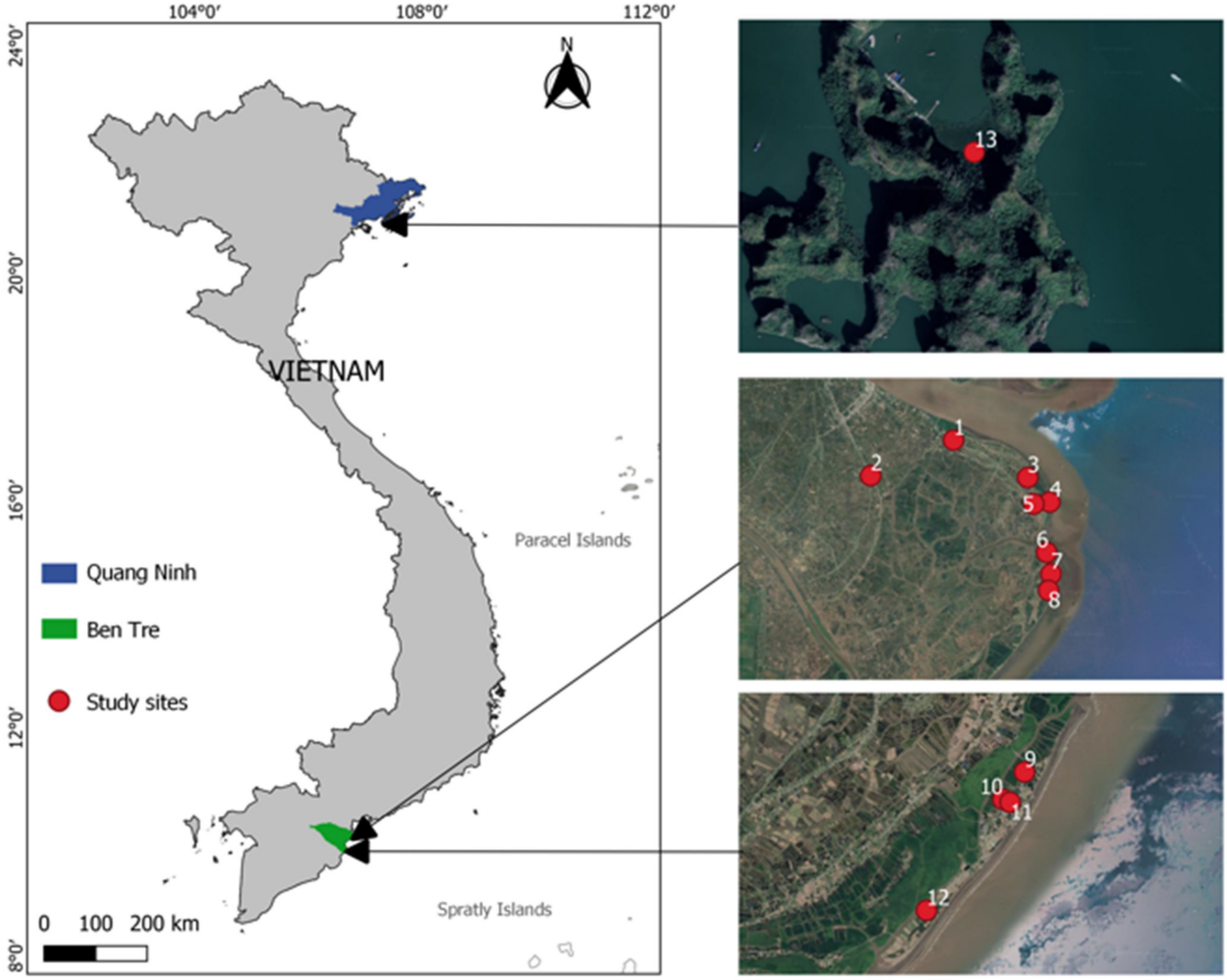


**Figure 1.** Study sites in Ha Long Bay, Quang Ninh province, northern Vietnam, and Ben Tre province, southern Vietnam. The background images were based on Google satellite (Available online: https://www.google.com/maps/ (accessed on 27 February 2022)).

**Table 1.** Bat species from each netting site in the studied mangrove in Ha Long Bay and Ben Tre province. The study sites are numbered following the localities in the Figure 1.

| Study Site | Locality Name | Coordinates | Captured Species (Individuals) |
|---|---|---|---|
| 1 | Thua My 2 (Cay Bang), Binh Dai District | 10°10′37″ N; 106°44′38″ E | *M. hasselti* (6♂, 8♀); *Myotis pilosus* (1♂, 1♀) |
| 2 | Binh Thuan (30–4), Binh Dai District | 10°09′39″ N; 106°42′23″ E | *M. hasselti* (3♂, 9♀); *T. melanopogon* (3♂, 3♀) |
| 3 | Thua Tien 1, Binh Dai District | 10°09′36″ N; 106°46′39″ E | *C. brachyotis* (9♂, 12♀); *M. minimus* (7♂, 15♀); *M. hasselti* (6♂, 9♀); *T. melanopogon* (1♂, 2♀) |
| 4 | Thua Tien 5, Binh Dai District | 10°08′56″ N; 106°47′15″ E | *M. minimus* (2♂, 2♀) |
| 5 | Thua Tien 11, Binh Dai District | 10°08′53″ N; 106°46′49″ E | *C. brachyotis* (1♂, 1♀); *M. minimus* (2♂, 3♀) |

