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SHORT COMMUNICATION

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A NEW SUBSPECIES OF THE MALAYAN BAMBOO BAT (CHIROPTERA: VESPERTILIONIDAE: TYLONYCTERIS MALAYANA EREMTAGA) FROM THE ANDAMAN ISLANDS, INDIA

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Abstract: The bamboo bats belonging to the genus *Tylonycteris* are unique due to their morphology and ecology, and are known from few locations in South Asia. We collected voucher specimens of *Tylonycteris malayana* from North Andaman Island, which forms the basis of the first specimen-based record of this species from the Andaman & Nicobar Islands, and the second record from India. Our analyses based on morphometrics, craniodental measurements, bacular morphology and molecular phylogeny based on cytochrome c oxidase subunit I gene indicate that the insular population of *T. malayana* may have diversified in isolation to differ from the mainland forms, and could represent putative new subspecies, described herein. In light of the new findings, we discuss the importance of conducting detailed study on the specimens of the Lesser Bamboo Bat earlier reported from the Andaman & Nicobar Islands to ascertain their taxonomic status.

Keywords: Bamboo Bat, cryptic diversity, molecular phylogeny, North Andaman Island, *Tylonycteris pachypus*.

The chiropteran fauna of the Andaman Islands has been the subject of study since 1902 (Miller 1902; Hill 1967; Aul et al. 2014). The family Vespertilionidae is represented by several species, including the bamboo bats of the genus *Tylonycteris*. Hitherto, only the Lesser Bamboo Bat *Tylonycteris pachypus* (Temminck, 1840) was reported from the Andaman Islands (Dobson 1876; Hill 1967; Aul 2014). While studying the chiropteran diversity of the Andaman Islands, we collected two specimens of bamboo bats of the genus *Tylonycteris* from North Andaman Island. Tu et al. (2017) while describing a new species, *T. tonkinensis*, from northeastern Laos and northern Viet Nam, reassigned

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the forms of *Tylonycteris robustula* Thomas, 1915 from northeastern India across mainland Southeast Asia to *Tylonycteris malayana* Chasen, 1940, and restricted *T. robustula* s. str. to Sumatra. Detailed study of the specimens from the Andaman Islands, supported by phylogenetic analysis, revealed them to be *T. malayana*, albeit differing from the nominate form, thus prompting the description of a new insular subspecies.

STUDY AREA AND METHODS

As part of an Indo-UK bat survey and conservation project, we surveyed the Andaman Islands between 2014 and 2016. In November 2015, we mist-netted two small male bamboo bats near Bamboo Tekri, Chipo Village, North Andaman Island, and because we were unsure of their identity, preserved them as vouchers. We took external measurements on live specimens and craniodental measurements of the extracted skulls of the vouchers using digital Vernier calipers (Mitutoyo™, to the nearest 0.01 mm). The following external and craniodental measurements were taken-External: FA, forearm length; E, ear length; Tl, tail length; Tib, tibia length; Hf, hindfoot length; 3mt, third metacarpal; 4mt, fourth metacarpal; 5mt, fifth metacarpal; 1ph3mt, first phalange of third metacarpal; 2ph3mt, second phalange of third metacarpal; 1ph4mt, first phalange of fourth metacarpal; 2ph4mt, second phalange of fourth

metacarpal; Craniodental: GTL, greatest length of the skull; CBL, condylobasal length; CCL, condylocanine length; CM³, maxillary toothrow; C¹-C¹, anterior palatal width; M³-M³, posterior palatal width; ZB, zygomatic breadth; BB, braincase breadth; CM₃, mandibular toothrow; M, mandible length. Bacula were extracted and stained following the standard method (Topal 1958). We compared our specimens with two specimens of *T. malayana* (labelled as *T. robustula* pre-Tu et al. 2017) from Malaysia, housed in the Harrison Zoological Museum, UK (specimen nos. 2.7443 and 3.7444), and also with external and craniodental measurements of the nominate form provided by Vuong Tan Tu (pers. comm., October 31, 2017).

