



# New Locality Records of the Zunheboto Horned Frog, *Xenophrys zunhebotoensis* Mathew and Sen 2007 (Anura: Megophryidae: Megophryinae), from India

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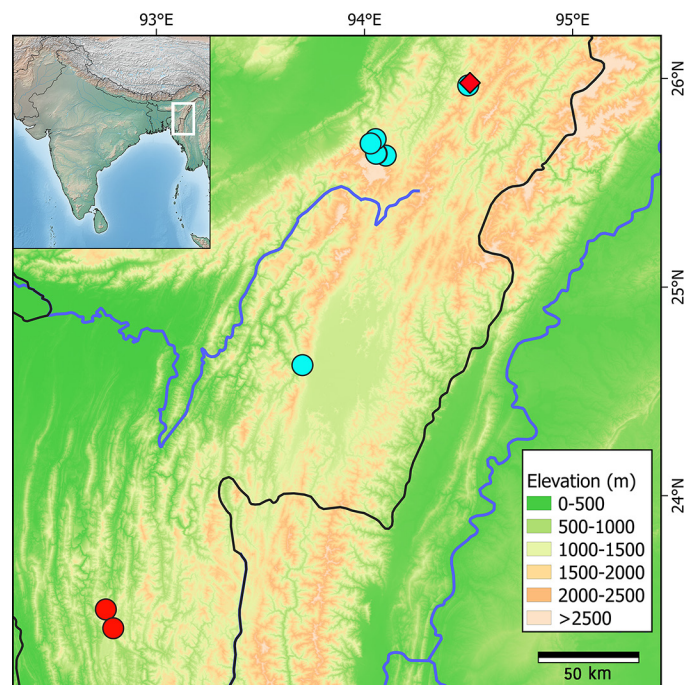
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The Zunheboto Horned Frog (*Xenophrys zunhebotoensis*) was described from Nguti (Sukhalu), Zunheboto District, Nagaland, India (Mathew and Sen 2007), and has been recorded from Meghalaya, Manipur, and Arunachal Pradesh (Saikia and Sen 2012; Sangma and Saikia 2015; Saikia et al. 2017; Kharkongor et al. 2018; Mahony et al. 2020). However, the records of this species from Meghalaya (Saikia and Sen 2012; Sangma and Saikia 2015; Kharkongor et al. 2018) and Arunachal Pradesh (Saikia et al. 2017) were erroneous and based on misidentifications (see Mahony et al. 2020). The species has been recorded with certainty only from Nagaland and western Manipur (Mathew and Sen 2007; Mahony et al. 2020). Mahony et al. (2020) discussed



**Fig. 2.** Map showing the known localities of the Zunheboto Horned Frog (*Xenophrys zunhebotoensis*). The type locality is indicated by the red diamond, previously published locality records by blue circles, and the new records by red circles.



**Fig. 1.** A male Zunheboto Horned Frog (*Xenophrys zunhebotoensis*) from Hmuifang, Mizoram, India (MZUHC 30036). Photograph by Samuel Lalronunga.

the high genetic diversity between *X. zunhebotoensis* from Nagaland and Manipur. However, without any diagnosable morphological distinction between the two populations, the Manipur population is treated as conspecific with *X. zunhebotoensis* (Mahony et al. 2020). Herein, we present the first record of this species from the state of Mizoram and also

discuss the record of the Little Karen Hills Horned Frog (*X. parva*) from Mizoram, India.

We collected three male Zunheboto Horned Frogs (Fig. 1) from the Hmuifang Community Forest Reserve (23°27.288'N, 92°45.4427'E; 1,384 m asl.) at Hmuifang Village on 17 July 2020 and from the Sailam Community Forest Reserve (23°21.8747'N, 92°47.578'E; 1,128 m asl) at Sailam Village on 7 September 2018 (Fig. 2). Frogs were humanely euthanized with Benzocaine, fixed in 10% formalin for 24 h, and then transferred to 70% ethanol. Prior to fixation, we excised femoral muscle tissue from some specimens and stored it in absolute ethanol for genomic DNA extraction. Specimens were deposited in the herpetological collection of the Systematics and Toxicology Laboratory, Department of Zoology, Mizoram University, India (MZUHC 20035–20037).

