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First Records of Bats (Mammalia: Chiroptera) from the World's Largest Cave in Vietnam

Vu Dinh Thong [1,2,*], Howard Limbert [3] and Debora Limbert [3]

[1] Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Road, Cau Giay District, Hanoi 11307, Vietnam
[2] Faculty of Ecology and Biological Resources, Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Road, Cau Giay District, Hanoi 11307, Vietnam
[3] Oxalis Adventure, Phong Nha Town, Bo Trach District 511860, Quang Binh Province, Vietnam; howard.limbert@oxalis.com.vn (H.L.); deb.limbert@oxalisadventure.com (D.L.)
* Correspondence: thongvudinh@gmail.com; Tel.: +84-(94)-5560108

Abstract: Located within the Phong Nha-Ke Bang National Park, Central Vietnam, Son Doong Cave is the world's largest cave and has become one of the most famous sites for caving tourists and scientists worldwide. It contains highly diverse landscapes and ecosystems with special value in many natural aspects including biodiversity. Bat species are usually regarded as key and fascinating dwellers in cave ecosystems. We recently conducted a bat survey in Son Doong Cave in May 2022 for an initial understanding of the bat species inhabiting this special cave. Bats were captured using mist nets. Echolocation calls were recorded and analyzed using the PCTape system and Batman and Selena software, respectively. Results from the survey confirmed that Son Doong Cave is a home for at least six echolocating bat species of five genera and four families: Hipposideridae (*Aselliscus stoliczkanus*, *Hipposideros scutinares*), Rhinolophidae (*Rhinolophus thomasi*), Molossidae (*Mops plicatus*) and Vespertilionidae (*Myotis pilosus* and *Myotis* cf. *muricola*). Of these five species, *H. scutinares* and *M. pilosus* are listed as globally "vulnerable" by the IUCN Red List of Threatened Species. Among the six captured and recorded species from the Son Doong Cave, each species is clearly distinguishable from the others in terms of morphological features, echolocation call structure and frequencies.

Keywords: bat; biodiversity; echolocation; Phong Nha-Ke Bang; Son Doong Cave

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1. Introduction

Son Doong Cave (17°27′25″ N 106°17′15″ E) is located in a remote tropical forest area of Phong Nha-Ke Bang National Park, Quang Binh Province, Central Vietnam. It was first found by a local man named Ho Khanh in 1990 and fully discovered in 2009 by Howard Limbert and other scientists from the British Vietnamese Caving Expedition (www.sondoongcave.org, accessed on 12 May 2022). It is also known as "Hang Sơn Đoòng" in Vietnamese language, meaning a cave on a mountain within the Đoòng tropical forest area. This name is derived from "Hang" (cave), "Sơn" (mountain) and "Đoòng" (local name of the forest area and a village) (Hồ Khanh, personal communication, accessed on 12 May 2022).

With an entire volume of more than 1,600,000 cubic meters (approximately 9000 meters long, 150 m wide and more than 200 m high), Son Doong Cave has been classified as the world's largest cave by the Guinness Book of Records in 2009 (www.guinnessworldrecords.com, accessed on 12 May 2022). It was also selected as one of the most beautiful places in the globe by BBC (British Broadcasting Corporation) news and has become one of the most eagerly anticipated destinations for caving tourists and scientists. It contains highly diverse and special ecosystems, including rivers, forests, flatland and others, which are home to interesting flora and fauna. However, because of the difficulty in travelling to a remote location within a primary forest area together with strict regulations by local authorities

to conserve the pristine ecosystems inside the cave, only a few scientists have entered the cave for scientific research. Although many animals and plants have been observed in the cave by caving scientists and tourists, data on the flora and fauna of Son Doong Cave are still poorly documented. To our knowledge, at least two new invertebrate animal species, a troglobiotic freshwater prawn (*Macrobrachium phongnhaense*) and a stygobiotic cyclopoid species (*Mesocyclops sondoongensis*), were discovered in Son Doong Cave [1,2]. Another 16 animal species and 158 plant species were also recorded from the cave [3,4]. In fact, many other animal species including bats, which inhabit the cave, have been observed by tourists and caving specialists but are still unstudied by biological scientists (www.oxalisadventure.com, accessed on 12 May 2022). In May 2022, we conducted a survey throughout the cave and obtained the first record of bats inhabiting this special ecosystem.