Wing punches from the specimens were taken and preserved in silica gel. Genomic DNA was then extracted using DNEasy Blood and Tissue kit (QIAGEN). A PCR was conducted to amplify partial cytochrome c oxidase subunit1(COI) gene sequences using forward and reverse primers VF1d (5'-TTCTCAACCAACCACAARGAYATYGG-3') and VR1d (5'-TAGACTTCTGGGTGGCCRAARAAYCA-3') (Ivanova et al. 2006). The PCR reaction was performed in a 25µl reaction volume containing 2µl of template DNA, 12.5µl of 2X reaction buffer (0.05U/µL Taq DNA polymerase, reaction buffer, 4mM MgCl₂, 0.4mM of each dNTPs), 0.5µl of each primer, and 9.5µl nuclease free water. The thermal profile followed included: 94°C

Table 1. List of cytochrome C oxidase subunit I (COI) sequences used to conduct phylogenetic analysis, with collection localities and GenBank accession numbers.

	Species	Collection locality	GenBank accession no.		
1.	Tylonycteris malayana eremtaga ssp. nov.	Near Bamboo Tekri, Chipo Village, North Andaman Island, Andaman and Nicobar Islands, India	MG807173		
2.	Tylonycteris malayana	Northeastern India, India	KX496400		
3.	Tylonycteris malayana	Northeastern India, India	KX496398		
4.	Tylonycteris malayana	Vu Quang National Park, Ha Tinh, Viet Nam	KX496402		
5.	Tylonycteris malayana	Virachey National Park, Taveng Village, Rattanakiri, Cambodia	KX496370		
6.	Tylonycteris malayana	Kbal Spean, Koulen NP, Siem Reap, Cambodia	KX496420		
7.	Tylonycteris malayana	Ulu Kenas Recreational Forest, Perak, Malaysia	KX496394		
8.	Tylonycteris pachypus	Pane Bandar Barbu 1,200m, Sumatra, Indonesia	KX496366		
9.	Tylonycteris pachypus	Deli, Sumatra, Indonesia	KX496536		
10.	Tylonycteris fulvida	Vu Quang NP, Ha Tinh, Viet Nam	KX496474		
11.	Tylonycteris fulvida	Na Don, Phuong Vien, Cho Don, Bac Kan Prov., Viet Nam	KX496506		
12.	Tylonycteris robustula	Berastagi, Sumatra, Indonesia	KX496367		
13.	Tylonycteris robustula	Pane Bandar Barbu 1200m, Sumatra, Indonesia	KX496368		
14.	Tylonycteris tonkinensis	Hat Hin, Nam Sing River, Phongsaly Prov., Laos	KX496442		
15.	Tylonycteris tonkinensis	Na Don, Phuong Vien, Cho Don, Bac Kan Prov., Viet Nam	KX496431		
16.	Pipistrellus javanicus	1km Sw Of Ban Houana, Khammouan, Laos	HM541255		
17.	Hypsugo cadornae	Along Banks Of Xekaman, Attapu, Laos	HM540636		

for 1 min, five cycles of 94°C for 30s, 50°C for 40s, and 72°C for 1 min, followed by 35 cycles of 94°C for 30s, 55°C for 40s, and 72°C for 1 min, with a final extension at 72°C for 10 min. The amplified PCR products were sequenced using an ABI prism 3730 sequencer (Applied Biosystems, USA) and big dye terminator sequencing kit (ABI Prism, USA). The gene sequence (633 base pairs, GenBank accession number MG807173) obtained in the study was analyzed using BLAST (Altschul et al. 1990) to double-check the quality of the sequence. Additional sequences of other related species were retrieved from the NCBI GenBank database for phylogenetic analysis (Table 1). The sequences were aligned using MUSCLE (Edgar 2004) incorporated in MEGA6 (Tamura et al. 2013) using default parameters. JModelTest 2 was used to choose the best-fitting maximum likelihood DNA substitution model for the dataset, based on Bayesian Information Criterion (BIC) scores for each model type (Guindon & Gascuel 2003; Darriba et al. 2012). The analysis used the Hasegawa-Kishino-Yano + Gamma [ncat=4] (HKY+G, BIC = 5144.08) nucleotide substitution model (Hasegawa et al. 1985). We constructed a Bayesian Inference of phylogeny in the BEAST 1.8.2 package (Drummond et al. 2012), with default priors, using the nucleotide substitution model obtained for a chain length of 10 million generations, sampling every 1000 generations. The tree was rooted using Pipistrellus javanicus and Hypsugo cadornae as outgroup taxa. We double-checked convergence by plotting likelihood