**Table 1.** *Cont.*

| Study Site | Locality Name | Coordinates | Captured Species (Individuals) |
|---|---|---|---|
| 6 | Thua Loi 8, Binh Dai District | 10°07′34″ N; 106°47′10″ E | *C. brachyotis* (3♂, 3♀); *M. minimus* (1♂, 3♀) |
| 7 | Thua Loi 2, Binh Dai District | 10°06′59″ N; 106°47′17″ E | *C. brachyotis* (3♂, 6♀); *M. minimus* (3♂, 3♀) |
| 8 | Thua Loi 11, Binh Dai District | 10°06′32″ N; 106°47′13″ E | *C. brachyotis* (1♂, 1♀) |
| 9 | Thanh Hai 8, Thanh Phu District | 09°50′46″ N; 106°39′47″ E | *C. brachyotis* (3♂, 9♀); *M. minimus* (2♂, 6♀) |
| 10 | Thanh Hai 1, Thanh Phu District | 09°50′27″ N; 106°39′32″ E | *M. hasselti* (6♂, 6♀); *Myotis pilosus* (1♀) |
| 11 | Thanh Hai 2, Thanh Phu District | 09°50′25″ N; 106°39′37″ E | *C. brachyotis* (2♂, 5♀); *M. minimus* (3♂, 3♀) |
| 12 | Thanh Hai 5, Thanh Phu District | 09°49′10″ N; 106°38′39″ E | *C. brachyotis* (3♂, 3♀); *Myotis pilosus* (1♂) |
| 13 | Dau Go Area, Ha Long Bay | 20°54′37″ N; 107°01′13″ E | *M. hasselti* (4♂, 6♀); *Myotis pilosus* (3♂, 3♀), *T. melanopogon* (7♀) |

### 2.2. Echolocation Recording and Analyses

Echolocation calls were recorded using the PCTape system (480 kHz, 16 bit) in two situations: inside a flight tent (5.0 m (length) × 5.0 m (width) × 3.5 m (height)) and after the release of bats. Echolocation calls were also recorded at emergence time at the roosting sites and when bats were foraging in their natural habitats where the PCTape system was set up next to a mist net to record echolocation calls bats coming to the net. Batman software, which is connected to the PCTape system and displays all detected bat calls, was used to select and record high-quality sound sequences. The tent is appropriate for recording echolocation calls of *Myotis hasseltii* because this species is relatively small and flies well within the tent. The tent is less suited for recording the calls of *Myotis pilosus* and *Taphozous melanopogon* because these are rather large and fast-flying species. High-quality sound sequences of each species were selected for sound analysis using the Selena software. Signals were displayed as sonograms with a FFT (Fast Fourier Transformation) of 256, Hann-window and zero-padding. The following call parameters were manually measured: initial frequency (iF), terminal frequency (tF), bandwidth (BW) and pulse duration (PD). Sound parameters were measured from the prominent first (1st) harmonic with the exception of *Taphozous melanopogon*, where we used the more prominent second (2nd) harmonic. The beginning and the end of the signals were set at −30 dB below the maximal amplitude of the signal. The PCTape system, and the Batman and Selena software are custom-made by the University of Tuebingen, Germany.

### 2.3. Tissue Sampling and Genetic Analysis

Punched wing samples were obtained from selected individuals and preserved in absolute ethanol. DNA samples for genetic analyses were extracted from wing punches using G-spin™ Total DNA Extraction Mini Kit (iNtRON, Korea) following the manufacturer's recommendations. The cytochrome c oxidase subunit 1 (COI), which was widely adopted for species identification, was used for genetic analysis in this study. The 657 bp COI fragments were amplified using PCR with the universal primer pair: VF1d: 5′-TTC TCA ACC AAC CAC AAR GAY ATY GG-3′; VR1d: 5′-TAG ACT TCT GGG TGG CCR AAR AAY CA-3′ [14]. The PCR reactions were performed in 20 µL of solution containing 1x Phusion buffer, 0.2 mM dNTP mix, 0.3 µM each primer (VF1d and VR1d), 1.5 U Phusion™ High-Fidelity DNA Polymerase (Thermo Scientific, Waltham, MA, USA) and 10–20 ng of DNA template. PCR reactions were run using Mastercycler nexus GSX1 (Eppendorf, Hamburg, Germany) with thermal cycle conditions as follows: an initial denaturation at 95 °C for 2 min, 35 cycles of 95 °C for 20 s, 50–57 °C for 20 s and 72 °C for 1 min, and a final extension at 72 °C for 5 min. The PCR products were purified and sequenced in both directions by the Sanger sequencing method using the BigDye Terminator v.3.1 Cycle Sequencing Kit (1st BASE company, Selangor, Malaysia). The obtained COI sequences were submitted to GenBank (accession numbers MZ604119, MZ604120 and MZ604121). The COI sequences were trimmed and aligned with BioEdit software using the ClustalW algorithm [15]. The phylogenetic tree based on the COI sequences was built by MEGA 7.0 software using the neighbor-joining method [16] and the Kimura-2-parameter model [17]. The bootstrap values were obtained using 1000 replicates.

## 3. Results

Five species, four genera and three families were captured over the surveys: *Cynopterus brachyotis*, *Macroglossus minimus*, *Myotis hasselti*, *Myotis pilosus* and *Taphozous melanopogon* (Tables 1 and 2). Almost all adult females captured in January and March were either pregnant or lactating. *Cynopterus brachyotis* and *M. minimus* were only captured by mist nets while *M. hasselti*, *M. pilosus* and *T. melanopogon* were captured by the mobile V shaped net and mist nets. Morphological measurements of each species do not exhibit either sexual dimorphism or geographic variation (Table 2). The ear height (EH) of captured *Cynopterus* individuals from Ben Tre province is in the range of 15.6–16.2 mm which differs from that of either *C. horsfieldii* or *C. sphinx* and conforms to the morphological diagnoses of *C. brachyotis*. In Ben Tre province, *C. brachyotis*, *M. minimus* and *Myotis hasselti* were very common with high numbers of captured individuals at almost all netting sites (Figure 1 and Tables 1 and 2) while *M. pilosus* and *T. melanopogon* were only recorded and captured at three and two sites, respectively (Table 1). In Ha Long Bay, all three bat species (*M. hasselti*, *M. pilosus* and *T. melanopogon*) were commonly recorded and captured over the survey.