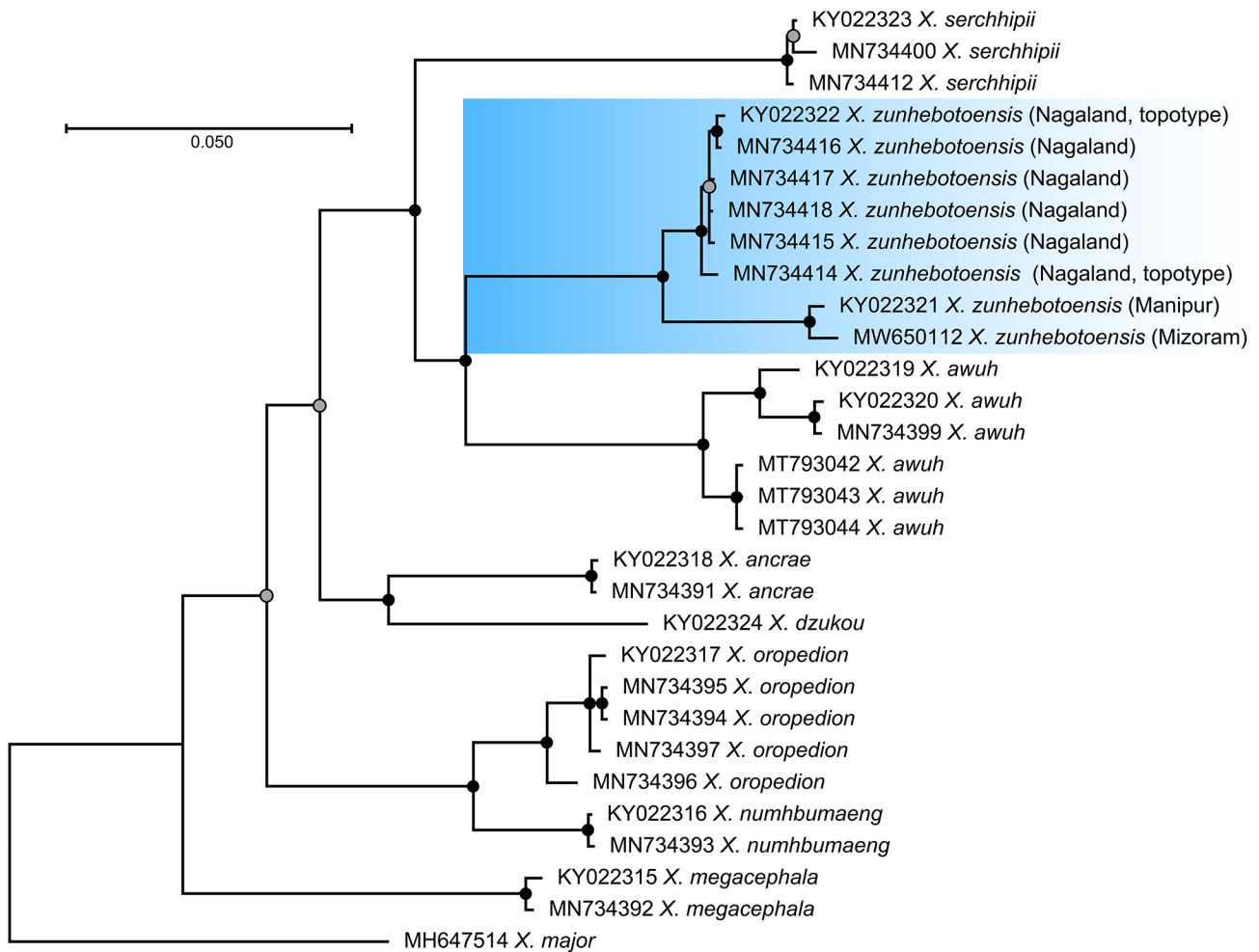
Following Lalronunga and Lalrinchhana (2017), we generated a fragment of the 6S rRNA mitochondrial gene sequence from one specimen (MZUHC 20036) for comparison with members of the *Xenophrys megacephala* species group

