



# On the Phylogenetic Relationships of the Indian Gliding Frog, *Pterorana khare* Kiyasetuo and Khare 1986 (Anura: Ranidae), with New Distributional Records from Mizoram, India

Lal Muansanga<sup>1</sup>, Ht Decemson<sup>1</sup>, Lal Biakzuala<sup>1</sup>, LH Laltlanhlui<sup>1</sup>, Fanai Malsawmdawngliana<sup>1</sup>, Gospel Zothanmawia Hmar<sup>1</sup>, Mathipi Vabeiryureilai<sup>1</sup>, Nachimuthu Senthil Kumar<sup>2</sup>, and Hmar Tlawmte Lalremsanga<sup>1</sup>

<sup>1</sup>Developmental Biology and Herpetology Laboratory, Department of Zoology, Mizoram University, Aizawl, 796004, India (htlrsa@yahoo.co.in)

<sup>2</sup>Department of Biotechnology, Mizoram University, Aizawl, 796004, India

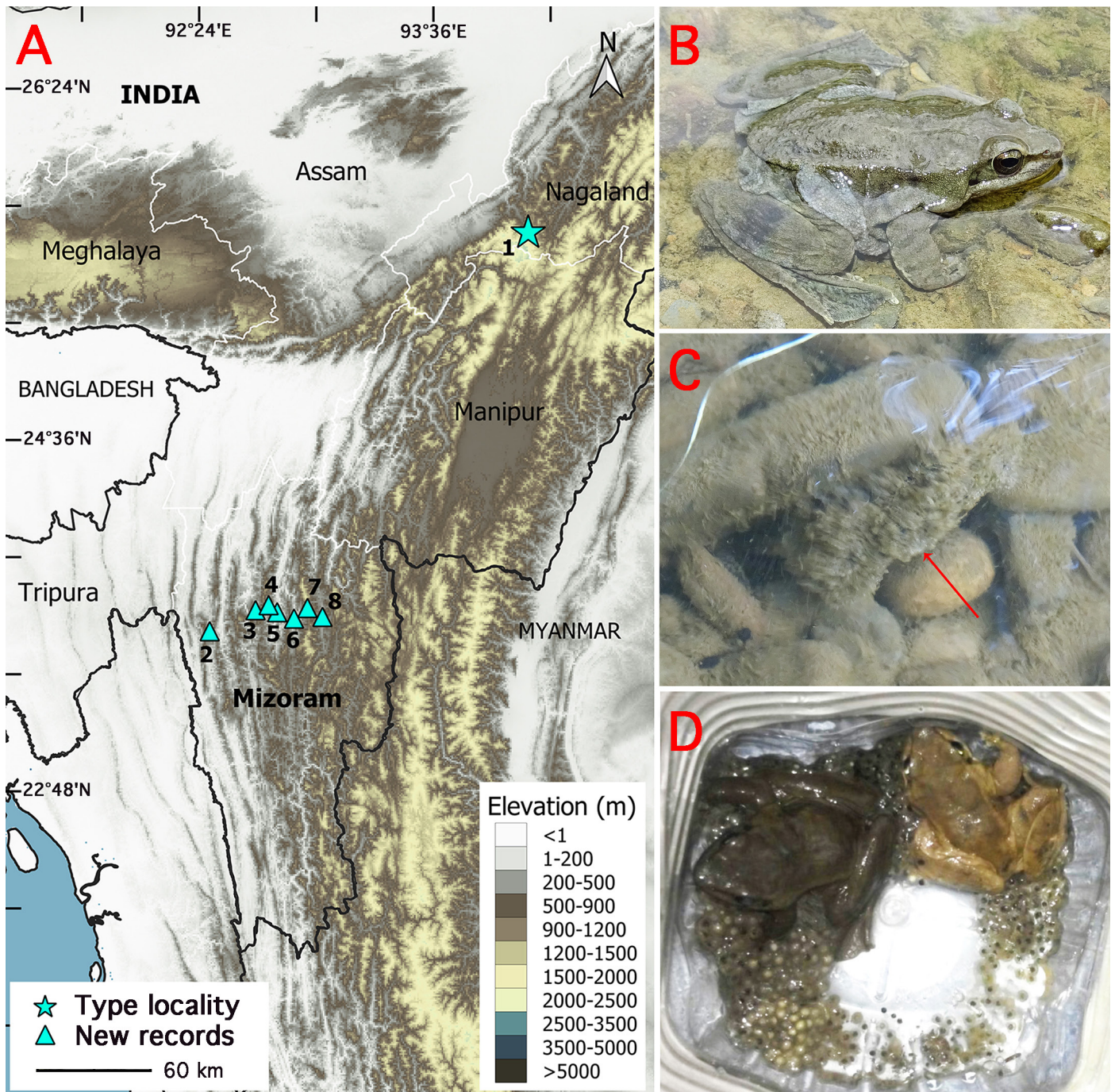
The Indian Gliding Frog (*Pterorana khare* Kiyasetuo and Khare 1986) was originally described from the Sanuoru and Rukhroma Rivers, Kohima, Nagaland, in northeastern India based on two males. Local people in the state considered this species to be an edible frog (Kiyasetuo and Khare 1986). *Pterorana* was considered a subgenus of *Rana* by Dubois (1992) but was later reassigned to the genus *Pterorana* based upon the distinct patagium (= lateral fold of skin in gliding animals) by Chanda et al. (2000). Sen and Mathew (2006) reported a female from Arunachal Pradesh, and they noted that the dermal skin flaps on the sides of the body and thighs in females are not as distinct as those of males. The range of *P. khare* extends from northeastern India to Myanmar (Frost 2021) and Bangladesh (Khan 2013). In India, it has been recorded from Arunachal Pradesh (Sen and Mathew 2006), Assam (Dutta, 2004), Nagaland (Kiyasetuo and Khare 1986), Manipur (Devi and Shamungou 2006), Meghalaya (Rangad et al. 2007), and Mizoram (Dey and Ramanujam 2003; Sen and Mathew 2003). Generally nocturnal and frequently associated with boulders, they are capable of gliding considerable distances (Chanda 2002). This species is listed as Vulnerable (VU) on the IUCN Red List (Dinesh et al. 2020). However, relatively little is known about the biology of this species and no genetic data have been available until now.

