

First documented bat records on the Hon Tre Island, Nha Trang Bay, Vietnam

Nguyen Truong Son, Alexander P. Yuzefovich & Sergei V. Krusko*

ABSTRACT. During the 2020–2021 trips, the bat fauna of Hon Tre Island, located in Nha Trang Bay in Khanh Hoa Province, Vietnam, was studied for the first time. Despite the small size of the island and the lack of fresh water sources on it, we revealed the presence of at least nine bat species from four families, which were captured and studied in the hand. The family Pteropodidae is represented by *Cynopterus sphinx*, an extremely ecologically flexible species, and also by *Rousettus leschenaulti* and *Eonycteris spelaea*; family Hipposideridae — by three species of *Hipposideros*: *H. grandis*, *H. galeritus* and *H. gentilis*; family Rhinolophidae — by *Rhinolophus* cf. *pusillus* and *R. pearsonii*; family Megadermatidae — by *Megaderma spasma*. Mitochondrial DNA sequences were obtained for six individuals of three species, which made it possible to confirm morphology-based identifications of the captured animals. In particular, this concerned *R. pearsonii*, whose specimens differed from those known from the mainland, primarily by their smaller size. The presence of a multi-species bat community on Hon Tre Island necessitates careful planning of tourism and infrastructure development, in order to minimize impact on the natural habitats of the island.

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Первые документированные находки рукокрылых на острове Хон Че, залив Нячанг, Вьетнам

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РЕЗЮМЕ. В ходе поездок 2020–2021 гг. впервые исследована фауна рукокрылых острова Хон Че, расположенного в заливе Нячанг в провинции Кханьхоа, Вьетнам. Несмотря на небольшие размеры острова и дефицит на нем источников пресной воды, нами выявлено присутствие по крайней мере девяти видов рукокрылых (из четырех семейств), представители которых были отловлены и изучены в руках. Семейство Pteropodidae представлено *Cynopterus sphinx* — экологически очень пластичным видом, а также *Rousettus leschenaulti* и *Eonycteris spelaea*; семейство Hipposideridae — тремя видами из рода *Hipposideros*: *H. grandis*, *H. galeritus* и *H. gentilis*; семейство Rhinolophidae — двумя видами: *Rhinolophus* cf. *pusillus* и *R. pearsonii*; семейство Megadermatidae — одним видом, *Megaderma spasma*. Для шести особей трех видов получены последовательности митохондриальной ДНК, позволившие подтвердить видовую принадлежность отловленных животных. Это в частности касается *R. pearsonii*, экземпляры которого отличались от известных с материка в первую очередь меньшими размерами. Наличие достаточно разнообразной фауны создает необходимость тщательного планирования при развитии туризма и рекреационного строительства для снижения пресса на естественные местообитания острова.

КЛЮЧЕВЫЕ СЛОВА: Chiroptera, новые находки, зоогеография, прибрежные острова, провинция Кханьхоа.

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Introduction

The fauna of the islands is of particular interest. For many terrestrial animals, island habitats, even separated from the coast by a narrow strait, are at least partially isolated, what determines their unique evolutionary destiny (Patiño *et al.*, 2017). In this regard, island populations, even if they do not represent distinct taxa, may possess specific features; islands also may house animal assemblages uncommon for inland territories. Both of these tendencies are most pronounced on islands remote from the mainland (Millien, 2006; Hebert *et al.*, 2020), but are also noted on coastal islands, in particular, in relation to Vietnam (Abramov & Kruskop, 2012; Kruskop, 2015; Vu Tuy Duong *et al.*, 2021; Vu Dinh Thong *et al.*, 2021).

There is reason to believe that the coastal part of Vietnam is a relic of the Pleistocene Sundaland (e.g., Grismer *et al.*, 2021; Poyarkov *et al.*, 2021). In this context, coastal islands — with their at least partial isolation — are important for understanding the history of the formation of the regional fauna.

Over the past decade and a half, studies of the bat fauna have been carried out on many islands and archipelagos in Vietnam, partly confirming notions about the specifics of island communities and populations (Vu Dinh Thong & Furey, 2008; Vu Dinh Thong *et al.*, 2010, 2018, 2020, 2021; Kruskop, 2011; Abramov & Kruskop, 2012). However, Hon Tre Island, located near Nha Trang, apparently has never been studied for bats. This is likely due to its location next to a large city (Nha Trang) and resort status, which make the island relatively unappealing for prospective bat and other mammal surveys. Furthermore, the proximity of Hon Tre Island to the coast (2.1 km) may have led to an assumption that its mammalian fauna would not have any distinct features from the mainland. However, the natural conditions of the island are rather peculiar in the context of Vietnam (see below), and at least two endemic species of reptiles are known from here (Grismer & Ngo Van Tri, 2007; Ngo

Van Tri *et al.*, 2008), which indicates a certain degree of distinctiveness of the island ecosystem.