2. Materials and Methods

2.1. Bat Capture and Morphological Measurements

The survey was conducted throughout Son Doong Cave between 14 and 18 May 2022. The cave is approximately 9000 m long including two dolines that are unique and contain primary forest areas (www.sondoongcave.org, accessed on 12 May 2022). Bats were captured and handled following guidelines recommended by the American Society of Mammalogists [5,6]. Two mist nets of 6.0 m (height) × 6.0–12.0 m (length) with a mesh size of 16 × 16 mm were set up across the cave passages and rivers nearby doline 1 and doline 2 between 17:30 h and 21:30 h (Figure 1). The remaining places inside the cave are so large to make them unsuitable for setting either mist nets or any trap.

Figure 1. Mist netting at the site inside Son Doong Cave near doline 1.

A mobile "flap-trap", which was made of two fishing rods and a short mist net, was also employed to capture bats foraging around the netting sites inside the cave [7]. Each captured bat was removed carefully from the net and placed in a cotton bag. Selected external measurements were made using a digital caliper (Absolute Coolant Proof Series 500, Mitutoyo America Corporation, Aurora, IL, USA): 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 [8], Borissenko and Kruskop [7] and Kruskop [9]. Reproductive status and age were assessed following Racey [10] and Brunet-Rossinni and Wilkinson [11], respectively. Every captured bat was released after taking morphological measurements and photographs for identification.

2.2. Echolocation Recording and Analyses

A PCTape system (480 kHz, 16 bit) was set up next to a mist net to record echolocation calls when bats were foraging in their natural habitats with an emphasis on the individuals coming to the nets (Figure 2). Echolocation calls of each captured bat were also recorded when it was released after taking the photographs and measurements necessary for identification. 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. A sound sequence of 5000 ms with a good ratio between background noise and echolocation signals of each species was selected for sound analysis using Selena software. Signals were displayed as sonograms with a FFT (fast Fourier transformation) of 256, Hann-window and zero-padding. The PCTape system, Batman and Selena software are custom-made by the University of Tuebingen, Germany. Echolocation calls recorded in natural habitats were carefully compared with the calls of the captured bats when they were released for identification. All recorded calls were also compared with an unpublished database of the first author and data in selected publications [12–21].

Figure 2. Echolocation recording beside a mist netting site inside Son Doong Cave.

3. Results

Six bat species belonging to five genera and four families were captured during the surveys: Hipposideridae (*Aselliscus stoliczkanus*, *Hipposideros scutinares*), Rhinolophidae (*Rhinolophus thomasi*), Molossidae (*Mops plicatus*) and Vespertilionidae (*Myotis pilosus*, *M.* cf. *muricola*). Each of the six species was clearly distinguished from the others on the basis

of morphological features and measurements (Figures 3 and 4; Table 1). Four species (*H. scutinares*, *M. plicatus*, *M. pilosus* and *M.* cf. *muricola*) were captured and recorded at netting site 1 in between the entrance and doline 1, and two species (*A. stoliczkanus* and *R. thomasi*) were captured at the netting site 2 near doline 2 (Figure 5; Table 1). All captured individuals in the survey were adults. A colony up to approximately 200 individuals of *H. scutinares* was observed when the bats were flying through netting site 1 following the direction from the entrance to doline 1. The number of individuals belonging to *M. plicatus*, *M. pilosus*, and *M.* cf. *muricola* was not counted because they did not fly as a colony. In particular, it was almost impossible to count *M. pilosus* and *M.* cf. *muricola* when they were flying around to forage above the water surface and ground inside the cave. At netting site 2, a colony up to approximately 50 individuals of *R. thomasi* and a colony of approximately 26 individuals of *A. stoliczkanus* were observed and recorded. Both captured females of *A. stoliczkanus* were pregnant. Each of these females carried a large embryo that was very likely to be born around the last week of May or first week of June. The remaining captured bats were reproductively inactive.