scores against generations in Tracer v1.6 (Rambaut et al. 2014). The first 20% of the trees were discarded as burnin, and chronograms were created using TreeAnnotator v1.8.2 (as part of the BEAST package) and visualized in FigTree v1.4.1 (Rambaut 2009). Kimura 2-parameter (K2P) pairwise distances were calculated using MEGA6.

RESULTS

We confirmed the identity of the specimens as *T. malayana*, based on external and craniodental measurements, diagnostic characters, and molecular phylogeny using partial cytochrome c oxidase subunit I gene sequence. Owing to discernible variation in morphological and craniodental measurements, bacular structure, and genetic distance, we describe a new insular subspecies of *T. malayana*.

Tylonycteris malayana eremtaga ssp. nov. (Images 1a,b, 2a-g, 3a-c)

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Andaman Bamboo Bat

Holotype: NHMOU.CHI.151.2015, adult male, 06.xi.2015, near Bamboo Tekri (13.373°N & 92.999°E), Chipo Village, Diglipur, North Andaman Island, Andaman & Nicobar Islands, India; coll. A. Gopi & Tauseef Hamid Dar.

Paratype: NHMOU.CHI.152.2015, adult male, other details same as holotype.





Image 1. Tylonycteris malayana eremtaga ssp. nov., holotype (NHMOU.CHI.151.2015) from Chipo Village, North Andaman Island, Andaman Islands, India, showing lateral view of head (a) and ventral view, showing characteristic club-shaped foot (b).

Table 2. External and craniodental measurements of *Tylonycteris malayana eremtaga* ssp. nov. from Chipo Village, North Andaman Island, Andaman Islands, India with comparison of *T. malayana* following Tu et al. (2017), and *T. robustula sensu lato*, following Bates and Harrison (1997), from Northern Myanmar (now included under *T. malayana* as per Tu et al. 2017). For *T. malayana* and *T. robustula sensu lato* the values are mean (range). Measurements are in mm. Abbreviations as stated in text.

	T. malayana erem	taga ssp. nov.	T. malayana (n=29)	T. robustula sensu lato	
Specimen Details	Holotype NHMOU.CHI.151.2015 Adult Male	Paratype NHMOU.CHI.152.2015 Adult Male			
	External				
НВ	37.3	36.0	_	41.1 (40.0–44.0) (n=6)	
FA	26.2	23.3	26.4 (25.5–27.5)	27.5 (26.6–28.1) (n=6)	
Е	10.2	10.2	_	9.7 (8.5–10.5) (n=6)	
TI	27.8	26.3	-	28.3 (26.0–31.0) (n=6)	
Tib	12.1	10.3	-	_	
Hf	6.2	5.8	-	5.3 (5.0–5.5) (n=6)	
3mt	25.5	22.9	-	26.1 (25.8–26.4) (n=6)	
4mt	25.7	22.5	-	25.8 (25.4–26.0) (n=6)	
5mt	24.7	22.2	-	25.0 (24.8–25.6) (n=6)	
1ph3mt	11.0	10.4	-	-	
2ph3mt	12.3	11.4	-	-	
1ph4mt	9.6	8.4	-	-	
2ph4mt	5.4	5.5	-	-	
	Craniodental		•		
GTL	11.7	11.5	12.34 (11.73–12.90)	12.3 (12.0–12.7) (n=4)	
CBL	11.6	11.4	-	-	
CCL	10.9	10.7	11.23 (10.66–11.82)	11.3 (11.1–11.7) (n=5)	
ZB	8.8	9.0	-	9.0 (8.9–9.1) (n=2)	
BB	6.7	6.5	6.78 (6.40–7.36)	7.0 (6.9–7.0) (n=5)	
C1-C1	3.8	4.0	4.09 (3.81–4.46)	-	
CM ³	4.1	3.7	4.06 (3.74–4.31)	4.0 (3.9–4.1) (n=6)	
M³-M³	5.5	5.5	5.54 (5.13–5.90)	4.3 (4.3–4.4) (n=4)	
CM ₃	3.9	3.8	4.32 (4.08–4.53) 5.6 (5.4–5.8) (1		
М	8.1	8.0	8.71 (8.25–9.19)	8.7 (8.5–9.0) (n=6)	
RW	6.1	5.7	_	5.5 (5.3–5.6) (n=5)	