In 2020–2021, we conducted two short-term surveys of bats on Hon Tre Island, which revealed an unexpectedly diverse local fauna.

Material and methods

Study site

Hon Tre Island is located in Nha Trang Bay between N 12.2365°–12.18026°, and E 109.2306°–109.3357°, and administratively is a part of the city of Nha Trang. It has a length of 12 km from west to east and 5.67 km from north to south and is separated from the coast by a 2.1 km wide strait. Its westward portion facing the coast and the city is heavily urbanized and occupied by tourist complexes and an amusement park; however, the remainder two-thirds of its area has been relatively undeveloped. Much of these natural habitats is preserved and covered by a xerophilic shrubs and low (3–5 meters tall) maquis-like forest (M. Nuraliev, pers. comm.). During the dry season, freshwater sources here are mostly limited to small natural barrels in dry temporary brook beds. Artificial tree plantations are also present throughout the island, including fruit trees which may create an additional food base for fruit bats.

The first survey was conducted for six days between in September 24–29, 2020, as a part of the field work of the Institute of Ecology and Biological Resources. During this period, various habitats were surveyed along the coastline of Hon Tre Island, several habitats in the interior of the island, as well as on Hon Mot Island, located near its southern coast (Fig. 1; points 1–31). Movement between points on the coast and on the island of Hon Mot was carried out using a motor boat.

In 2021, bats were studied from May, 21 till June, 06, in the vicinity of the research station in Dombay Bay (N 12.1973°, E 109.2902°; Fig. 1, points 32–36) as part of the field work organized by the Joint Vietnamese-

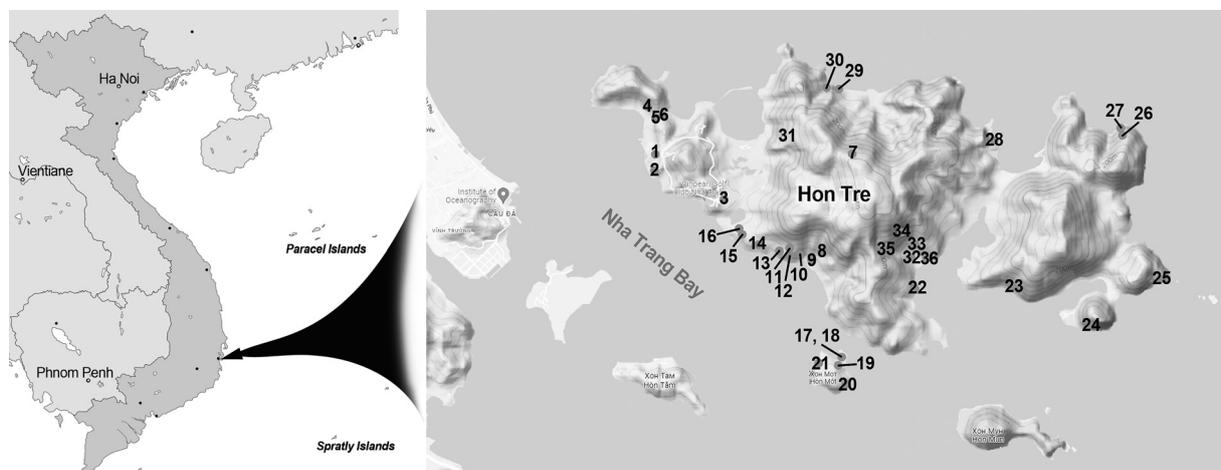


Fig. 1. Schematic map of the Hon Tre Island and localities covered by survey. Localities 1–31 were examined by NTS in September, 2020; localities 32–36 were examined by SVK in May–June, 2021.

Russian Tropical Research and Technological Centre. This latter area is among the least anthropogenically altered and least exposed to recreational pressure; therefore we posit that it could be considered representative of the natural communities of Hon Tre Island.

