

New distribution record of *Hipposideros grandis* G.M. Allen, 1936, Grand Leaf-nosed Bat (Chiroptera, Hipposideridae), from Bangladesh based on morphometric and molecular evidence

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Abstract. We confirm the presence of *Hipposideros grandis* G.M. Allen, 1936 from Baraiyadhala National Park, Bangladesh. The identification was based on morphometric attributes of two captured individuals and sequencing of the mtDNA cytochrome oxidase subunit I (COI) gene. The roosting habitat was chiefly degraded mixed evergreen forests characterized by steep and undulating terrain with many waterfalls and hilly streams. Our new record in Bangladesh extends the westernmost known range of *H. grandis*. The ecology of this bat species should be studied, as very little is known about it.

Key words. Baraiyadhala National Park, cave bat, cytochrome oxidase subunit I gene, Data Deficient species, status and distribution, taxonomy

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INTRODUCTION

No comprehensive studies have been conducted on the status and distribution of chiropteran fauna of Bangladesh. According to a report by the International Union for Conservation of Nature (IUCN) Bangladesh, 35 bat species have been identified, with 18 of these species classified as Data Deficient (IUCN Bangladesh 2015). Since that assessment, eight additional bat species have been added to the national inventory: *Hipposideros lankadiva* (Kellart, 1850), *Hipposideros Pomona* (K. Andersen, 1918), *Rhinolophus pusillus* (Temminck, 1834), *Pipistrellus javanicus* (Gray, 1838), *Macroglossus sobrinus* (K. Andersen, 1911), *Coelops frithii* (Blyth, 1848), *Rhinolophus luctus* (Temminck, 1834), and *Hipposideros armiger* (Hodgson, 1835) (Saha et al. 2015, 2017a, 2017b, 2017c, 2021; Mia et al. 2019; Ahmed et al. 2020; Aziz et al. 2024).

Recently UI Hasan and Kingston (2022) systematically reviewed the diversity and distribution of bats in Bangladesh. They confirmed 31 species of bat and indicated that additional 43 species (38 of which highly probable) are expected to occur in Bangladesh due to their confirmed distribution in neighbouring countries.

Among the recorded species in Bangladesh, the family Hipposideridae constitutes six species: *Hipposideros pomona*, *Hipposideros larvatus* (Horsfield, 1823), *Hipposideros lankadiva*, *Hipposideros cineraceus* (Blyth, 1853), *Coelops frithii*, and *Hipposideros armiger* (IUCN Bangladesh 2015). *Hipposideros grandis* has been so far recorded from China, Myanmar, Thailand, and Vietnam (Bates et al. 2016). *Hipposideros grandis*, Grand Leaf-nosed Bat, has been regarded as a distinct species since 2006 (Thabah et al. 2006), but has since been considered to be conspecific with *larvatus* group (Yuzefovich et al. 2022). The taxonomic status of this species requires further clarification to distinguish it from other taxa in the “*larvatus* species complex”, as well as to define its distributional range (Yuzefovich et al. 2022; Bates et al. 2016). Recently *H. grandis* has been recorded from Bandarban Sadar Upazila of Bangladesh (Aziz et al. 2024). In the present study, we extend the westernmost distribution of this species from Baraiyadhala National Park of Bangladesh.

METHODS

As a part of the wildlife diversity survey in Baraiyadhala National Park (Figure 1), we conducted mist netting in various habitats including near waterfalls and streams, in valleys, on steep hills, in natural forests, in bushy and bamboo thickets, in caves and in a hilly cleft, and near abandoned human structures. The first individual (JUMB0004) was captured from a hill cleft at the evening time on 16 August 2022. Standard protocol was