Three of the five species used echolocation calls for foraging and spatial orientation: *M. hasselti*, *M. pilosus* and *T. melanopogon*. Both *M. hasselti* and *M. pilosus* produced downward frequency-modulated search calls with the most energy in the 1st harmonic (Figure 2). The search call structure of these two species is typical for the genus *Myotis* with an initial steeper frequency-modulated (FM) sweep followed by a shallower sweep and ending with a steeper terminal part (Figure 2). *Taphozous melanopogon* produced shallowly modulated multi-harmonic search calls with most energy in the second harmonic, which is typical for the genus (Figure 2). The number of visible harmonics depended on the recording equipment and situations. The four sound parameters (iF, tF, BW and PD) were measured from the first harmonic of *M. hasselti* and *M. pilosus* and from the second harmonic of *Taphozous melanopogon* (Table 3).

While foraging, *M. hasselti* produced high energy search calls (Figure 3) which swept in about 5 ms from about 96 to about 24 kHz (exact value see Table 3). The approach to prey is characterized by a decrease in pulse interval and duration, a reduction of bandwidth and the emission of the typical terminal group (the buzz) consisting of buzz I and buzz II. The beginning of buzz II is indicated by a distinct lowering of the emission frequency, which is typical for *Myotis* species (Figure 4). Sometimes the foraging *M. hasselti* emitted search signals together with social signals (Figure 5). The frequencies of the social calls had an inverted horseshoe appearance which last about 15 ms and were emitted about 26 ms in front of a search call. Each social call thus comprised two frequency modulated components: beginning with downward and followed with an upward component. The iFM, lowest frequency, tFM and PD of the social calls are in a range of 43.7–65.8 kHz, 25.4–33.8 kHz, 41.4–44.6 kHz and 9.9–18.5 milliseconds, respectively.

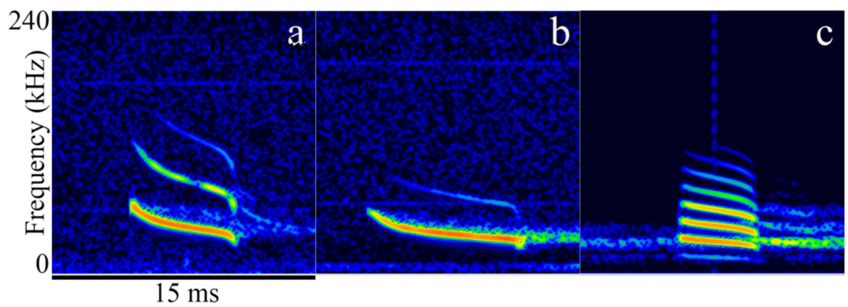


**Figure 2.** Search calls of *Myotis hasseltii* (**a**), *Myotis pilosus* (**b**) and *Taphozous melanopogon* (**c**) from the study sites.

