Short note

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First records of *Hypsugo cadornae* (*Chiroptera*: *Vespertilionidae*) in China

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Abstract: *Hypsugo cadornae* bats have been found in India, Myanmar, Thailand, Vietnam, Laos, and Cambodia. In 2017 and 2018, 15 medium size *Hypsugo* bats were collected from Shaoguan, Guangzhou, and Huizhou in Guangdong, China. Molecular and morphological examinations identified them as *H. cadornae*. This is the first record of *H. cadornae* in China. Morphological and ultrasonic characteristics of *H. cadornae* were compared with its close relative, *Hypsugo pulveratus*.

Keywords: echolocation calls; *Hypsugo*; morphology; phylogeny.

Hypsugo cadornae was formerly classified as a subspecies of *Hypsugo savii* (Ellerman and Morrison-Scott 1951) or *Hypsugo lophurus* (Corbet and Hill 1992). Because of strikingly differences in skull and baculum, it was later separated from *H. savii* and *H. lophurus* and considered as a distinct species (Bates et al. 1997, 2019; Corbet and Hill 1992; Hill and Harrison 1987). In 2005, Bates et al. described *Hypsugo pulveratus* as a species morphologically similar to *H. cadornae* (Bates et al. 2005). Whereas *H. pulveratus* is widely distributed in China (IUCNredlist, Wilson and Reeder 2005), *H. cadornae* was not found there yet.

H. cadornae was first discovered in north-eastern India (Thomas 1916) and later found in India, Myanmar (Bates and Harrison 1997), Thailand (Hill and Thonglongya 1972), Vietnam (Kruskop and Shchinov 2010), Laos (Görföl et al. 2014), and Cambodia (Furey et al. 2012). In 2017 and 2018, we captured 15 medium size *Hypsugo* bats from Shaoguan, Guangzhou, and Huizhou in Guangdong, China. Three individuals from the three different cities were carefully examined.

Nine body and 12 skull morphological features were measured using a vernier caliper (0.01 mm) according to Bates and Harrison (1997); Furey et al. (2012). Compared with *H. pulveratus*, *H. cadornae* (Figure 1A) bats have a less distinctive dorsal pelage (Bates et al. 2005; Görföl et al. 2014). The forearm length ranged from 31.7 to 37.1 mm. The fifth metacarpal was nearly as long as the third and fourth metacarpal (Table 1). On the skull, the rostrum was short and narrow; the nasal notch was broad and round (Figure 1B–E, Table 1). The braincase was full and rounded with a shallow sagittal crest in the midpart. The cranial profile lacked a frontal depression between the rostrum and braincase. The palate was short, but zygomata were relatively robust with postorbital eminences. The first upper premolar (P2) was very short and was smaller than that of *H. pulveratus*.

Genomic DNA was isolated from the muscle cells of each of the three bats. Polymerase chain reaction was then performed to amplify a portion of the mitochondrial cytochrome *b* gene (*CYTB*; 1140 bp) using primers CY1 (5′-TAG AAT ATC AGC TTT GGG TG-3′) and CY2 (5′-AAA TCA CCG TTG TAC TTC AAC-3′) (Li et al. 2006) and a fragment of the cytochrome oxidase *c* subunit I gene (*COI*; 657 bp) using primers Bat5310 (5′-CCT ACT CRG CCA TTT TAC CTA TG-3′) and R6036 (5′-ACT TCT GGG TGT CCA AAG AAT CA-3′) (Robins et al. 2007). The PCR products were sequenced (GenBank accession numbers of the

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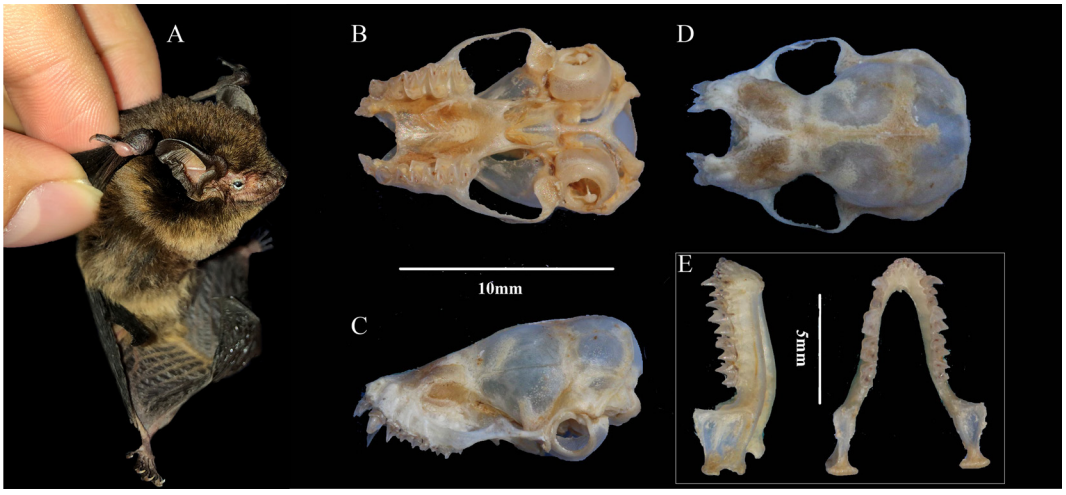