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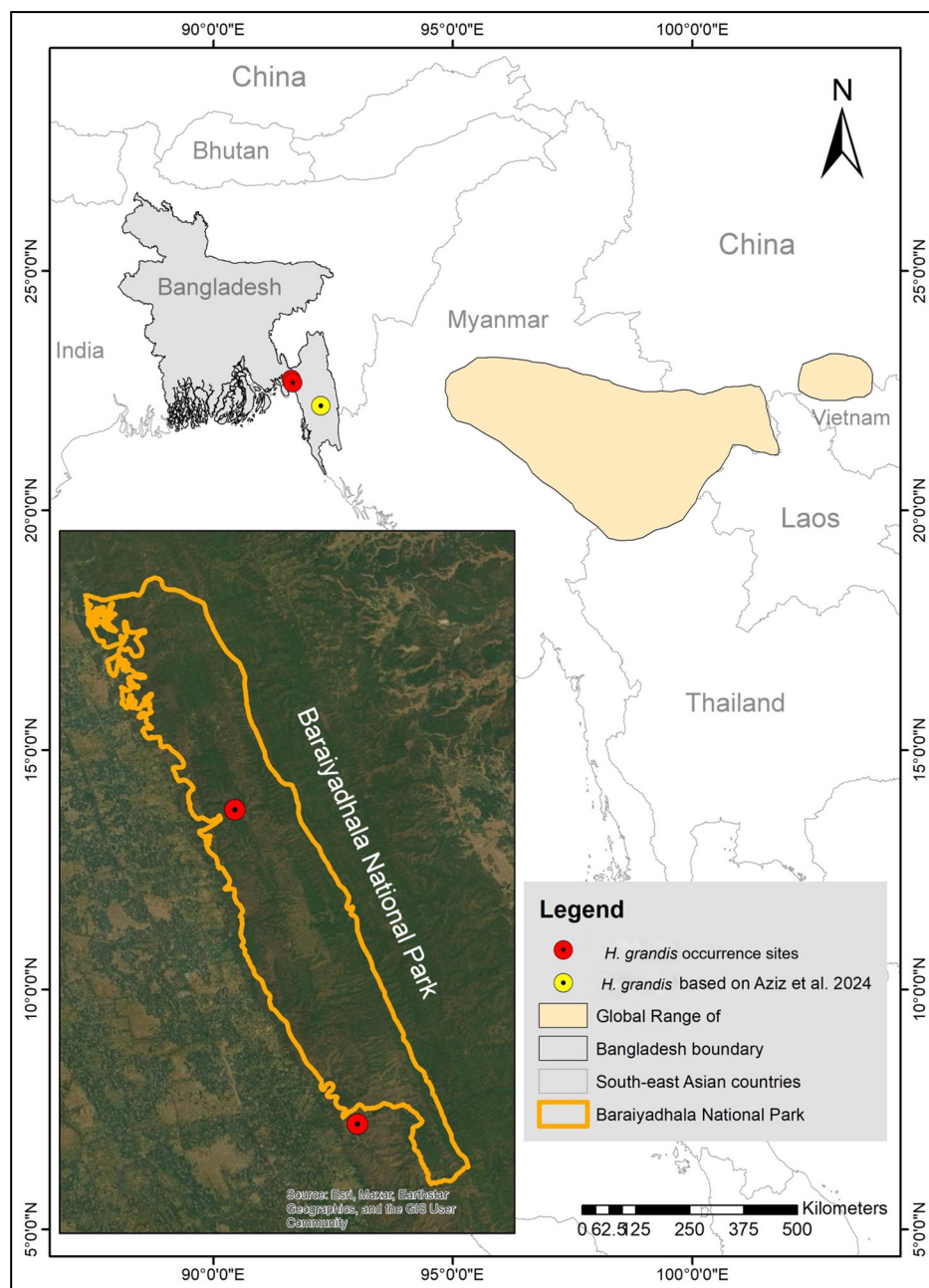


Figure 1. Location of *Hipposideros grandis* sites in Baraiyadhala National Park, Bangladesh and global range of this species according to Bates et al. (2016) and Aziz et al. 2024.