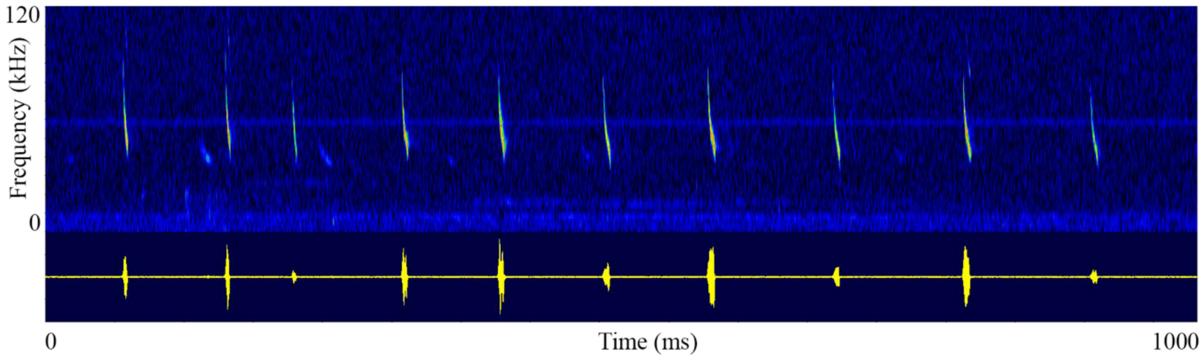


**Figure 3.** Sequence of search calls of *Myotis hasselti* from Ha Long Bay.

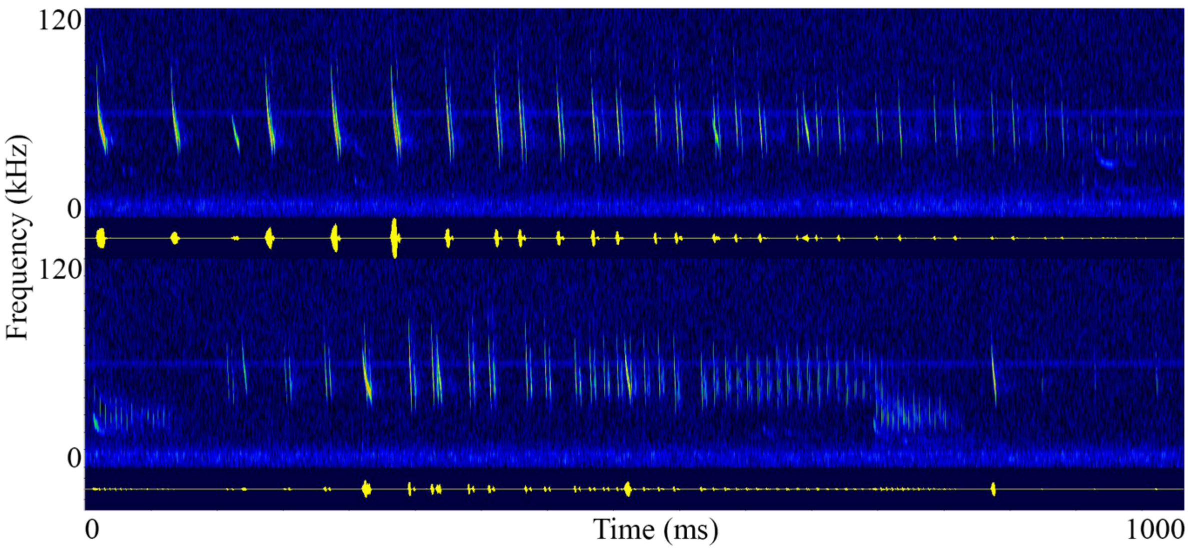


**Figure 4.** Echolocation calls of *Myotis hasselti* with two approach sequences including buzzes.

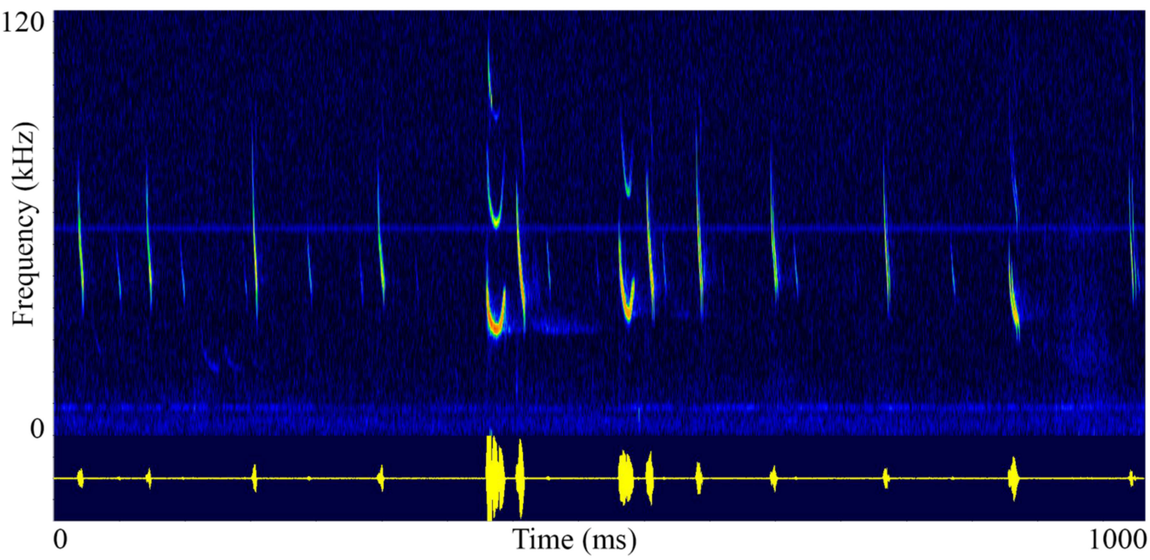


**Figure 5.** Two social calls of *Myotis hasselti* emitted in a fixed temporal pattern in front of a search call.

**Table 2.** Five external measurements (in mm) of bat species recorded from Ben Tre province. Abbreviations are defined in the Section 2. Sample sizes differing from those reported under n are given in parentheses. Data are presented as range (minimum and maximum), mean and standard deviation.

| Species | n | Sex | FA | EH | TIB | HF | Tail |
|---|---|---|---|---|---|---|---|
| *Cynopterus brachyotis* | 40 | ♀♀ | 65.8 ± 1.0<br>63.8–66.9 | 16.0 ± 0.2<br>15.8–16.2 (3) | 23.9 ± 0.3<br>23.6–24.1 (3) | 12.5 ± 0.2<br>12.3–12.6 (3) | 11.1 ± 0.5<br>10.6–11.5 (3) |
| | 25 | ♂♂ | 65.4 ± 1.0<br>63.5–66.8 | 15.7 ± 0.1<br>15.6–15.8 (3) | 25.1 ± 0.5<br>24.6–25.6 (3) | 13.0 ± 0.9<br>12.2–13.9 (3) | 13.3 ± 1.6<br>11.5–14.7 (3) |
| *Macroglossus minimus* | 35 | ♀♀ | 40.6 ± 1.0<br>39.1–44.5 | 16.3 (1) | 16.1 (1) | 10.3 (1) | 2.5 (1) |
| | 20 | ♂♂ | 40.3 ± 0.5<br>39.5–41.5 | 15.8; 16.2 (2) | 15.9; 16.4 (2) | 10.0; 10.5 (2) | 2.3; 2.5 (1) |
| *Taphozous melano-pogon* | 12 | ♀♀ | 65.0 ± 1.3<br>63.3–66.8 | 18.8 ± 0.8<br>17.5–19.8 | 24.7 ± 1.1<br>23.5–26.8 | 16.8 ± 0.8<br>15.7–18.0 | 27.8 ± 0.6<br>26.8–28.5 |
| | 4 | ♂♂ | 64.4 ± 0.9<br>63.8–65.8 | 19.1 ± 0.4<br>18.6–19.6 | 25.1 ± 0.8<br>24.3–25.8 | 17.1 ± 0.5<br>16.5–17.6 | 27.8 ± 1.5<br>26.5–29.9 |
| *Myotis hasseltii* | 38 | ♀♀ | 38.7 ± 0.4<br>37.5–39.8 | 13.9 ± 0.4<br>13.3–14.5 (8) | 16.3 ± 0.4<br>15.8–16.8 (8) | 9.6 ± 0.2<br>9.4–9.8 (8) | 39.8 ± 0.8<br>37.5–40.0 (8) |
| | 25 | ♂♂ | 38.0 ± 0.4<br>37.3–38.9 | 12.2 ± 0.5<br>11.5–12.6 (5) | 16.6 ± 0.4<br>15.9–16.9 (5) | 9.2 ± 0.4<br>8.8–9.6 (5) | 38.3 ± 0.5<br>37.5–38.7 (5) |
| *Myotis pilosus* | 9 | ♀♀ | 56.8 ± 0.7<br>55.6–57.9 | 16.6 ± 0.7<br>15.5–17.5 | 20.0 ± 0.9<br>18.6–21.5 | 16.3 ± 0.7<br>15.3–17.5 | 41.2 ± 3.1<br>38.6–46.8 |
| | 6 | ♂♂ | 55.7 ± 1.3<br>53.8–56.8 | 16.9 ± 0.4<br>16.3–17.5 | 19.1 ± 0.5<br>18.5–19.8 | 15.9 ± 0.6<br>15.3–16.8 | 42.3 ± 1.5<br>39.8–43.8 |