Figure 3. Frontolateral view of CF bat species: *A. stoliczkanus* (**left**), *H. scutinares* (**center**) and *R. thomasi* (**right**). Photos: Vu Dinh Thong.

Figure 4. Frontolateral view of FM bat species: *M. pilosus* (**left**), *M.* cf. *muricola* (**center**) and *M. plicatus* (**right**). Photos: Vu Dinh Thong.

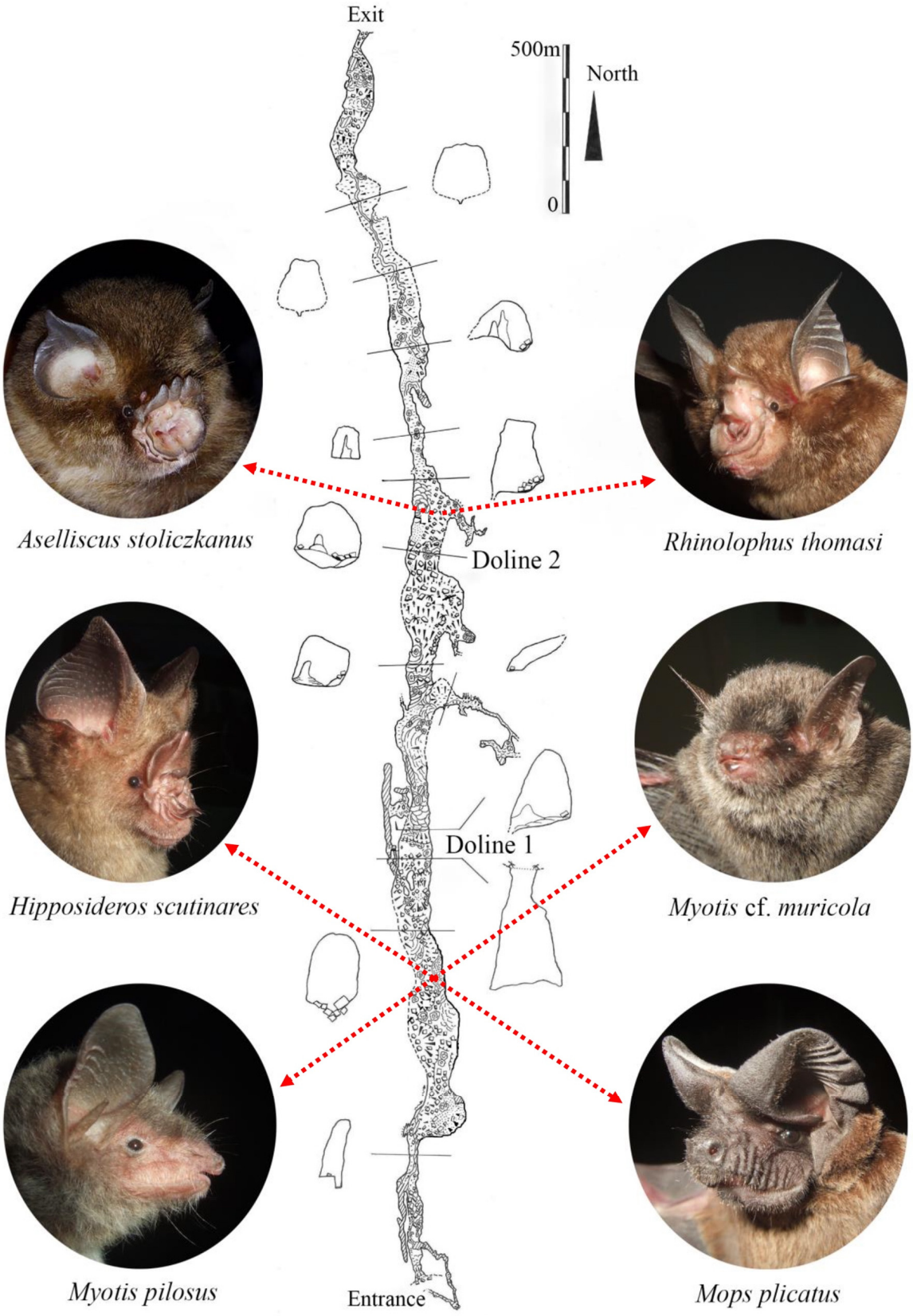

Figure 5. A map of Son Doong Cave with bat species captured at each netting site. Background map: Debora Limbert.