available in GenBank. The generated sequence was deposited at GenBank (NCBI) with the accession number MW650112 (Mahoney et al. 2020). Sequences were aligned with the CLUSTALW algorithm (Thompson et al. 1994) in BioEdit software (Hall 1999). Ambiguously aligned regions were further adjusted by eye where necessary to ascertain homology. A Bayesian inference phylogenetic reconstruction (Huelsenbeck et al. 2001) was carried out using the Phylogeny.fr web service (Dereeper et al. 2008). Uncorrected pairwise genetic distances (p-distance) between sequences were determined with MEGA 7 (Kumar et al. 2018).

The three males (Table 1) collected from Mizoram matched the descriptions of *X. zunhebotoensis* in Mathew and Sen (2007) and Mahony et al. (2020) and the generated sequence formed a clade with sequences of *X. zunhebotoensis* in the phylogenetic tree (Fig. 3). The genetic distance between the sequence from Mizoram and the sequence of a topotype (GenBank KY022319) was 2.8%, whereas the genetic distance between the sequences from Mizoram and Manipur

**Table 1.** Morphometric measurements of three male Zunheboto Horned Frogs (*Xenophrys zunhebotoensis*) from Mizoram, India. Abbreviations: SVL = snout-to-vent length (SVL); HW = head width, measured at the posterior angle of the jaws; HL = head length, measured from the rear of the mandible to the tip of the snout; SL = snout length, measured from the tip of the snout to the anterior border of the orbit; SN = distance from the center of the nostril to the tip of the snout; EL = eye length, the horizontal distance between the anterior and posterior borders of the orbit; IUE = inter-upper eyelid width, the shortest distance between the upper eyelids; UEW = maximum upper eyelid width; TYD = maximum tympanum diameter; TYE = distance from the anterior border of the tympanum to the posterior orbital border; FAL = forearm length, measured from the elbow to the wrist; HAL = hand length, measured from the wrist to the tip of the third digit; FIL = first finger length, measured from the tip of the first digit to its base where it joins the second digit; FIIL = second finger length, measured from the tip of the second digit to its base where it joins the first digit; FIIIL = third finger length, measured from the tip of the third digit to its base where it joins the second digit; FIVL = fourth finger length, measured from the tip of the fourth digit to its base where it joins the third digit; FIIIW = minimum third finger width taken at the base of the terminal portion of the digit, which is expanded in some species; FIIIDW = maximum width of the third fingertip; TIVW = minimum fourth toe width taken at the base of the terminal portion of the digit, which is expanded on some species; TIVDW = maximum width of the fourth toe tip; TL = thigh length, from the cloaca to the knee; SHL = shank length, from the knee to the ankle; TFOl = tarsus and foot length, from the ankle to the tip of the fourth digit; FOL = foot length, measured from the proximal end of the inner metatarsal tubercle to the tip of the fourth digit; IMT = length of the inner metatarsal tubercle.