We collected *P. khare* from different parts of Mizoram (Fig. 1A) and deposited specimens in the Departmental Museum of Zoology, Mizoram University (MZMU). Individuals were identified using Kiyasetuo and Khare (1986) and Chanda (2002). Measurements to the nearest 0.1 mm were taken using a dial caliper (Mitutoyo™ 505-507).

We extracted genomic DNA from liver tissues using QIAamp DNA Mini Kit (Cat No. ID:51306) following the standard protocol provided by the manufacturer, performed

PCR amplification using forward (L02510) (Palumbi 1996) and reverse (H03063) (Rassmann 1997) primers, and generated partial 16S rRNA sequences from *P. khare* (MZMU 2068; accession number: MW547411) along with those of five other anuran species that were deposited in the GenBank repository as follows: Assam Forest Frog (*Hydrophylax leptoglossa*; MZMU 1818; accession number: MW165470), Indoburman Stream Frog (*Sylvirana lacrima*; MZMU 1632; accession number: MW440531), Indoburman Torrent Frog (*Amolops indoburmanensis*; MZMU 1753, MZMU 1784; accession numbers: MW165450, MW165459), Assam Hill Frog (*Clinotarsus alticola*; MZMU 1775; accession number: MW165453), and Asian Black-spined Toad (*Duttaphrynus melanostictus*; MZMU 1821; accession number: MW165455), which we used as an out-group. In our dataset, we included the seven sequences generated in addition to 35 sequences obtained from the NCBI database. All sequences were aligned by using the Muscle algorithm, and uncorrected p-distances were calculated using MEGA X software (Kumar et al. 2018). The Bayesian Inference (BI) phylogenetic reconstruction was carried out in Mr.Bayes 3.2.5 using the GTR+I+G model (Ronquist and Huelsenbeck 2003). The MCMC (one cold and three hot chains) was run for 20 million generations, terminating the analysis when the standard deviation of split frequencies become less than 0.01, sampling every 1,000 generations, the burn-in set to 25%, and the remaining trees used to assess Bayesian posterior probabilities (BPP) for nodal support.