Every bat species captured at Son Doong Cave used multi-harmonic echolocation calls for foraging and spatial orientation. However, the number of visible harmonics depended on the recording equipment and situation. The sound parameters were measured from selected calls of bats flying inside the cave before coming to the mist nets (Figures 6 and 7; Table 2).

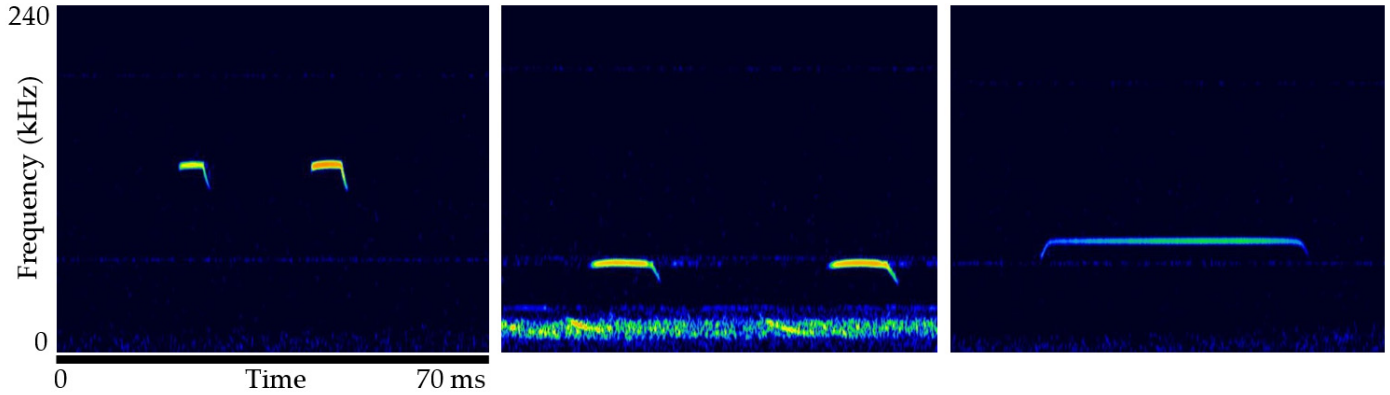

Figure 6. Echolocation calls of CF bat species: *A. stoliczkanus* (**left**), *H. scutinares* (**center**) and *R. thomasi* (**right**).

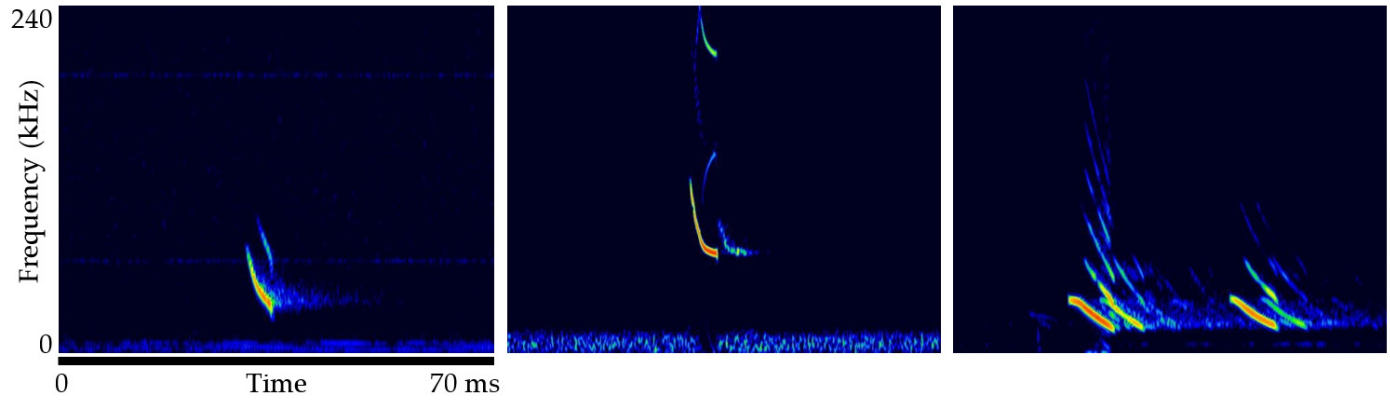

Figure 7. Echolocation calls of FM bat species: *M. pilosus* (**left**), *M.* cf. *muricola* (**center**) and *M. plicatus* (**right**).