**Table 3.** Sound parameters of echolocating bat species from Ha Long Bay and Ben Tre Province. Abbreviations are defined in the Section 2. Sample sizes are the numbers of signals measured within 1000 selected milliseconds of each species. Data are presented as range (minimum and maximum), and mean and standard deviation.

| Species | n | iFM | tFM | BW | PD |
|---|---|---|---|---|---|
| *Myotis hasseltii* | 49<br>(first harmonic) | 76.4 ± 9.6<br>60.1–95.8 | 33.9 ± 2.4<br>24.9–38.0 | 42.3 ± 9.4<br>25.8–66.2 | 5.1 ± 1.1<br>2.6–6.6 |
| *Myotis pilosus* | 14<br>(first harmonic) | 62.1 ± 4.3<br>56.4–70.0 | 24.2 ± 1.7<br>21.6–26.8 | 37.9 ± 4.6<br>31.0–46.5 | 8.4 ± 0.5<br>7.6–9.3 |
| *Taphozous melanopogon* | 10<br>(second harmonic) | 32.5 ± 1.7<br>30.1–35.2 | 20.6 ± 0.6<br>19.7–21.6 | 11.9 ± 2.0<br>9.4–15.5 | 9.3 ± 2.4<br>4.8–12.2 |

## 4. Discussion

To date, the known bat fauna in Vietnam comprise three species of *Cynopterus* (*C. brachyotis*, *C. horsefieldii*, *C. sphinx*), two species of *Macroglossus* (*M. minimus* and *M. sorbrinus*), three species of *Taphozous* (*T. longimanus*, *T. melanopogon*, *T. theobaldi*) and at least 20 species of *Myotis*, including *M. hasselti* and *M. pilosus* [11]. Three species (*C. brachyotis*, *M. minimus* and *T. melanopogon*) are morphologically distinguishable from other species of their respective genus [11]. *Myotis pilosus* is morphologically distinguishable from other *Myotis* species in Vietnam by its long hindfoot with long and sharp claws [11,18]. The morphological identification of *M. hasselti* is sometimes uncertain because body size and many other external characteristics of this species are quite similar to those of *Myotis horsfieldii* and other species. Therefore, identification of *M. hasselti* from the study sites was based on both morphological and genetic features (Figure 6 and Table 2). Captured individuals of *M. hasselti* from Ha Long Bay and Ben Tre Province are identical in morphological features. With the bootstrap value of 100, three representative samples (IEBR-T.05012021.3, IEBR-T.06012021.1 and IEBR-T.06012021.2) from Ben Tre province were phylogenetically grouped into a clade, which supports morphological identification of *M. hasseltii* (Figure 5).