used in mist netting and handling bats (Kunz and Kurta 1988), and the morphological measurements were taken with digital slide calipers with an accuracy of 0.01 mm. The specimen was initially identified as *Hipposideros* sp. Additionally, we found an old abandon temple with a roost of about 300 *Hipposideros* individuals. The second individual (JUMB0005) was captured from the temple with a hand net on 3 June 2023. Morphometric measurements were taken from the live specimen (Figure 2) following Bates and Harrison (1997). The specimens were euthanized and tissue samples were collected for molecular identification. The voucher specimens (JUMB0004 and JUMB0005) were preserved at the Wildlife Museum, Department of Zoology, Jahangirnagar University under the permit (FD/SUFAL/SIG/17/2020/53).

Genomic DNA was extracted from arm muscle tissue using the Wizard Genomic® DNA Purification Kit (Promega, Madison, WI, USA). PCR amplification of 525 bp of the mitochondrial cytochrome c oxidase subunit I (COI) gene was performed using the primers VF1d 5'-TTCTCAACCAACCACAARGAYATYGG-3' and VR1d 5'-TAGACTTCTGGGTGGCCRAARAAYCA-3' (Ivanova et al. 2007). PCR amplification was performed in 25 µl of G2 Green PCR Master Mix (Promega, USA) in a thermal cycle. PCR conditions were as follows: initial denaturation of 1 min at 94 °C, followed by five cycles of denaturation for 30 s at 94 °C, annealing for 40 s at 50 °C and extension for 1 min at 72 °C, followed by 35 cycles of denaturation for 30 s at 94 °C, annealing for 40 s at 55 °C and extension for 1 min at 72 °C, with a final extension for 10 min at 72 °C (Lim et al. 2016). PCR amplification was evaluated by 1% agarose gel electrophoresis under ultraviolet illuminator. The successfully amplified PCR product was sequenced using an ABI 3500 sequencer.



Figure 2. *Hipposideros grandis* **A.** Head with supplementary nose-leaf. **B.** Mixed colony of *Hipposideros* spp.

DNA sequences were edited using Chromas v. 2.6.2. The assembled sequences were aligned using the ClustalW multiple alignment function in BioEdit v. 7.0 (Hall 1999).

Nucleotide compositions were calculated and summarized, and pairwise distances were estimated using K2P model (Kimura 1980). Phylogenetic trees were constructed in MEGA v. 11.0.13 using Tamura-Nei model and 1,000 bootstrap replications with neighbour-joining method (Kumar et al. 2018). *Rhinolophus rouxii* (JQ316214) from India was used as an outgroup.

RESULTS

Hipposideros grandis G.M. Allen, 1936

Figure 2

New record. BANGLADESH – CHATTOGRAM • Sitakunda, Baraiyadhala; 22.6537°N, 091.6643°E; 29 m alt.; 16.VIII.2022 and 3.VI.2023; M.K. Hasan, A. Saha, A.K. Datta leg.; JUMB0004 and JUMB0005.

The first individual was captured from a hill cleft in the evening, and the second individual was captured from the temple.

Identification. Morphological inspection suggested that both specimens belong to the *larvatus* group (Murray et al. 2012), but they are smaller than *H. larvatus* and closely match *H. grandis*; head body 65.00 ± 0.28 mm, forearm 60.10 ± 0.14 mm, ear 20.35 ± 0.21 mm, and tail 32.60 ± 0.14 mm (Table 1). Physical appearance showed that both specimens were male individuals and had dark-brown coloration with pale tips (Figure 2) aligns with the characteristics of *H. grandis*, as reported by Kruskop (2015). In contrast, the ventral fur was blandly pale grayish.