Table 1. External measurements (in mm) of captured bats in Son Dong Cave. Abbreviations are defined in the Material and Methods.

Species	*n*	Sex	FA	EH	TIB	HF	T	Capture Site Zone
A. stoliczkanus	2	♀♀	43.4; 42.9	11.3; 9.2	19.5; 18.1	6.2; 6.3	38.8; 36.2	Doline 2
H. scutinares	1	♂	79.4	28.5	37.5	18.5	49.9	Doline 1
R. thomasi	2	♀♀	43.5; 46.9	16.5; 20.3	17.8; 18.2	8.6; 8.9	23.6; 26.8	Doline 2
	1	♂	46.6	20	17.5	8.9	26.6	Doline 2
M. pilosus	1	♀	56.8	17.9	19.8	16.9	46.8	Doline 1
	2	♂♂	53.5; 58.6	16.5; 18.2	18.8; 22.6	15.9; 16.8	46.2; 46.9	Doline 1
M. cf. *muricola*	2	♂♂	32.1; 32.3	10.0; 12.3	13.9; 14.5	7.1; 7.5	32.7; 33.6	Doline 1
M. plicatus	1	♂	51.5	22.8	18.6	13.5	41.6	Doline 1

Table 2. Sound parameters of echolocating bat species from Ha Long Bay and Ben Tre province. Sample sizes are the numbers of signals measured within 5000 selected millisecond recordings for each species. Data are presented as range (minimum and maximum), mean and standard deviation.

Species	*n*	iFM	CF	tFM	BW	PD
A. stoliczkanus	31 (second harmonic)	-	128.7 ± 1.0 125.4–130.1	113.6 ± 2.5 109.9–120.7	-	5.3 ± 0.8 3.9–6.6
H. scutinares	32 (second harmonic)	-	60.6 ± 0.9 59.2–62.9	45.1 ± 2.1 40.9–49.8	-	11.0 ± 1.5 7.0–13.5
R. thomasi	5 (second harmonic)	62.9 ± 2.3 59.2–65.3	76.9 ± 0.2 76.6–77.0	64.5 ± 2.1 62.9–68.1	-	49.3 ± 6.0 43.8–58.4
M. pilosus	16 (first harmonic)	69.3 ± 4.0 61.5–75.6	-	25.5 ± 1.7 23.0–28.6	43.7 ± 5.2 32.9–50.3	5.0 ± 0.3 4.6–5.6
M. cf. *muricola*	42 (first harmonic)	105.2 ± 10.2 87.8–127.7	-	63.3 ± 1.3 61.1–65.8	41.8 ± 10.5 25.4–65.8	4.2 ± 0.6 2.7–5.0
M. plicatus	47 (first harmonic)	38.4 ± 2.6 33.3–42.7	-	12.6 ± 2.1 8.5–16.9	25.8 ± 3.6 18.3–31.9	7.0 ± 3.4 3.4–17.1

Three species, *R. thomasi*, *A. stoliczkanus* and *H. scutinares*, use multi-harmonic echolocation calls with the most energy in the second harmonic. The call structure of *R. thomasi* was typical for species of the family Rhinolophidae with a shallow upward frequency modulation (iFM) followed by a CF component and ending with a shallow terminal frequency modulation (tFM). The call structure of *A. stoliczkanus* and *H. scutinares* was typical for species of the family Hipposideridae with a constant frequency (CF) followed by a steep terminal frequency modulation (tFM). Therefore, four sound parameters (iFM, CF, tFM and PD (pulse duration)) of *R. thomasi* and three sound parameters (CF, tFM and PD) of *A. stoliczkanus* and *H. scutinares* were measured from the second harmonic of each "CF bat" species. *Myotis pilosus*, *M.* cf. *muricola* and *M. plicatus* produced echolocation calls with a dominant downward frequency modulation (FM) component and the most energy in the first harmonic. Therefore, four sound parameters (iFM, tFM, BW and PD) of *M. plicatus*, *M. pilosus* and *M.* cf. *muricola* were measured from the first harmonic of each "FM bat" species. The beginning and the end of the signals were set at −30 dB below the maximal amplitude of the signal.