Diagnosis: A small bat with a forearm length up to 23.3–26.25 mm. Dorsal pelage uniformly grey-brown, venter slightly paler; wings and interfemoral membrane dark brown. Although the new subspecies is essentially similar to nominotypic *T. m. malayana* (see Tu et al. 2017), it varies from the nominate form slightly in having smaller craniodental features (GTL: 11.5 in *T. m. eremtaga* ssp. nov. vs 11.73–12.90 in *T. m. malayana*). The rostrum is robust in *T. m. eremtaga* ssp. nov., shorter and broader than that of the nominate subspecies (CM³: 3.7–4.1 in *T. m. eremtaga* ssp. nov. vs 3.74–4.31 in *T. m. malayana*; RW: 5.7–6.1 in *T. m. eremtaga* ssp. nov. vs 5.3–5.6 in *T. robustula* sensu lato (see Bates & Harrison

1997). Externally, the fur color of *T. m. eremtaga* ssp. nov. appears to be uniform grey-brown while that of *T. m. malayana* is dark brown.

Description: A small bat with a forearm length up to 26.25mm. Head characteristically flat, projecting forwards and downwards (Image 1a). Ears roughly triangular with broadly rounded tips. Tragus short and broad. Base of the thumb and the soles of the feet have broad fleshy pads (Image 1b). Wings short with 3rd, 4th and 5th metacarpals roughly equal in length. Wing and interfemoral membranes dark brown (Image 1a,b), pelage fine and dense, uniformly grey-brown on the dorsum, slightly paler on the ventral surface.



Image 2. Dorsal (a), ventral (b), lateral (c) and frontal (d) view of the skull and dorsal (e), lateral (f) and frontal (g) view of the mandible of *Tylonycteris malayana eremtaga* ssp. nov. (NHMOU.CHI.151.2015) from Chipo Village, North Andaman Island, Andaman Islands, India with detail of size and shape of the upper canine and incisors. Scale = a–c: 10 mm; d–g: 5 mm.

Species		1	2	3	4	5	6
1	T. m. eremtaga ssp. nov.	0					
2	T. m. malayana	2.2–4%	0				

Table 3. K2P pairwise distance matrix of the taxa of genus Tylonycteris (Chiroptera: Vespertilionidae)