Molecular analysis. COI gene sequences of our two bat specimens were generated and deposited in GenBank (accession no. PQ483291 and PQ483292). BLAST results show that the COI gene of these two specimens matched (99–96%) with *H. grandis* from other geographical areas. The maximum-likelihood phylogenetic tree showed that *H. grandis* from other geographical areas form two distinct clades (Clades

Table 1. Comparative measurements of *Hipposideros grandis* and *H. larvatus* in South and Southeast Asia. HB = head–body length, FA = forearm, 3mt = third metacarpal, 1ph3mt = first phalanx of the third metacarpal, 2ph3mt = second phalanx of the third metacarpal, TIB = length of tibia, HF = hind feet.

Attributes (mm)	<i>H. grandis</i> , Bangladesh (JUMB004)	<i>H. grandis</i> , Bangladesh (JUMB005)	<i>H. grandis</i> , Vietnam (Thanh et al. 2019)	<i>H. grandis</i> , Vietnam (Tu et al. 2019)	<i>H. larvatus</i> , India (Srinivasulu et al. 2010)
HB	64.8	65.2	64.3±3	61.7–68.9	74.0–78.0
FA	60.2	60	63.4±2.4	64.3–64.7	61.2–64.8
3mt	48.7	49.3	NA	NA	NA
1ph3mt	18	18.2	NA	NA	NA
2ph3mt	16.9	17.5	NA	NA	NA
TIB	25.2	25.8	25.3±1.6	23.4–26	NA
HF	11.6	12.2	10.7±0.6	NA	NA
Tail	32.7	32.5	34.4±1.7	31–37	37.0–44.0
Ear	20.5	20.2	21.3±1.4	23–24.4	23.0–26.0

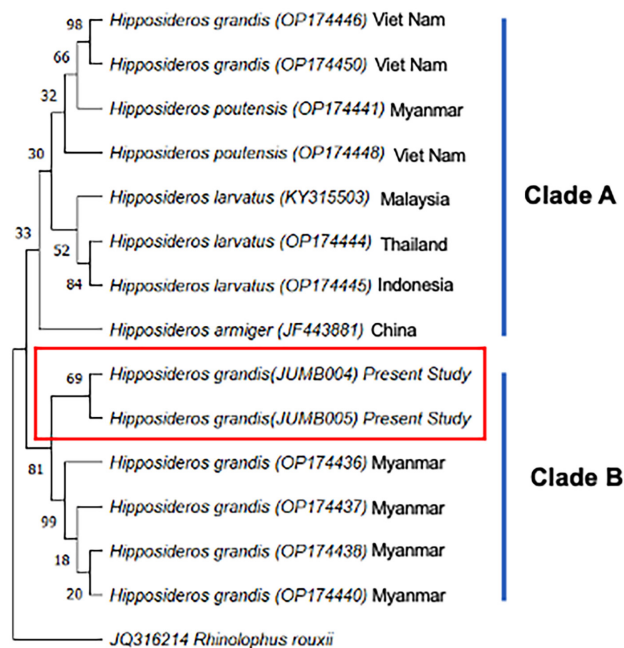


Figure 3. Maximum-likelihood phylogenetic tree based on the COI gene. The specimens outlines in red are those from the present study.

A and B; Figure 3). *Hipposideros grandis* from Vietnam formed Clade A with *H. larvatus*, *H. poutensis*, and *H. armiger*. Our *H. grandis* specimens formed a monophyletic clade (Clade B) with the *H. grandis* from Myanmar.

Nucleotide divergence values of *Hipposideros* species based on the Kimura-2 parameter model ranged from 0.02 to 0.11 (Table 2). Our *H. grandis* showed the shortest distance, 0.03, to *H. grandis* from Myanmar, and they showed greatest distance (0.09) to *H. grandis* from Vietnam.

Additional records. At the two study sites, we observed additional individuals of *Hipposideros*, which we suggest may be *H. grandis*.