Survey methods

In September, 2020 bats were captured using monofilament mist nets (3×6, 3.5×9 and 3.5×12 m) and harp traps (1.8×1.8 m) set at ground level across trails, over small ponds and streams, or near edges of forest (Jones *et al.*, 1996). Mist nets were set for 2–12 hours nightly, whereas harp traps were left open all night. In total, the sampling effort during six nights counted 544.32 m² harp trap × h (m²hth; three traps per night) and 3.180 m² mist net × h (m²nh; 1–5 nets per night).

In 2021, in the vicinity of the Dombay Station, observations were carried out in the dark using electric LED headlamps and an ultrasound bat detector D-230 (Pettersson Elektronik AB). For bat trapping, monofilament mist nets 4×11 and 3.5×9 meters in size were used, put in places of the supposed flight paths of animals on fiberglass folding poles, simultaneously with a mobile trap with a 2.5×2.5 meter net, affixed to five-meter carbon fiber rods (Borissenko, 1999). The work itself (observations and trapping), as a rule, began from the time when the animals emerged (around 17:45–18:00) and continued until 22:00–24:00. Total sampling effort by mist nets was ca. 6852.5 m²nh.

Each captured bat was identified, examined to determine sex and reproductive condition; most of them were weighed, measured externally (see Table), and photographed. Standard external measurements were taken in the field: body mass (M), head and body length (L), tail length (C), hind foot length (without claws; Pl), ear length (A) and forearm length (FA). External parasites, most of which were parasitic flies of the families Streblidae and Nycterebiidae, were collected and fixed in 70% ethanol. Selected 1–4 specimens per bat species were euthanized and preserved as collection vouchers for further laboratory study (totally preserved in 70% ethanol); their tissue samples (pectoral muscle, liver or kidney) for DNA analysis were fixed separately in 95% ethanol. The remainder bats were released at the capture site following examination.

Laboratory analyses

Mitochondrial DNA was sequenced and analyzed for selected six specimens (individuals of *Rhinolophus pearsonii* and two species of *Hipposideros*) to clarify their identification and taxonomic relations. Total DNA was extracted from ethanol preserved muscle samples using standard protocol of proteinase K digestion, phenol-chloroform deproteinization and isopropanol precipitation (Sambrook *et al.*, 1989). The cytochrome *b* (*cytb*) gene (1140 bp) was amplified with primers previously used by us for *Hipposideros* species, following the protocol described in Yuzefovich *et al.* (2021). Partial cytochrome oxidase subunit I (*cox1*)

gene (657 bp) was sequenced using standard DNA barcoding protocols for mammals (Ivanova *et al.*, 2012). Preliminary genetic identification was conducted with the use of BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>), and then the Maximum Likelihood phylogenetic trees (ML trees) were obtained with the sequences of appropriate species. Sequences for comparison were taken from GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>; for *cytb* gene) and from the public projects of the Barcode of Life Data Systems (BOLD, <https://www.boldsystems.org/>; for *cox1*).

Results

The dry, rugged crooked forest covering the slopes, and the extremely small number of fresh water sources (of which many are unstable) allowed us to tentatively assume that the local bat diversity and density will be quite low. However, the observed density and activity of bats were relatively high. We have recorded presence of nine bat species from four families. Full list of captured species and number of captured specimens are provided in Table.

Cynopterus sphinx (Vahl, 1797)

This extremely ecologically flexible species was the most numerous bat we captured on Hon Tre Island. A total of 22 individuals were captured and examined in the hands. The animals were caught both in a dry riverbed at different distances from the coast, on a trail in the crooked forest and near the station buildings. It is noteworthy that we did not see any fruit bats near mango trees (despite the presence of ripe fruits) and did not find mango fruits eaten by fruit bats. Probably, they feed on some naturally growing fruit-bearing trees (most likely — wild fig trees). More than half of the captured animals of both sexes were juveniles that had not yet reached adult size. Meantime four captured adult females were lactating or in post-lactation. This suggests at least two (possibly more) breeding peaks in this species.

Rousettus leschenaulti (Desmarest, 1820)

Three males captured into mist net around 19:57 on September, 25, and examined in hand. All the animals were of an adult size. Animals were captured above the stream surrounded by secondary forest and adjacent to the fruit tree planting area such as mango fruits. During the survey we also observed a local net with one dead individual of the same species.

Eonycteris spelaea (Dobson, 1871)

Two dead individuals (one adult female and one immature) were found in a local net on September, 26 and 28. An adult animal was captured over a small stream surrounded by secondary liana forest. The immature animal was captured over a local trail surrounded by secondary sparse forest on a small Hon Mot Island, separated from Hon Tre Island by about 300 m of water (Fig. 1).