Species	1	2	3	4	5	6
T. m. eremtaga ssp. nov.	0					
T. m. malayana	2.2-4%	0				
T. tonkinensis	6.4–7%	5.2-5.8%	0			
T. robustula	9.50%	6.90%	7.6–8.2%	0		
T. fulvida	17.7–19.2%	17.70%	17–19.3%	17.9–19.4%	0	
T. pachypus	17.8–19.9%	14.8–16.9%	14.2–15.5%	16.40%	6.4-7.0%	0
	T. m. eremtaga ssp. nov. T. m. malayana T. tonkinensis T. robustula T. fulvida	T. m. eremtaga ssp. nov. 0 T. m. malayana 2.2–4% T. tonkinensis 6.4–7% T. robustula 9.50% T. fulvida 17.7–19.2%	T. m. eremtaga ssp. nov. 0 T. m. malayana 2.2–4% 0 T. tonkinensis 6.4–7% 5.2–5.8% T. robustula 9.50% 6.90% T. fulvida 17.7–19.2% 17.70%	T. m. eremtaga ssp. nov. 0 T. m. malayana 2.2–4% 0 T. tonkinensis 6.4–7% 5.2–5.8% 0 T. robustula 9.50% 6.90% 7.6–8.2% T. fulvida 17.7–19.2% 17.70% 17–19.3%	T. m. eremtaga ssp. nov. 0 2 3 4 T. m. malayana 2.2-4% 0 0 0 T. tonkinensis 6.4-7% 5.2-5.8% 0 0 T. robustula 9.50% 6.90% 7.6-8.2% 0 T. fulvida 17.7-19.2% 17.70% 17-19.3% 17.9-19.4%	T. m. eremtaga ssp. nov. 0 T. m. malayana 2.2-4% 0 T. tonkinensis 6.4-7% 5.2-5.8% 0 T. robustula 9.50% 6.90% 7.6-8.2% 0 T. fulvida 17.7-19.2% 17.70% 17-19.3% 17.9-19.4% 0

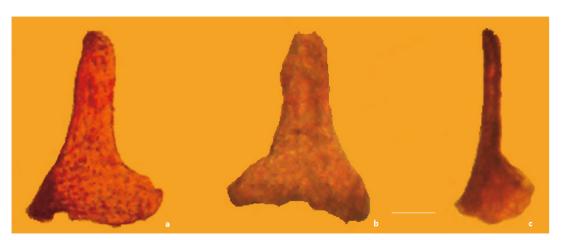


Image 3. Dorsal (a), ventral (b) and lateral (c) view of the baculum of Tylonycteris malayana eremtaga ssp. nov. (NHMOU.CHI.151.2015) from Chipo Village, North Andaman Island, Andaman Islands, India. Scale = 0.1 mm.

The skull is small, with a length (GTL) up to 11.7mm. The whole skull is characteristically flattened and broad (Image 2a,c,d). The breadth of the braincase exceeds twice the occipital height, and the rostrum is short and broad (Image 2a), with distinct supraorbital projections. Sagittal crest absent. Lambdoid crest weakly developed. Mesopterygoid space wide and short. Horizontal ramus of each half-mandible is relatively thick, coronoid process large, condyle and angular process well developed (Image 2f).

Upper toothrow length (CM3) up to 3.8mm. The first incisor (i3) is large, narrow and bicuspidate; second is unicuspidate and roughly equal to the length of the outer cusp of i2. The upper canine is separated from i³ by a short diastema, and is double the length of i². Upper canine has a distinct posterior secondary cusp. Upper premolar (pm4) has a crown area approximately half that of m1 and slightly exceeds half of the height of the upper canine. M1 slightly exceeds m2 in breadth, and has a distinct protocone. M3 lacks a metastyle but it is not greatly reduced. The three lower incisors are tricuspidate and located adjoining the lower canine. Lower canine weak, not as robust as the upper canine

and slightly exceeding the protoconid of m, in height. Pm, and pm, are equal in size and compressed in the toothrow, anterior part of pm, positioned on the posterior cingular cusp of the canine. In m, and m, the talonid exceeds the trigonid in size, in mathey are roughly equal.

The baculum of the new subspecies is located at the tip of the short penis, which has a globular glans. It has a broad, flared base (0.3mm) (Image 3), which is concave in the holotype and slightly notched in the paratype. The shaft of the baculum of the holotype is long (0.4mm in length), straight, with a slight ventral concavity beneath the broadly rounded tip. In the lateral aspect, the shaft of the baculum is long and straight, and the base is flared, unlike that of the nominate subspecies, in which the shaft curves dorsally along its length, and the base is knob-like (Hill & Harrison 1987). The paratype matches the holotype in all characters excepting size (Table 2).