Myotis pilosus produced high energy search calls that swept in about five milliseconds (ms) from about 75 to about 23 kHz while foraging above rivers inside Son Doong Cave (Figure 7; Table 2). *Myotis* cf. *muricola* also foraged inside the cave and produced search calls of about 4 ms with frequencies ranging from about 127 to 61 kHz (Table 2). *Mops plicatus* produced its calls with a wide range of pulse duration, from about three to seventeen ms (Table 2). Among the six echolocating bat species in Son Doong Cave, *R. thomasi* used CF calls with the longest pulse duration, from about 43 to 58 ms (Table 2). Remarkably, each bat species inhabiting Son Doong Cave are clearly distinguishable from each other on the basis of echolocation call structure and frequency, which are important and helpful for either acoustic identification or monitoring (Figures 6 and 7; Table 2).

4. Discussion

Among the six species captured from Son Doong Cave during a recent survey, morphological features and measurements of the captured individuals of five species fit the assignment of the identified taxa (Figures 3 and 4; Table 1). Two species of *Aselliscus* are known in Vietnam, *A. dongbacanus* and *A. stoliczkanus* [9,13]. These two species are quite similar in body size and almost all morphological features. However, they are distinguishable on the basis of genetics with selected morphological and dental measurements [13,22]. Externally, the posterior nose leaf of *A. dongbacanus* is more fleshy than that of *A. stoliczkanus* [13] and the upper canine of *A. dongbacanus* is longer than that of *A. stoliczkanus* [13]. To date,

A. dongbacanus is only known in karst areas in northern Vietnam, whereas *A. stoliczkanus* is a widespread species with records from various areas and a wide range of ecosystems in northern and central Vietnam [9,13] as well as Phong Nha-Ke Bang National Park over the past decades [9,14–16,23]. Therefore, the characteristics of the posterior noseleaf and echolocation calls of the captured and observed individuals in Son Doong Cave can be assigned to *A. stoliczkanus*.

Vietnam has 19 *Myotis* species including *M. pilosus* and *M. muricola* [13]. *Myotis pilosus* is distinguishable from other *Myotis* species in Vietnam by its body size with a forearm length of over 50 mm and a hindfoot including claws that is approximately three-quarters of the tibia length [9,13,15,16,22]. The hindfoot with long claws and other morphological features of the three *Myotis* individuals captured at Son Dong Cave fit the identification of *M. pilosus*. Echolocation call structure and frequency of *M. pilosus* in Son Dong Cave are also similar to those of the species from other areas of Vietnam [13,15,16]. The external characteristics and measurements of two other *Myotis* individuals are almost identical to those of *Myotis muricola* (Figure 3; Table 1). Dorsal and ventral pelages of these individuals are dark grey and whitish, respectively. These bats and other individuals of the same taxon recorded inside Son Doong Cave use FM echolocation calls with an iFM and tFM of about 105 and 63 kHz, respectively. These sound parameters are higher than those of *Myotis muricola* described in previous publications [17,18]. Taxonomically, *Myotis muricola* has been regarded as a species complex and its taxonomic status requires extensive studies with data from morphology, acoustics and genetics. Therefore, the two small *Myotis* males captured at Son Doon Cave were provisionally identified as *M.* cf. *muricola*. Further study with expected genetic data for a more comprehensive comparison is required to confirm this species taxonomy in the future.