Rhinolophus cf. pusillus Temminck, 1834

Seven individuals (six males and a female) of small horseshoe bats from the «*pusillus*» species group were

Table. List of bat species captured on the Hon Tre Island, with number of specimens examined in hands (number of measured specimens in parentheses), and limits (min–max) of external measurements for the measured specimens (body mass in grams, other measurements in mm).

Species	Number	Capture sites	M	L	C	PI	A	FA
<i>Cynopterus sphinx</i>	22 (11)	1, 16, 32, 33, 35, 36	38–50	87–100	10.0–17.0	11.0–14.3	19.2–23.7	67.7–72.4
<i>Rousettus leschenaulti</i>	3 (2)	1, 31	75–93	112–117	12–13	15.9–20.2	17.5–21.9	75.2–77.6
<i>Eonycteris spelaea</i>	2	17, 31	34–54	80–100	12–15	18–21	14–16	60–70
<i>Hipposideros cf. grandis</i>	3 (2)	29, 36	18.5–24.0	61–76	34–35	10.0–10.8	20.0–20.5	62.0–63.0
<i>Hipposideros galeritus</i>	3	29, 35	6.1–6.4	50.5–55.0	40.0–40.7	5.4–6.2	12.0–14.0	45.9–47.8
<i>Hipposideros gentilis</i>	4	30, 32, 35	5.8–6.4	43.7–48.0	30.0–32.5	5.6–6.6	22.2–23.6	40.7–43.0
<i>Megaderma spasma</i>	6 (5)	32–36	17.6–20.6	70.5–74.5	0	14.3–15.2	38.4–40.6	53.2–57.6
<i>Rhinolophus pearsonii</i>	2	32, 35	10.0–10.5	59–59	18–22	8.9–9.4	23.8–24.7	45.4–46.7
<i>Rhinolophus cf. pusillus</i>	7	2, 32–36	4.2–5.4	36.7–47.0	16.5–24.0	6.0–6.8	15.5–18.2	36.9–38.8

captured. The animals were caught near buildings, among plantations of trees (mainly mango trees 4–6 meters tall), on a forest path and above a dry brook bed. Current knowledge on taxonomy of the «*pusillus*» species group, including Vietnam, is inconclusive (Li *et al.*, 2006; Francis *et al.*, 2010; Soisook *et al.*, 2016). Within *R. pusillus*, the existence of up to six «putative species» is assumed (Chornelia *et al.*, 2022); their relationships with previously described taxa of small horseshoe bats require further revision. External measurements of the specimens from Hon Tre Island (Table) mainly fall within the overlap between *R. pusillus* and *R. lepidus* (Kruskop, 2013). The only exception is female captured on September, 2020, with forearm 36.9 mm, which, however, only slightly exceeds the lower size limit of *R. lepidus*. The length of the upper tooth row in four measured specimens is 6.14–6.48 mm, which is somewhat better consistent with *R. lepidus*, but also lies near the overlap area. Therefore, until more thorough research is carried out, we refrain from final identification of the small horseshoe bat from Hon Tre Island.

Rhinolophus pearsonii Horsfield, 1851

Two adult females of relatively horseshoe bats large (forearm length 45.4–46.7 mm) were caught above a dry riverbed (Fig. 2). According to the size and noseleaf structure, they were preliminarily identified in the field as *R. cf. affinis*; however, they differed from *R. affinis* from other regions of Vietnam in their smaller size and tone of fur color. *Cytb* sequences (GenBank Nos. OP320923–OP320924) showed 96% similarity to *R. pearsonii* (Fig. 3). However, the sizes of specimens from Hon Tre Island are significantly smaller (forearm length of *R. pearsonii* in central Vietnam 47.8–53.2 mm, mean 50.7±1.56; $n=17$). The texture of fur is also slightly different from previously examined mainland specimens. On the obtained ML tree, Hon Tre specimens form a distinct haplogroup. Unfortunately, most of the available sequences taken for comparison originate from different parts of China; therefore, were unable to compare with *cytb* data for geographically proximal *R. pearsonii*. This species is reported to exhibit pronounced genetic



Fig. 2. *Rhinolophus pearsonii*, adult female. Hon Tre Island, May, 2021. Photo by S.V. Kruskop.

variation (Francis *et al.*, 2010; Chornelia *et al.*, 2022), and the identification of related haplogroups may be the key to understanding the origin of the fauna of Hon Tre Island. The frequency of the main CF component of echolocation calls in captured animals was about 61 kHz, which corresponds to the upper threshold of the norm for populations of this species from northern Vietnam and southern China (Zhang *et al.*, 2009; Vu Dinh Thong *et al.*, 2021).