We encountered *P. khare* most frequently as individuals or in small groups of four or five frogs in swift-flowing rivers and streams, clefts of rocks, and along riverbeds during the beginning of the dry season (mid-September to November). We heard advertisement calls of males (Fig. 1B) and encoun-



**Fig. 1.** Map of northeastern India (A) created using open-source QGIS version 3.16.2. The type locality of the Indian Gliding Frog (*Pterorana khare*) at the Sanuoru River, Kohima, Nagaland, India (1), is indicated by the blue star. New records of the species at the Teirei Stream, Dampa Tiger Reserve (2), Tuithum Stream (3), Tuipawl Stream (4), Tuiriial River (5), Tuirini River (6), Tamdil National Wetland (7), and Tuivawl River (8), Mizoram, India, are marked by blue triangles. (B) An adult male Indian Gliding Frog (*Pterorana khare*) (MZMU 2257) from Tuithum Stream, Mizoram. (C) A submerged egg mass from Tuithum Stream, Mizoram. (D) A breeding pair of Indian Gliding Frogs (*Pterorana khare*) from Tuithum Stream m, Mizoram. Photographs by Hmar Tlawmte Lalremsanga.

tered them among bushes, rock crevices, underneath boulders along flowing stream water, and in swift-flowing water from about 1600 h onward. The calls, an “onkk-onkk-onkk,” resemble a pig grunt and could be heard from a distance of about 20 m. On 27 October 2015, we found a breeding site with egg clutches (Fig. 1C) at Tuithum Stream in Aizawl District.

A pair in axillary amplexus was held overnight in a plastic container and the female laid eggs at about 1900 h (Fig. 1D). The eggs (n = 984) were pigmented, soft, and delicate, with approximate diameters of 1.0–1.2 mm. After taking measurements (male SVL = 63.1 mm; female = 64.0 mm), we released both adults and the egg mass in the same natural habitat the