Figure 1: (A) Portrait of a living *Hypsugo cadornae* (B) Dorsal, (C) lateral, and (D) ventral views of the skull and occlusal and lateral views of the mandible of an *H. cadornae* bat (GD-181117 from Huizhou City, China).

newly generated sequences; *CYTB* ds625, MN508627, MN508628; *COI* MN508629, MN508631, MN508632). The alignment of sequences was performed using concatenated *CYTB* and *COI* sequences of known *H. cadornae* and other five related bat species (Figure 2) with MEGA X (Kumar et al. 2018). The Maximum-likelihood phylogenetic tree was constructed based on the Hasegawa-Kishino-Yano model. Bootstrap support with 1000 replicates was analyzed with the percentage cut-off of 50%. All three examined individuals (GD-172497, GD-180937, and GD-181117) were found to be phylogenetically clustered with *H. cadornae* bats with 100% bootstrap value but were separated from other species of *Hypsugo* bats (Figure 2). Therefore, based on morphological features and molecular data, examined individuals were identified as *H. cadornae*.

Echolocation calls of the three *H.cardonae* bats were recorded using an Avisoft Bioacoustics USG 116(e) detector equipped with an Avisoft FG microphone. The spectrograms were generated by 512 consecutive fast Fourier transforms (FFT) at 96.87% frequency overlapped with a Hamming window using the Avisoft-SASLab Pro software. Echolocation calls of *H. cadornae* were found to be in frequency-modulation (FM), usually with multiple harmonics. Thirty relatively clear sound waves of each bat were randomly selected for analysis. The maximum energy of calls was mostly in the first harmonic. The peak frequency with the maximum energy was 38.81 ± 1.34 kHz. This is a relatively low frequency suitable for hunting insects with thick exoskeletons such as beetles, bugs, and butterflies (Weterings et al. 2015). The highest frequency was found at 65.60 ± 4.89 kHz, and the pulse duration was 3.10 ± 0.77 ms.

Adaptation to environments is a possible reason for the distribution of *H. cadornae* bats throughout the Indomalayan region. It has been reported that *H. cadornae* can roost on banana trees or a dry bamboos in a lowland forest (Bates et al. 2005). In our surveys, *H. cadornae* bats were captured from an old building, a concrete bridge, and sabal trees in villages, which were partly or entirely surrounded by lowland forests. More surveys with a wider scope are warranted to discover new species of bats and to understand their ecological conditions. Presented first records of *H. cadornae* in China enlarged the range of the species distribution in south-eastern Asia (IUCNredlist, Lim et al. 2016). This study advanced our knowledge of bat distribution and provided important information for further studies of various aspects of *H. cadornae* in China.

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Table 1: Selected morphological and craniodental measurements (in mm) of *Hypsugo* bats.