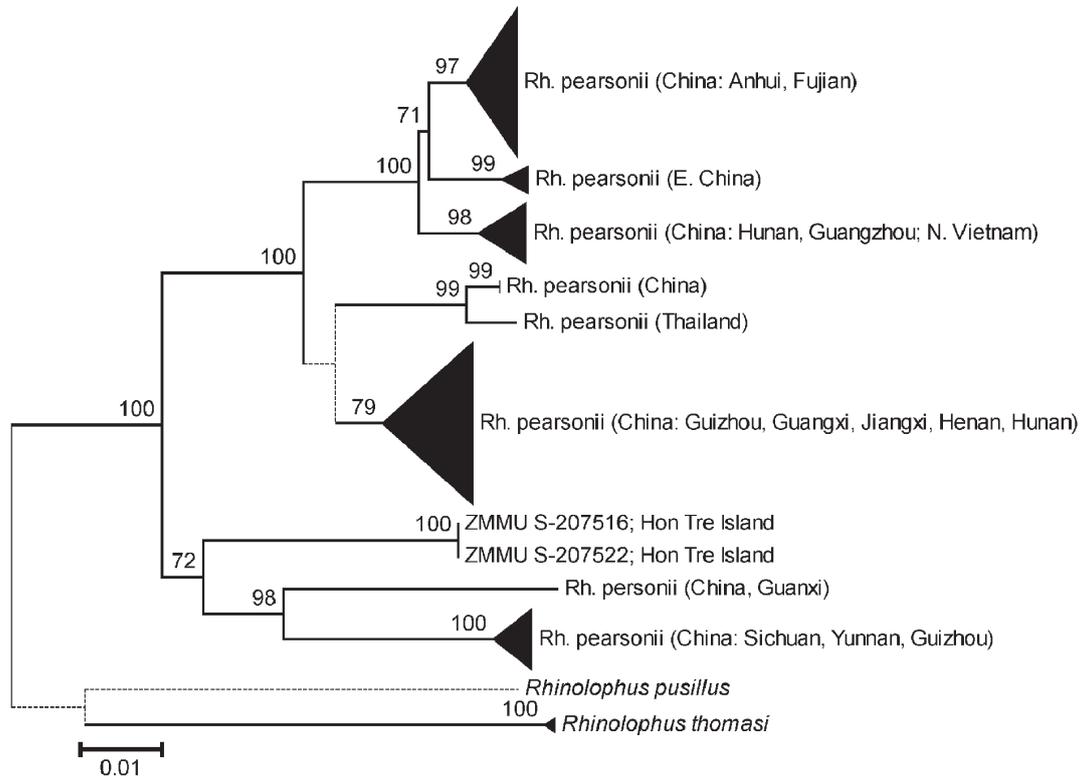


Fig. 3. Relationships of the Hon Tre *Rhinolophus pearsonii* based on sequences of the *cytb* gene (Maximum Likelihood method). Values of bootstrap support (500 iterations) are shown at nodes; only supports over 70% are shown.

Hipposideros grandis Allen, 1936

The three specimens (two males and one female) were captured in a mango plantation on September, 26, and May, 22. The two measured animals (male and female) have forearm length 62–63 mm, which is slightly beyond the upper size limit for individuals of this species from the lowland forests of South Vietnam (56.5–61.4 mm: Kruskop, 2013). According to the sequence of the cytochrome *c* oxidase (*COI*) gene (GenBank No. OP004325), the analyzed specimen closely matches with haplotypes common in the Central Highlands of Vietnam and Thailand (Yuzefovich *et al.*, in press). This indicates a possible phylogeographic connection of Hon Tre Island with the highlands of central Indochina, and not with the south of Vietnam, which, however, requires verification using similar data on other species. Despite the very few individuals of this species captured on the Island, the presence of wingless flies from the genus *Ascodipteron* on the animal caught in 2021 indicates a colonial rather than a solitary lifestyle (e.g. Hastriter, 2007).