DISCUSSION

We use both morphological and molecular data to identify *Hipposideros grandis* because the complexity of morphological parameters make it hard to identify cryptic species of the *larvatus* group. We not only confirm the occurrence of *H. larvatus* in Baraiyadhala National Park but also extend this species' geographic distribution westwards (Figure 1). The nearest population in Bangladesh is at Bandarban (Aziz et al. 2024), which is

Table 2. Genetic distance among different *Hipposideros* species based on Kimura 2-parameter model on the COI gene fragment (525 bp).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
<i>H. grandis</i>_Bangladesh (JUMB004)														
<i>H. grandis</i>_Bangladesh (JUMB005)	0.03													
<i>H. grandis</i>_Vietnam (OP174446)	0.09	0.09												
<i>H. grandis</i>_Myanmar (OP174436)	0.03	0.03	0.08											
<i>H. grandis</i>_Myanmar (OP174437)	0.03	0.03	0.08	0.00										
<i>H. grandis</i>_Myanmar (OP174438)	0.03	0.03	0.08	0.00	0.00									
<i>H. grandis</i>_Vietnam (OP174450)	0.09	0.10	0.00	0.08	0.08	0.08								
<i>H. larvatus</i>_Thailand (OP174444)	0.11	0.11	0.08	0.09	0.09	0.09	0.08							
<i>H. larvatus</i>_Indonesia (OP174445)	0.10	0.10	0.07	0.08	0.08	0.08	0.07	0.02						
<i>H. poutensis</i>_Vietnam (OP174448)	0.10	0.11	0.08	0.09	0.09	0.09	0.08	0.07	0.08					
<i>H. grandis</i>_Myanmar (OP174440)	0.03	0.03	0.08	0.00	0.00	0.00	0.08	0.09	0.08	0.09				
<i>H. armiger</i>_Laos (HM540308)	0.10	0.09	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.08	0.07			
<i>H. armiger</i>_China (JF443881)	0.10	0.10	0.07	0.08	0.08	0.08	0.07	0.07	0.07	0.09	0.08	0.02		
<i>H. poutensis</i>_Myanmar (OP174441)	0.09	0.09	0.04	0.07	0.07	0.07	0.04	0.05	0.05	0.05	0.07	0.05	0.06	
<i>H. larvatus</i>_Malaysia (KY315503)	0.10	0.10	0.06	0.08	0.08	0.08	0.07	0.05	0.05	0.07	0.08	0.06	0.06	0.05

about 78 km south-east of our new record. Distant phylogenetic analysis is used to identify the proximity of nearest population where, the nearest population should have low genetic distance than the distant population (Nei 1996). Likewise, our specimens show shorter genetic distances with the Myanmar population (0.03) than to the more distant Vietnam population (0.09).

This species occurs in caves and subterranean habitats in Bangladesh and other parts of its range (Bates et al. 2016; Aziz et al. 2024), but we recorded from a hill cleft and an abandoned temple surrounded by mixed evergreen forest. Baraiyadhala National Park supports 55 species of mammals, including 13 species of bat (Hasan et al. in press). Our bat study was undertaken as a part of a larger wildlife survey in this national park.

More study of the bat fauna of Bangladesh is needed to help conserve bat species in the country. We observed no threats to *H. grandis* at either of our study sites. Very little information is available on this species' habitat preference, food, feeding behavior, and other ecological aspects; thus, we recommend additional research in these areas.

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ADDITIONAL INFORMATION

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical statement

No ethical statement is reported.

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Author contributions

Conceptualization: MKH, AKD, AS, SB. Data curation: MKH, AKD. Formal analysis: MKH, AKD, AS. Funding acquisition: MKH. Investigation: MKH, AKD, AS, SB. Methodology: MKH, AKD, AS, SB. Resources: MKH. Supervision: MKH. Visualization: MKH, AKD, AS. Project administration: MKH. Software: MKH, AKD, AS. Validation: MKH, AKD, AS, SB. Writing – original draft: MKH, AKD, AS, SB. Writing – review and editing: MKH, AKD, AS, SB.

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Data availability

All data that support the findings of this study are available in the main text and Supplementary materials

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