	Hypsugo cadornae				*Hypsugo pulveratus*		
	China	Vietnam	India and Myanmar	Cambodia	Myanmar	Laos and Vietnam	China
Character	This study	Bates et al. 1997	Bates and Harrison 1997	Furey et al. 2012	Bates et al. 2005	Görföl et al. 2014	James et al. 2008
HB	43.5 ± 1.87(40.1–46.9) (15)	46.6 M/44.1 F	49.3(47–52.5) (5)	45.8	43.7 ± 3.3(38.2–49.4) (9)		44
FA	34 ± 1.26(31.7–37.1) (15)	33 M/36.6 F	35.1(32.6–36.5) (5)	36.1	34.1 ± 1.2(32.0–35.2) (9)	34.6 ± 1.39(32.4–37.6) (32)	33.5
T	39.7 ± 2.89(36.3–45) (15)	34.3 M/32.1 F	39.7(34–49) (5)	36	34.4 ± 2.0(32.0–38.0) (9)		22
E	12.1 ± 0.97(11.1–14.1) (15)	14.1 M/12.4 F	14.5(14–15) (5)	15.4	11.4 ± 1.3(10.2–13.8) (9)		7
HF	5.8 ± 0.47(5.2–6.7) (15)	7 M/5.9 F	6.9(6.5–7) (5)	7.3	6.4 ± 0.8(5.5–8.0) (9)	6.1 ± 0.79(4.7–8.2) (24)	8
TIB	13.9 ± 0.80(12.7–15.7) (15)	15 M/15.6 F		16	13.8 ± 0.3(13.4–14.1) (9)	13.9 ± 0.53(12.9–14.7) (11)	
5MET	31.2 ± 1.41(28.2–34.3) (15)	30.9 M/32.8 F	32.3(30.2–34.4) (5)		30.5 ± 0.8(29.4–31.7) (9)		
4MET	32.2 ± 1.38(29.8–35.4) (15)	31.7 M/33.4 F	33.5(31.2–35.7) (5)		32.1 ± 0.9(30.6–33.3) (9)		
3MET	33.1 ± 1.18(31.6–35.7) (15)	32.7 M/33.8 F	34.2(32.5–36) (5)		32.7 ± 0.8(31.6–33.6) (9)		
GTL	14.0 ± 0.22(13.6–14.3) (8)	13.4 M/13.6 F	13.8(13.6–14) (3)	13.9	14.0 ± 0.2(13.6–14.2) (9)	14.01 ± 0.38(13.26–14.64) (26)	13.74
CCL	13.1 ± 0.21(12.8–13.4) (8)	12.5 M/12.7 F	12.7(12.6–12.8) (5)	12.79	12.6 ± 0.2(12.4–12.9) (9)	12.63 ± 0.30(12.00–13.20) (28)	11.06
ZB	9.0 ± 0.27(8.5–9.3) (6)	8.4 M/8.6 F		8.95	8.5 ± 0.2(8.2–8.6) (9)	8.46 ± 0.18(8.15–8.80) (20)	8.04
BB	7.0 ± 0.15(6.8–7.2) (8)	6.9 M/6.7 F	7.1(6.7–7.5) (5)		6.7 ± 0.1(6.7–6.9) (9)	6.75 ± 0.16(6.18–6.97) (27)	
PC	3.9 ± 0.15(3.7–4.1) (8)	3.8 M/3.5 F	3.7(3.5–3.9) (5)		3.7 ± 0.1(3.6–3.8) (9)		3.76
C–M^3	4.8 ± 0.14(4.5–4.9) (8)	4.7 M/4.9 F	4.7(4.6–4.9) (5)	4.77	5.0 ± 0.1(4.7–5.3) (9)	4.88 ± 0.14(4.75–5.15) (33)	5.44
C–M_3	5.1 ± 0.11(4.9–5.2) (8)	4.8 M/4.9 F	5(4.8–5.1) (4)	5.21	5.5 ± 0.2(5.1–5.8) (9)	5.20 ± 0.14(4.86–5.47) (33)	6
M^3–M^3	6.0 ± 0.22(5.5–6.2) (8)	5.7 M/6.0 F	5.9(5.8–6) (5)	6.2	5.8 ± 0.2(5.6–6.2) (9)	5.73 ± 0.21(5.20–6.11) (29)	
M	10.1 ± 0.25(9.8–10.4) (8)	9.8 M/9.8 F	9.9(9.5–10.3) (5)	9.86	9.9 ± 0.3(9.5–10.4) (9)	9.56 ± 0.21(9.15–9.96) (31)	
RW	5.0 ± 0.14(4.8–5.2) (8)	5.1 M/5.3 F	5.1(4.9–5.4) (5)			4.95 ± 0.22(4.53–5.45) (30)	
C^1–C^1	4.8 ± 0.20(4.5–5) (8)	4.8 M/5.2 F		4.75	4.4 ± 0.2(4.1–4.7) (9)	4.22 ± 0.19(3.72–4.53) (30)	
MAW	7.9 ± 0.12(7.7–8.1) (8)			7.82		7.24 ± 0.13(6.94–7.56) (27)	

Values are given in mean ± SD, min-max (*n*).

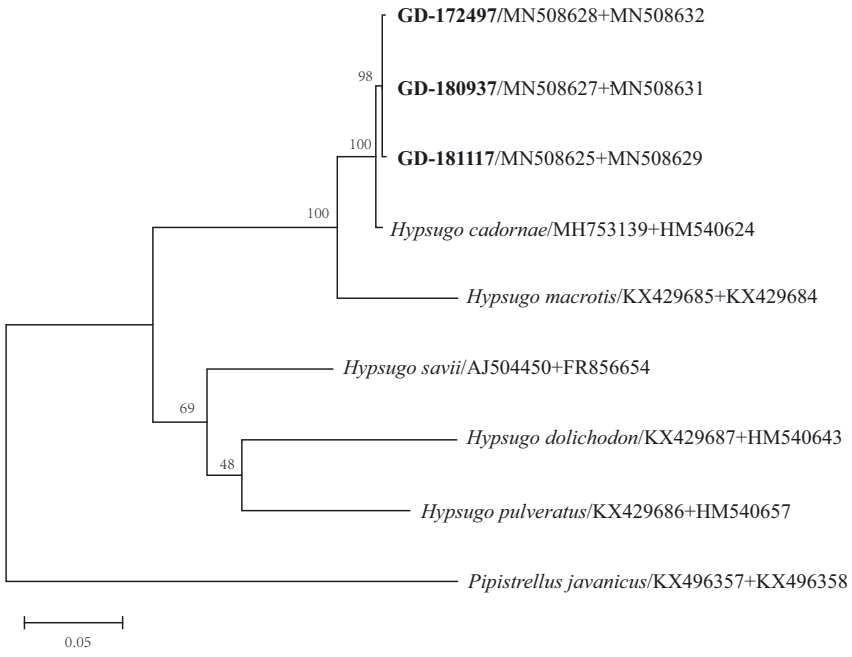

Figure 2: Maximum likelihood phylogenetic tree based on concatenated nucleotide sequences of fragment of *CYTB* and *COI* genes of different *Hypsugo* species. *Pipistrellus javanicus* was used as an outgroup taxon.

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Conflict of interest statement: The authors declare no conflicts of interest regarding this article.

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