**Table 1.** Data for ten male Indian Gliding Frogs (*Pterorana khare*) collected in Mizoram, India.

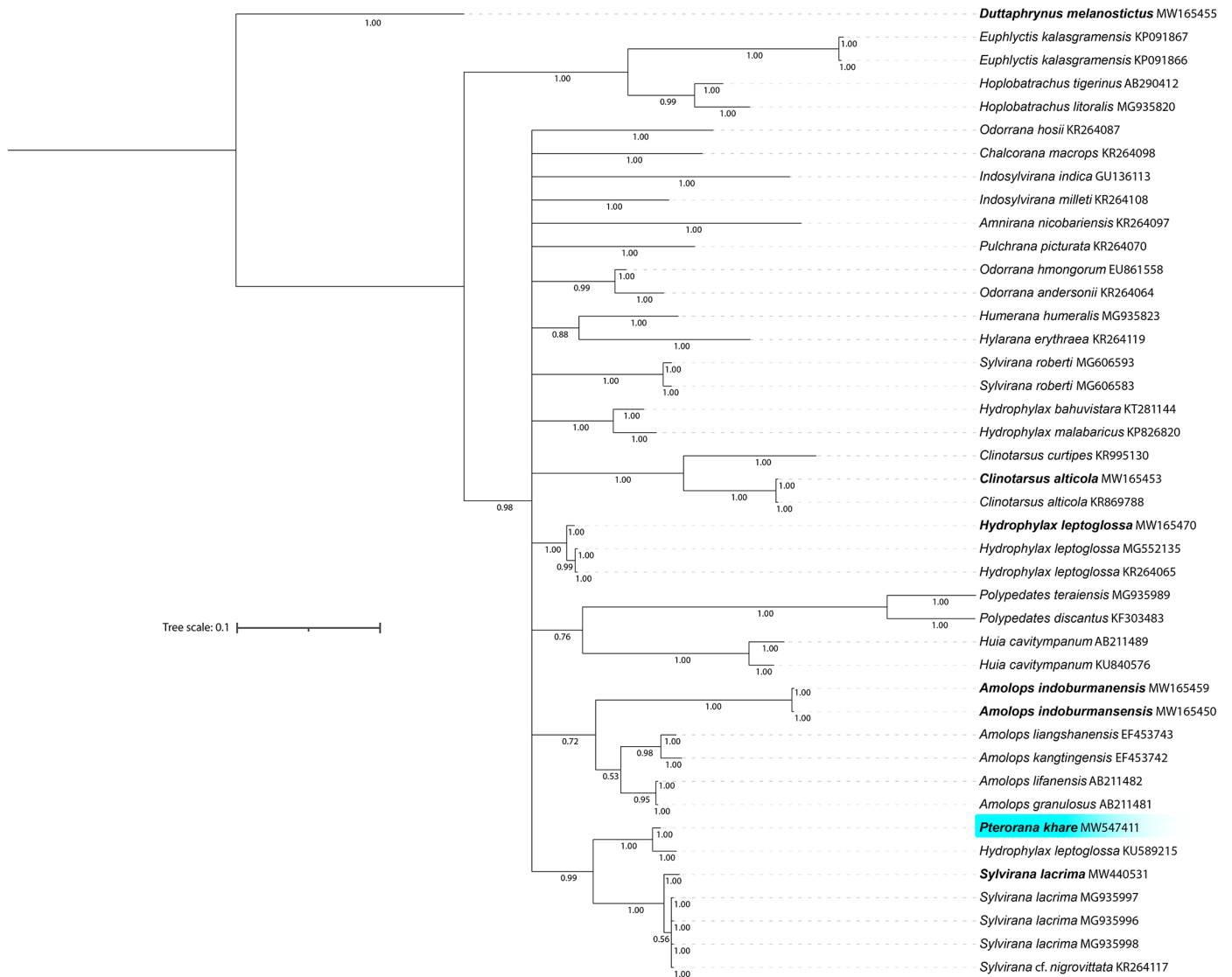
Locality	MZMU	MZMU	MZMU	MZMU	MZMU	MZMU	MZMU	MZMU	MZMU	MZMU	MZMU	MZMU	MZMU	MZMU	MZMU	
Tamdil National Wetland	2068	2147	2002	2144	2148	2146	1620	2145	2257	2258	Tuipawl Stream	Tuirei Stream, Dampa Tiger Reserve	Tuivawl River	Tuirial River	Tuithum Stream, AICS	
Elevation (m asl)	779	572	254	462	454	145	713									
Location	23°44'22.29"N 92°57'15.52"E	23°45'14.10"N 92°45'19.58"E	23°41'7.68"N 92°53'9.33"E	23°37'13.74"N 92°27'13.01"E	23°41'38.83"N 93°1'52.94"E	23°43'4.93"N 92°47'58.44"E	23°43'41.78"N 92°41'19.12"E									
Snout-vent length	61.3	56.8	63.5	59.5	56.5	58.3	62.1	58.6	63.5	64.2						
Head length	26.2	24.4	28.1	27.2	23.7	25.6	28.6	21.9	22.9	25.9						
Head width	23.1	22.5	24.8	23.7	23.4	24.1	24.8	20.4	25.3	24.2						
Thigh-flap width	9.0	12.7	21.2	7.7	11.6	7.5	20.7	11.5	22.1	17.3						
Femur length	25.9	31.4	33.9	29.5	29.8	30.1	30.8	30.5	36.8	35.7						
Lateral body-flap width	19.6	9.6	21.3	9.5	10.1	7.6	22.6	12.5	24.2	17.3						

next day. Details of sample collection sites and morphological data for the ten specimens collected are in Table 1.