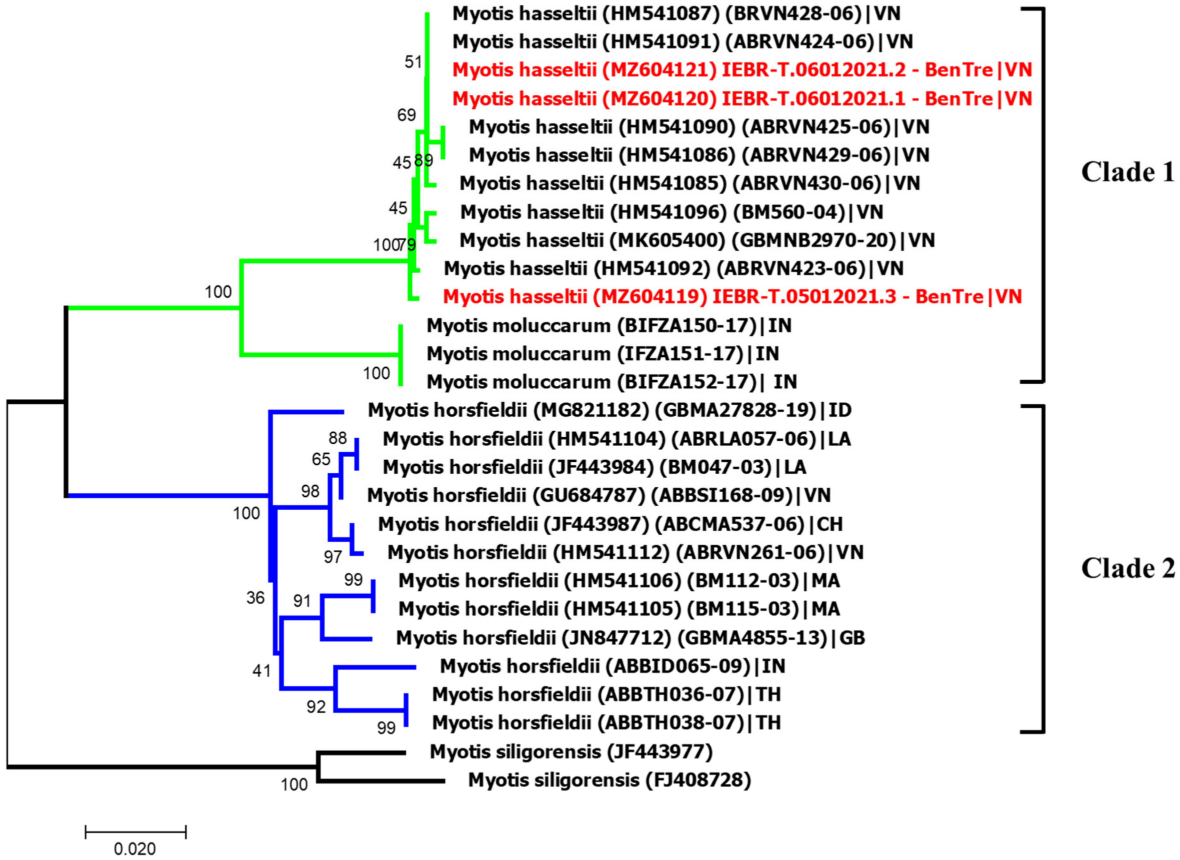


**Figure 6.** Phylogenetic analysis of *Myotis hasseltii* samples collected in Ben Tre. The number next to each branch indicates the bootstrap value with 1000 replicates. The scale bar indicates the number of nucleotide changes per site. Abbreviations: IN = Indonesia, ID = India, LA = Laos; MA = Malaysia, TH = Thailand, VN = Vietnam. GenBank accession numbers or BOLD IDs of DNA sequences are given in the blanket.

Morphologically, *C. brachyotis* differs from two other *Cynopterus* species by ear shape and size (Figure 7 and Table 2) [11,18]. The upper half of the posterior edge of the ear is almost straight in *C. brachyotis*, but clearly concave in either *C. horsfieldii* or *C. sphinx* (Figure 7). The ear height of all captured *Cynopterus* individuals from the study sites was in a range of 15.6–16.2 mm (Table 2) which was within the described range of *C. brachyotis* (13.0–20.0 mm) but distinctly shorter than the that of either *C. horsfieldii* (17.0–22.0 mm) or *C. sphinx* (18.0–24.0 mm) in previous publications [18,19]. Francis (2019) regarded *C. brachyotis* as a species complex, which contains at least one cryptic species in southeast Asia [19]. However, the taxonomy of this species complex is still unclear and requires further studies throughout its distributional range. Therefore, we here identified the captured individuals from Ben Tre province as *C. brachyotis*.

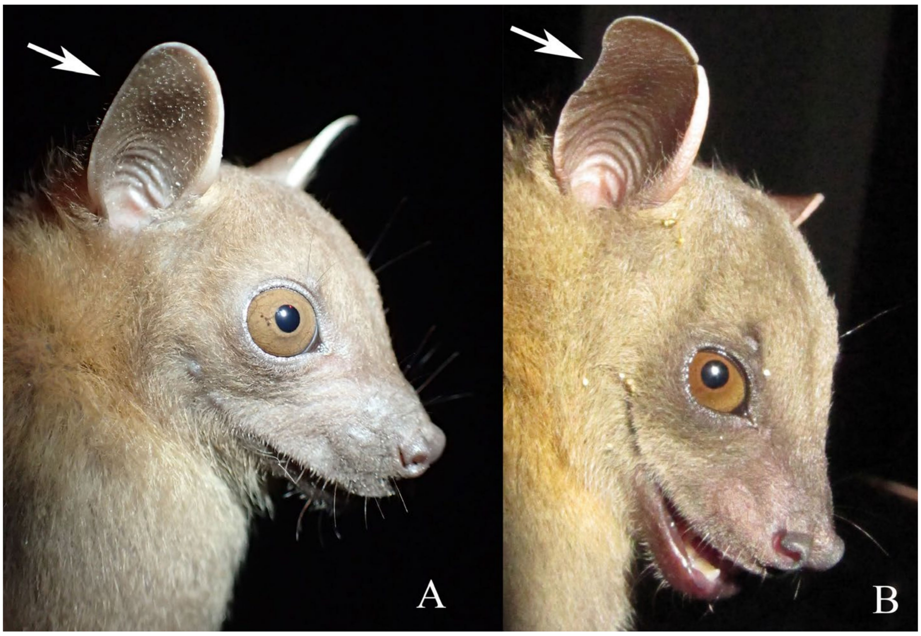


**Figure 7.** *Cynopterus brachyotis* (**A**), adult female (with straight posterior edge of the ear) from mangrove ecosystem in Ben Tre province and *C. sphinx* (**B**), adult female (with concave posterior edge of the ear) from the Cat Ba Biosphere Reserve. Photos: Vu Dinh Thong.

*Macroglossus minimus* is one of the two species of the genus *Macroglossus* worldwide: *M. minimus* and *M. sobrinus* [10,11,18,20]. *Macroglossus minimus* is distinguishable from *M. sobrinus* by its median groove on upper lip [10,11,19]. The captured bats from Ben Tre province showed this morphological diagnosis clearly (Figure 8). Their external measurements are also identical to previous descriptions (Table 2) [11,18,19].