	MZUHC 20035	MZUHC 20036	MZUHC 20037
SVL	29.3	31.0	30.1
HW	10.9	10.9	10.5
HL	10.8	11.1	11.2
SL	3.9	4.4	4.4
SN	2.2	2.8	2.9
EL	4.2	4.2	4.3
IUE	3.1	3.0	3.2
UEW	3.4	3.4	3.4
TYD	2.1	2.2	2.2
TYE	1.5	1.7	1.8
FAL	6.5	6.5	6.6
HAL	8.4	8.7	8.5
FIL	3.7	3.6	3.8
FIIL	3.4	3.4	3.5
FIIIL	5.7	5.8	5.9
FIVL	3.6	3.8	3.8
FIIIW	0.6	0.6	0.7
FIIIDW	0.9	0.9	0.9
TIVW	0.6	0.6	0.6
TIVDW	0.8	1.0	0.9
TL	14.2	15.5	15.0
SHL	14.6	15.6	16.2
TFOl	21.1	22.6	21.0
FOL	13.9	14.4	13.6
IMT	1.3	1.5	1.9



**Fig. 3.** Bayesian inference (BI) phylogeny of the 16S rRNA dataset. Posterior probability >90% marked with black dots; <90% indicated by gray dots.

**Table 2.** Uncorrected p-distances (%) between samples of Zunheboto Horned Frogs (*Xenophrys zunhebotoensis*) (16S rRNA) indicated by GenBank accession numbers (bold letters indicate topotypic samples) and locations.

	1	2	3	4	5	6	7	8
1 <b>KY022322</b> , Nagaland	—							
2 <b>MN734414</b> , Nagaland	0.0	—						
3 MN734416, Nagaland	0.0	0.0	—					
4 MN734417, Nagaland	0.0	0.0	0.0	—				
5 MN734418, Nagaland	0.0	0.0	0.0	0.0	—			
6 MN734415, Nagaland	0.0	0.0	0.0	0.0	0.0	—		
7 KY022321, Manipur	2.3	2.3	2.3	2.3	2.3	2.3	—	
8 MW650112, Mizoram	2.8	2.8	2.8	2.8	2.8	2.8	0.9	—

(GenBank KY022321) was only 0.9% (Table 2). As previously demonstrated by Mahony et al. (2020), the genetic distance between the samples from Nagaland and samples from Manipur and Mizoram is high (2.3–2.8%) but within the conventional threshold of species-level divergence for the 16S

rRNA gene (3.0%) used in other groups of anurans (Vences et al. 2005a, 2005b; Vieites et al. 2009).

The present record extends the range of this species about 160 km southwest of the closest known locality in Manipur. Recent studies on the amphibian fauna of Mizoram



**Fig. 4.** A female Common Warty Horned Frog (*Xenophrys serchhipii*) from Tamdil, Saitual District, Mizoram, India (MZUHC 20063). Photograph by Samuel Lalronunga.

(Lalronunga et al. 2020a, 2020b, 2020c) have led to a review of the faunal list. With the present record, six species of the genus *Xenophrys* have been recorded from Mizoram: Naga Hills Horned Frog (*X. awuh*), Jerdon's White-lipped Horned Frog (*X. major*), Tamenglong Horned Frog (*X. numhbumaeng*), Little Karen Hill Horned Frog (*X. parva*), Common Warty Horned Frog (*X. serchhipii*), and Zunheboto Horned Frog (*X. zunhebotoensis*) (Mathew and Sen 2007; Lalropeki, 2018; Lalronunga et al. 2020a, 2020b; Lalmuansanga 2020).

Lalropeki (2018) provided a brief account and photograph of *X. parva* (MZMU 1225) from the Tamdil wetland, Saitual District, Mizoram. However, the photograph and size (54.48 mm SVL) of the specimen correspond morphologically with *X. serchhipii*. In fact, we collected specimens of *X. serchhipii* (MZUHC 20057, MZUHC 20062–3; Fig. 4) during a herpetological survey at Tamdil. Furthermore, specimens identified as *X. parva* from India are erroneous (see Mahony et al. 2020) and the species is known with certainty only from the type locality (Karen Hills, southeastern Myanmar) (Mahony et al. 2020). Therefore, we remove *X. parva* from the amphibian faunal list of Mizoram.

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