Molecular phylogeny: The phylogenetic tree obtained from the analysis of COI gene of the Tylonycteris species supports the distinctness of the Andaman population with the taxa previously assigned to T. robustula sensu lato and T. pachypus sensu lato

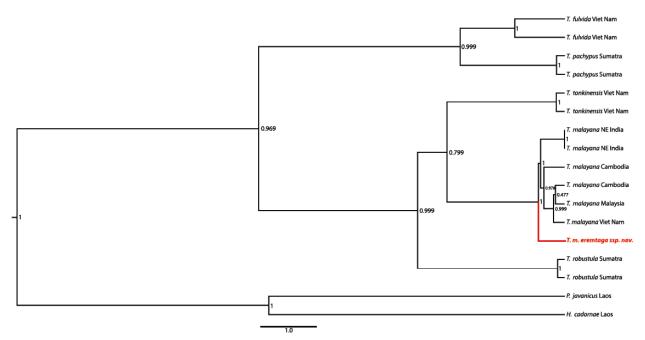


Figure 1. Bayesian Inference (BI) tree based on partial Cytochrome c oxidase subunit I (COI) gene sequences using Hasegawa-Kishino-Yano nucleotide substitution model with gamma distribution [4 discrete categories] (HKY+G, BICc = 5144.082). Values at the nodes are posterior probability values. *Pipistrellus tenuis* and *Hypsugo cadornae* are used as outgroup taxa.

clading separately with high posterior support, in agreement with Tu et al. (2017). Within the *T. robustula* sensu lato clade, five distinct subclades were observed: one subclade of *T. robustula* sensu stricto from Sumatra, one subclade of *T. tonkinensis* from Laos and Viet Nam, two separate subclades of *T. m. malayana* from Southeast Asia and India respectively, and one subclade of *T. m. eremtaga* ssp. nov. from the Andaman Islands, supported by high posterior probability (> 0.75) (Fig. 1). The distinctness of the new subspecies is also supported by K2P distances of 2.2–4% from the nominate subspecies for the COI gene (Table 3).

Etymology: The subspecific epithet, 'eremtaga,' is a Latinized noun derived from the Aka-Kora dialect of the Great Andamanese language, meaning 'forest-dweller'.

Ecological notes: The presence of multiple males in the area suggests the existence of a colony of these bats in the near vicinity of the village. The specimens collected from Chipo Village, Diglipur, North Andaman Island were found to share their habitat with other species such as the Greater Short-nosed Fruit Bat Cynopterus sphinx, the Lesser Short-nosed Fruit Bat Cynopterus brachyotis, Tickell's Bat Hesperoptenus tickellii, and the Andaman Intermediate Horseshoe Bat Rhinolophus affinis andamanensis. This subspecies is currently known only from Chipo Village, Diglipur, North Andaman Island, Andaman & Nicobar Islands, India.

Discussion

One taxon, *Tylonycteris pachypus* sensu lato, has so far been reported to be present on Andaman Islands, from Wimberleygunj, South Andaman Island (Hill 1967), and Webi and Karmatang in North Andaman Island (Aul 2014), principally based on external morphology and forearm length. In light of the recent taxonomic revision (Tu et al. 2017), and the discovery of the *T. malayana eremtaga* ssp. nov. from the Andaman Islands, it is imperative that the specimens collected earlier from the Andaman Islands (Dobson 1876; Hill 1967; Aul 2014) need to be re-examined further to ascertain their correct identity.

The bamboo bats belonging to genus *Tylonycteris* depend on bamboo forests, and harvesting of bamboo for commercial purpose, destruction of bamboo forests, forest fire, and increased use of pesticides in fringe villages might have a negative impact on the survival of these species.

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