**Figure 8.** *Macroglossus minimus*, adult female from a mangrove ecosystem in Ben Tre province, southern Vietnam. Photo: Vu Dinh Thong.

Among the three species of *Taphozous* in Vietnam, *T. melanopogon* is distinguishable from two other species, *T. longimanus*, and *T. theobaldi*, in morphology [11,18,19]. Of these, the wing membrane is attached at the ankle in *T. longimanus*, but at the tibia, above the ankle, in both *T. melanopogon* and *T. theobaldi* [19]. *Taphozous melanopogon* is distinctly smaller than *T. theobaldi* in body size [11,19]. Based on the forearm length of 63.3–66.8 mm and wing membrane attached at the tibia, we identified the captured bats from Ben Tre province as *T. melanopogon* (Figure 9).

**Figure 9.** *Taphozous melanopogon*, adult male from mangrove ecosystem in Ben Tre province, southern Vietnam. Photo: Vu Dinh Thong.

*Myotis pilosus* is distinguished from all other *Myotis* species in Vietnam and southeast Asia by its long hindfoot, which is equal to or larger than 80% of other tibia [11,19]. With a forearm length of 53.8–57.9 mm and a ratio of hindfeet/tibia larger than 80%, we identified the captured bats from Ben Tre province as *M. pilosus* (Figure 10). This species has been included in previous publications as *Myotis ricketti* [10,11]. However, *M. ricketti* is regarded as a synonym of *M. pilosus* [21]. *Myotis pilosus* has rarely been documented in previous publications. It is listed as globally "Vulnerable" in the current IUCN Red List of Threatened Species [21]. This species was also commonly recorded in the mangrove ecosystem at the Cat Ba Biosphere Reserve, northern Vietnam [22].

**Figure 10.** *Myotis pilosus*, adult male from the mangrove ecosystem in Ben Tre province, southern Vietnam. Photo: Vu Dinh Thong.

*Myotis hasseltii* is one of the 20 *Myotis* species distributed in Vietnam [11,18,19]. This species is quite similar to *Myotis horsfieldii* in morphology (Figure 11). These two species can be distinguished by wing membrane attachment at the ankle in *M. horsfieldii*, but at the side of the foot in *M. hasseltii* [19]. The captured bats from Ben Tre province exhibit this morphological diagnosis clearly (Figure 7). Genetic data also support morphological identification. Three COI sequences of *M. hasseltii* from Ben Tre province were aligned with 25 COI sequences of *M. hasseltii*, *M. moluccarum*, *M. horsfieldii* and *M. siligorensis* from the BOLD system and NCBI GenBank databases. The phylogenetic tree shows the high sequence similarity (>99%) in comparison with sequences of confirmed *M. hasseltii* in the reference databases (Figure 6). This species was regarded as a nationally rare species with few records over the past decades [11]. However, this species has been quite common in every studied mangrove area. It was also commonly recorded in the mangrove ecosystem at the Cat Ba Biosphere Reserve, northern Vietnam between 2015 and 2020 [22].

**Figure 11.** *Myotis hasseltii*, adult male from the mangrove ecosystem in Ben Tre province, southern Vietnam. Photo: Vu Dinh Thong.

Echolocation calls of *M. hasselti* have been poorly documented in previous publications. Wilson and Mittermeier (2019) described the calls as a steep FM sweep with the iFM, tFM and PD of 82.0–104.0 kHz, 23.0–30.0 kHz, and 2.5–5.5 ms, respectively [18]. We recorded hundreds of sound sequences containing entire signals of *M. hasselti* from both Ha Long Bay and Ben Tre province. However, the initial frequency of all the signals from three selected sound sequences and all remaining calls of *M. hasseltii* was lower than 96.0 kHz and the PD was up to 6.6 milliseconds (Table 3). To our knowledge, social calls of *M. hasselti* were not included in any publication. During the survey, we recorded the horseshoe-shaped social calls of this species. During the approach to prey *M. hasselti* changed the parameter of its echolocation signals. Therefore, it is important to use only search signals for species identification. Further investigations are needed to understand the role of this species in the highly endangered mangrove ecosystem.

Results from our surveys suggested the potential of mangroves for bat research and conservation. Annual weather in the Mekong Delta including Ben Tre province is characterized by two distinct seasons: rainy season from May to November and dry season from December to April. Our surveys were conducted in each district in both seasons. However, results from captures and observation did not exhibit any clear difference between the rainy and dry seasons. It is likely that the mangrove ecosystem in Ben Tre province or even in the whole Mekong Delta supplies adequate food and foraging habitats for bats during the whole year. Unfortunately, the total mangrove area in Ben Tre province has been critically decreased by the development of aquaculture, rice crops and other factors over recent decades, from 90 $km^2$ in 1998 to 42.7 $km^2$ in 2015 [1,5]. Remarkably, the remaining mangrove area of this province is still continuously decreasing [5]. In Quang Ninh province, including the Ha Long Bay, undated data on mangrove areas is inaccessible; however, it remarkably decreased between 2007 and 2014 (Figure 12). On the other hand, at least 11 wind power projects have been developed in the coastal zones of Ben Tre province. These projects may negatively impact bats, birds and other flying animals of this province and generally in the Mekong Delta [23,24]. Little is known about bat species foraging in nearshore and offshore regions in Vietnam. At least two species, *T. melanopogon* and *M. hasseltii*, were recorded at a recording station in a distance up to 4 km from the nearest seashore over our surveys in 2021. Although several studies have recorded bats at sea in other regions [25–27], no bat study was conducted in near-shore or off-shore environments in southeast Asia. Although results from studies on other regions should be interpreted with extreme caution, several predictions can be made regarding certain species of bats in Vietnam and in Ben Tre province. Bat species that can forage in the near-shore and off-shore environment in Ben Tre should be well-adapted to open space or have body structure sturdy enough to withstand oceanic winds. Among five species found in this study, *M. hasseltii*, *M. pilosus* and *T. melanopogon* possess these characteristics. Thus, the rapid developments of near-shore and off-shore wind farms in Ben Tre can potentially impact these species.