Hipposideros scutinares is a large leaf-nosed bat species belonging to the "*H. pratti*" group that is distinguishable from other species groups of the family Hipposideridae in Vietnam on the basis body size, noseleaf structure and echolocation parameters [9,12,13]. Among the three species (*H. lylei*, *H. scutinares* and *H. swinhoei*) of the "*H. pratti*" group in Vietnam, *H. lylei* and *H. scutinares* are distinguishable from *H. swinhoei* on the basis of a noseleaf structure with "three emarginations on the anterior leaf" [9,12,13,24]. *Hipposideros scutinares* is distinguished from *H. lylei* on the basis of a smaller "shield" and shallower medial emargination on the anterior noseleaf [9,13,24]. Records of this species in Phong Nha-Ke Bang National Park were also included in previous publications [8,9,12–14,23,24].

Rhinolophus thomasi is the only horseshoe bat species of the family Rhinolophidae captured and recorded in Son Doong Cave during the survey. This small horshoe bat species has been recorded in a wide range of habitats and landscapes in Vietnam [9,12,13]. Among the 19 horseshoe bat species in Vietnam, *R. thomasi* is distinguishable from almost all other species on the basis of body size and noseleaf structure. *R. thomasi* is most similar to *R. sinicus* in terms of noseleaf structure but can be distinguished from *R. sinicus* by a short and broad lancet [9,13]. Records of *R. thomasi* from Phong Nha-Ke Bang National Park were also included in previous publications [9,12–14,23].

To date, *M. plicatus* is the only species of the family Molossidae in Vietnam [9,13,19–21]. It is distinguishable from all other bat species of Vietnam on the basis of morphological characteristics, including a free tail with a thick and fleshy ear [9,13]. The nomenclature of this species is still under discussion. It was commonly known as either *Tadarida plicatus* or *Chaerephon plicatus* [8,9,22]. The single captured individual of a free-tailed bat from Son Doong Cave was identified as *Mops plicatus* following recent publications including records of this species in Vietnam (www.batnames.org, accessed on 18 May 2022) [13,19,20]. This individual is larger than other bats of *Mops plicatus* recorded in other areas of Vietnam [19–21] (Table 1).

It is very likely that many bat species forage within the dense forest areas and other habitats of the cave. Since 2013, the cave has been opened to the public and become one of the most attractive destinations for caving tourists and media agencies worldwide, including VTV (Vietnam National Television), Good Morning America and the BBC (British

Broadcasting Corporation). By April 2022, at least 15,200 people, including scientists, tourists, porters and others, from many different countries worldwide have visited Son Doong Cave.

In many localities, tourism development normally impacts negatively on natural environmental protection and biodiversity conservation. Having recognized the importance of conservation in the world's largest cave, Oxalis Adventure and local authorities have implemented a strict regulation system for visiting the cave. Every visitor has to follow a scientifically designed narrow route throughout the cave to avoid disturbing the natural environment (Figure 8). To date, all habitats and natural environments of Son Doong Cave are completely pristine and well conserved for the long-term and sustainable development of ecotourism. As a result, roosting sites and foraging habitats for bats within the cave are well conserved. In particular, Son Doong Cave contains highly diverse habitats and ecosystems, including rivers inside the cave and primary forest areas area at the two enormous dolines, which are home to at least 158 plant species (Figures 5 and 9) [4]. Six bat species were recorded over a short survey in one season. The cave is located in a locality in central Vietnam with four distinct seasons. Many sections and habitats of the cave have not been surveyed. Therefore, further inventories are needed to determine bat diversity in this special ecosystem.