*Pterorana khare* is most closely related to *H. leptoglossa* (KU589215; voucher IASST AR79) from Nagaland, with a minimal uncorrected p-distance of 0.019 (Table 2). Our BI inference phylogenetic tree (Fig. 2) revealed a well-supported clade (BPP = 1.00) of *P. khare* + *H. leptoglossa* and an inter-clade relationship between *P. khare* + *H. leptoglossa* and *S. lacrima* + *S. cf. nogrovittata* that also was well supported (BPP = 0.99). In the BI topology, other sequences from Mizoram also were demonstrated to be highly supported nodes with respect to their lineages: *H. leptoglossa* (MW165470) from Palak National Wetland (Mizoram) clustered with conspecific sequences from Myanmar (MG552135) and a specimen in the American Museum of Natural History (AMNH) (KR264065; voucher CAS239886) with a BPP of 0.99, and p-distances of 0.093, 0.096, and 0.101 were diagnosed between the *P. khare* and *H. hydrophylax* sequences from Myanmar, Mizoram (India), and a specimen from the AMNH (CAS239886), respectively; *S. lacrima* (MW440531) from the Dampa Tiger Reserve (Mizoram) was clustered with a group consisting of *S. lacrima* + *S. cf. nogrovittata* (BPP = 1.00) and an uncorrected p-distance of 0.073 from *P. khare*; two sequences of *A. indoburmanensis* sampled from Sialsuk (MW165459) and the MZU Campus (MW165450) in Mizoram formed a distinct lineage and are diagnosed as a sister species to the group of *A. liangshanensis* + *A. kangtingensis* + *A. lifanensis* + *A. granulatus* (BPP = 0.72) and the uncorrected p-distance between *P. khare* and the two samples of *A. indoburmanensis* was 0.169; *C. alticola* (MW165453) from the Pualreng Wildlife Sanctuary (Mizoram) grouped with a conspecific (KR869788) from Assam (BPP = 1.00) and had a p-distance of 0.158 from *P. khare*.

According to our genetic divergences and phylogenetic topology, *P. khare* is closely related to the genus *Hydrophylax*. This agrees with the morphologically based hypothesis of Ao et al. (2006), who stated that the genus *Pterorana* is closely related to the genus *Hydrophylax* in having humoral glands (lacking in *Sylvirana*), but does not fall within either *Hydrophylax* or *Sylvirana* due to the lack of beard-like papillae on the lower lips of larvae, which are an apomorphic character for *Hydrophylax* + *Sylvirana* (Grosjean 2004). Furthermore, our analysis suggests the existence of two different lineages within the 16S rRNA sequences of *H. leptoglossa*, which is suggestive of cryptic intraspecies genetic diversity between populations or, less likely, the presence of an unrecognized species within the known range of *H. leptoglossa*. Consequently, we recommend additional morphological and molecular investigations to elucidate the precise systematic position of *P. khare* and to address the taxonomic status of species of *Hydrophylax* in northeastern.





**Fig. 2.** Bayesian inference tree based on a 16S rRNA gene fragment showing the phylogenetic position of the Indian Gliding Frog (*Pterorana khare*). Sequences generated in this study are in bold type. Numbers at each node represent Bayesian posterior probability values.

Moreover, the location listed by Dey and Ramanujam (2003) at the Tlawng River, (Sairang; 23°36'N, 93°00'E; elev. 2,000–2,500 m asl), 21 km from Aizawl in north-western Mizoram, should be corrected. Using a Garmin Montana 650 GPS unit, we recorded the location of Sairang as 23°48'58.45"N, 92°39'9.26"E (elev. 60–65 m asl). Also, according to Pachuau (2013), no elevations in the vicinity of that site or along the entire length of Tlawng River drainage (185.5 km) exceed 1,395 m asl, and that elevation occurs only at the headwaters at Zobawk, Lunglei District, over 100 km aerial distance south of Sairang. Therefore, the lower elevation in the range for the species is likely to be 26 m asl (vs. 200–1,400 m asl in Frost 2021) at the Dhaleswari River, Bairabi, Mizoram (Sen and Mathew 2003).

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