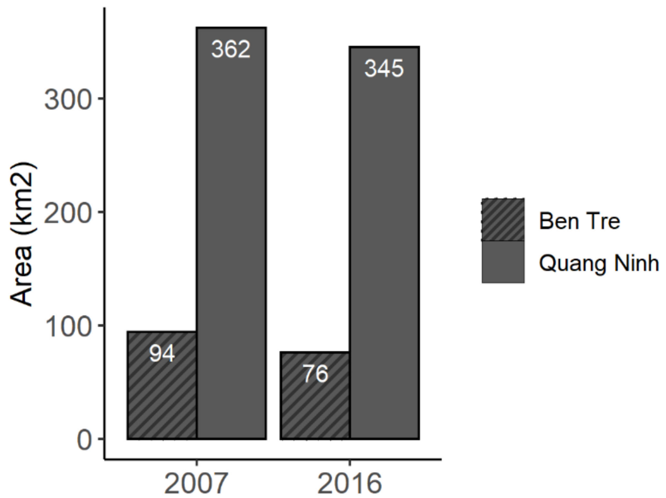


**Figure 12.** Mangrove area in Ha Long Bay and Ben Tre province decreased between 2007 and 2016. Data sources were extracted from https://www.eorc.jaxa.jp/ALOS/en/lulc/data/index.htm (accessed on 19 September 2021).

From a national view, the mangrove area of Vietnam declined dramatically from 408,500 ha in 1943 to 235,569 ha in 2019 [1]. The loss of mangrove areas is one of the critical threats to bat species inhabiting this ecosystem. To date, few areas of Vietnam have received attention from scientists for bat research. Results from this study in Ben Tre province and a recent one at the Cat Ba Biosphere Reserve indicate mangroves are an important ecosystem for bat research and conservation [22]. Globally, bats of the wetland areas, including mangrove, have been less well studied while this ecosystem area has "decreased by up to 33% over the past 10 years" [28]. Although mangrove areas have declined dramatically by human activities and climate change, bats in this ecosystem are still largely unknown. Extensive studies in mangroves are required for timely conservation of bats in the future.

During the surveys, echolocation calls were recorded using one PCTape system and Echo Meter Touch 2 PRO for transect recording, two SM4BAT FS for stational recording. Results from the recordings also indicated that all three echolocating bat species, *M. hasselti*, *M. pilosus* and *T. melanopogon*, commonly foraged in the mangrove. Echolocation calls of two uncaptured species forms were also recorded in the mangrove areas of Ben Tre province. One of the forms is similar to the call of *T. melanopogon* in signal structure but distinctly different in frequencies. The remaining form exhibits the signal structure of a

*Pipistrellus* species. These unidentified recordings indicate that bats inhabiting the mangrove ecosystem in Ben Tre province are more diverse than the captured species composition.

## 5. Conclusions

Mangroves in Ha Long Bay within Quang Ninh province and Ben Tre province provide roosting sites and foraging habitat for at least five bat species: *C. brachyotis*, *M. minimus*, *M. hasselti*, *M. pilosus* and *T. melanopogon*. Of these species, *M. pilosus* is a globally "Vulnerable" species. These species have been rarely recorded from other ecosystems but are very common in mangroves. Results from our surveys indicate that mangroves are an important ecosystem for future bat research and conservation.

**Author Contributions:** Conceptualization, V.D.T.; methodology, V.D.T., N.H.T. and V.L.; validation, V.D.T., N.V.S. and N.T.T.H.; formal analysis, V.D.T., A.D., H.-U.S., N.V.S. and N.T.T.H.; investigation, V.D.T. and V.L.; resources, V.D.T., N.H.T. and V.L.; data curation, V.D.T., L.Q.T., N.V.S. and N.T.T.H.; writing—original draft preparation, V.D.T., A.D., H.-U.S.; writing—review and editing, V.D.T., A.D., H.-U.S., V.L., N.V.S., N.T.T.H., N.H.T., N.K.L., L.Q.T., N.M.H. and N.T.L. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research is funded by the Vietnam Academy of Science and Technology under the grant number UQĐTCB.01/2021.

**Institutional Review Board Statement:** The study was approved by the Scientific Council of the Institute of Ecology and Biological Resources (protocol code 682/STTNSV approved on 15 September 2019).

**Data Availability Statement:** All data is available in the main text.

**Acknowledgments:** We thank the Vietnam Academy of Science and Technology under grant number UQĐTCB.01/2021; the following people and institutions for their various support: Paul A. Racey of the University of Exeter, UK; Tigga Kingston of the Texas Tech University, USA; Paul J.J. Bates of the Harrison Institute, UK; Christian Dietz; Marianne Carter, Stuart Paterson, Christina Imrich, Henry Rees, Leala Rosen, Kate Toiton and Sherilyn Bos of the Conservation Leadership Programme; Doctorates of the Institute of Ecology and Biological Resources, VAST; Managers and staff of Ha Long Bay and Ben Tre province; Truong Anh Tho of the Center for Biodiversity Conservation and Endangered Species.

**Conflicts of Interest:** The authors declare no conflict of interest.

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