Hipposideros gentilis K. Andersen, 1918

One female was captured in September 2020 and three adult males were caught in May, 2021. In 2021, animals were mist-netted over a dry riverbed nearby the Dombay Station (Fig. 4). In the narrowing of the bed with almost closing tree canopies, a high activity of bats was once observed, apparently with a predominance of this species; but in the following days this phenomenon did not recur. *Hipposideros gentilis* has a pronounced phylo-



Fig. 4. *Hipposideros gentilis*, adult male. Hon Tre Island, May, 2021. Photo by S.V. Kruskop.

genetic structure with deeply divergent genetic lineages (Yuzefovich *et al.*, 2021). A genetic analysis of the Hon Tre samples (GenBank Nos. OP019035–OP019037) show that they clearly belong to the «southern» mitochondrial lineage (*sensu* Yuzefovich *et al.*, 2021; together with animals from e.g. Dong Nai Province), not forming any special haplogroup. Our finds represent the first confirmed species records for the Khanh Hoa Province.

Hipposideros galeritus Cantor, 1846

One male was captured in 2020, and two adult males were captured in 2021. Animals were netted above a dry riverbed, surrounded by xerophilous maquis-like forest.

Megaderma spasma (Linnaeus, 1758)

Malaysian false vampires were the most common bats in direct proximity of the buildings and inside buildings of the scientific station. A small group of 3–6 individuals lived in the attic of the main residential building, and every night the animals flew out through the ceiling hatch and passed along the covered gallery of the building. The animals were also caught in various parts of the dry riverbed. In total, seven individuals were examined in the hand, including two adult females, which were lactating or recently postlactating. On May, 23, in the secondary growth thickets near the buildings and a water-purifying station, a juvenile *M. spasma* was found in a mist net, apparently dropped by its mother. The female flew around, attracted to the cries of the pup. Eventually, the female, hanging on a shrub branch, allowed the juvenile to be transplanted onto her. Thus, in this place, the second half of May is the birthing season for these bats. Remains of the large cicada (Cicadidae) were once dropped by an adult male, captured into a mist net in the riverbed.

Conclusion

In the course of our studies on Hon Tre Island, we revealed a diversity of bats comparable to that, for example, on the Con Dao Archipelago (9 and 8 species, respectively), which is noticeably larger both in area and the diversity of habitats (Vu Dinh Thong *et al.*, 2010; Kruskop, 2011). Comparable data were obtained for another group of coastal islands, Cu Lao Cham (9 species: Vu Dinh Thong *et al.*, 2018). At the same time, inland areas, which are not isolated from other forests and have a greater diversity of landscapes, are characterized by a much higher diversity of bats (e.g. more than 45 species in the Cat Tien National Park: Vu Dinh Thong, 2015). We did not meet on the island representatives of Emballonuridae, like *Taphozous melanopogon*, though this bat is quiet characteristic for coastal habitats (Vu Dinh Thong *et al.*, 2018, 2022). We also did not capture Vespertilionidae, like *Pipistrellus* or *Scotophyllus*, which undoubtedly live in the Nha Trang itself, and for which theoretically a two-kilometer strait does not represent a significant obstacle. Probably these species, demanding of water sources, can appear on the island in other seasons and leave when prolonged dry weather sets in. During our survey, FM echolocation calls of some undetermined vespertilionid bat (probably, *Pipistrellus*) were registered

on September, 27 in site 30, and on May, 21, in site 36, testifying at least facultative occurrence of this bat family on Hon Tre Island.

It is noteworthy that we did not observe the expected higher concentration of bats near the research station (where they could be attracted by artificial shelters in buildings and water sources in reservoirs). The only species that has been observed to be very active near the buildings, and which undoubtedly used the buildings as roosts was *Megaderma spasma*. However, in natural habitats (on a dry bed and on a forest trail), we also regularly observed false vampires, which, apparently, are generally quite abundant on the island.

In general, Hon Tre Island, despite being located close to the coast, has an ecosystem, which contains xerophilous plant communities uncommon for other parts of Vietnam (which are commonly much more humid: e.g. Averyanov *et al.*, 2003), and a relatively rich fauna and flora, including an unexpectedly speciose bat community. Being formally within the boundaries of a large city and being a known tourist site, the Island experiences high anthropogenic pressure, and a significant part of it is covered with tourist infrastructure. When using the existing structure and, moreover, expanding it with new constructions, thoughtful planning is necessary to preserve the natural ecosystem of the Island, including the habitats of the bats occurring there. We can recommend search for chiropteran day roosts (in natural hollows or in old buildings) and, if found, monitoring and protection of these sites, as well as all-round protection of fresh water sources, even seasonal ones.

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