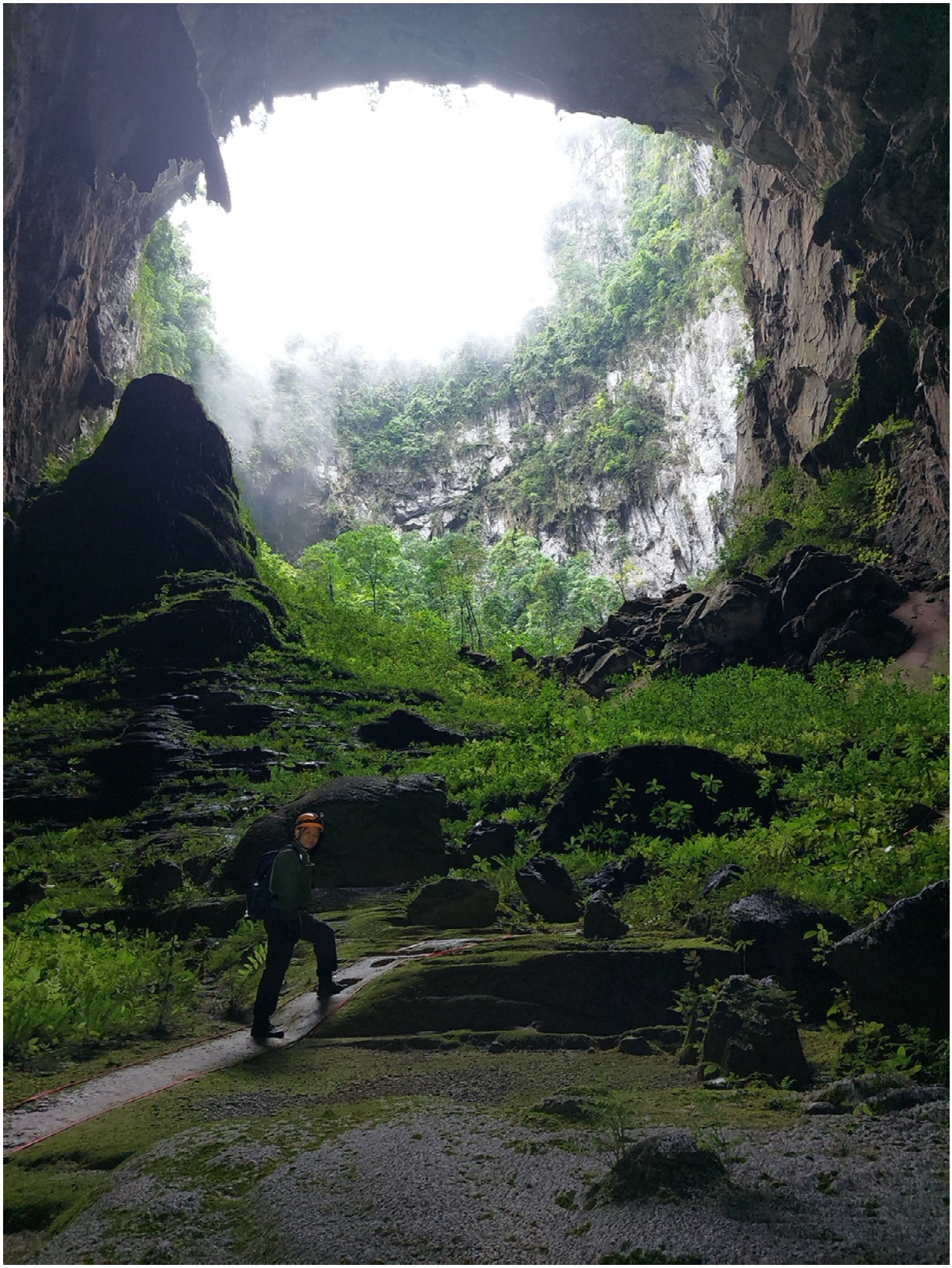

Figure 8. A scientifically designed narrow footpath inside Son Doong Cave must be followed by every visitor to avoid negative impact on the natural environment. Photo: Vu Dinh Thong.

Figure 9. Primary forest in doline 2 of Son Doong Cave. Photo: Vu Dinh Thong.

5. Conclusions

Son Doong Cave is a home to at least six echolocating bat species of five genera and four families, namely, Hipposideridae (*Aselliscus stoliczkanus*, *Hipposideros scutinares*), Rhinolophidae (*Rhinolophus thomasi*), Molossidae (*Mops plicatus*) and Vespertilionidae (*Myotis pilosus* and *Myotis* cf. *muricola*). Each bat species of Son Doong Cave is distinguishable from each other on the basis of morphological features, echolocation call structure and frequencies. Morphological characteristics of each captured species are almost similar to those of the respective species from other areas of Vietnam.

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