REPTILES & AMPHIBIANS

First Record of the Nagaland Montane Torrent Toad, *Duttaphrynus chandai* Das, Chetia, Dutta, and Sengupta 2013 (Anura: Bufonidae), from Mizoram, India, with Comments on Phylogenetic Relationships

Hmar Tlawmte Lalremsanga[1], Lal Muansanga[1], Mathipi Vabeiryureilai[1], Lal Biakzuala[1], Lal Rinsanga[1],
John Zothanzama[2], Nachimuthu Senthil Kumar[3], and Jayaditya Purkayastha[4]

[1]Department of Zoology, Mizoram University, Aizawl-76004, Mizoram India
[2]Department of Environmental Sciences, Mizoram University, Aizawl-76004, Mizoram India
[3]Department of Biotechnology, Mizoram University, Aizawl-76004, Mizoram India
[4]Help Earth, Raghunath Choudhury Path, Lachitnagar, Guwahati 781007, Assam, India (mail.jayaditya@gmail.com)

The genus *Duttaphrynus* is comprised of 26 currently recognized species of which 18 have been reported from India and ten occur in the northeastern part of the country (Frost 2020). The Nagaland Montane Torrent Toad (*Duttaphrynus chandai*) has been known only from a small area of southwestern Nagaland near the border of Manipur from Khonoma, Dzuleke, and Thekhekhwei Hill at elevations of 1,568–1,787 m asl (Das et al. 2013). Herein we report the first occurrence of the species from the state of Mizoram and establish its relation with congeners using morphological and genetic methods.

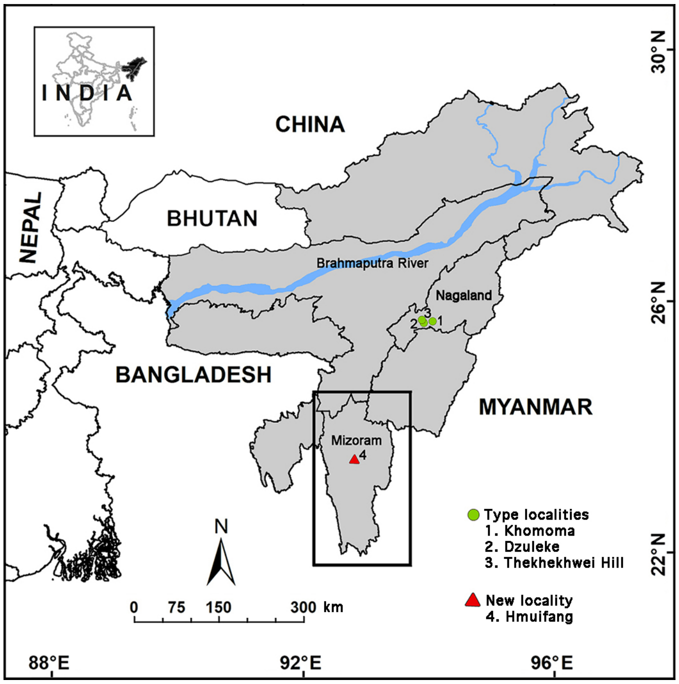

Fig. 1. Map showing documented locality records of the Nagaland Montane Torrent Toad (*Duttaphrynus chandai*) in India. Green dots mark the type localities in Nagaland and the red triangle indicates the new locality in Mizoram.

We collected five toads (Departmental Museum of Zoology, Mizoram University, MZMU 884–7, 1499) from the Hmuifang Community Reserve Forest, Mizoram (Fig. 1): MZMU 884 (Fig. 2) from a hole in a tree trunk (23°45'36.63"N; 92°75'20.20"E; 1,484 m asl) on 27 August 2010, MZMU 885 from a tree about 1.5 m above the ground (23°45'46.89"N; 92°75'20.69"E; 1,480 m asl) on 28 August 2010, MZMU 886 from the forest floor (23°45'39.16"N;

Fig. 2. A Nagaland Montane Torrent Toad (*Duttaphrynus chandai*; MZMU 884) from the Hmuifang Community Reserve Forest, Mizoram, India. Photograph by H.T. Lalremsanga.

Table 1. Morphometric data for Nagaland Montane Torrent Toads (*Duttaphrynus chandai*) from Hmuifang Community Reserve Forest, Mizoram, India. SVL, snout-vent length (from tip of snout to vent); SL, snout length (from the anterior corner of eye to snout tip); EN, eye to nostril distance (distance between anteriormost point of eyes and nostrils); INS, internarial space (distance between nostrils); IOS, interorbital space (least distance between upper eyelids); ED, eye diameter (horizontal diameter of the eyes); HL, head length (distance between angle of jaws and snout-tip); HWAJ, head width at angle of jaw (width of head at the level of jaw angle); HDN, head depth at nostril (depth of head at the level of nostril); PGL, paratoid length (distance between anterior corner to the posterior corner of paratoid gland); PGW, paratoid width (maximum width of the paratoid gland), P-P inter-paratoid distance (minimum distance between the paratoid glands); FLL, forelimb length (distance between elbow and base of outer tubercle); F1 to F4, length of 1st to 4th fingers (from the base of the outer metacarpal tubercle to the tip of the respective finger); IMC length of inner metacarpal tubercle (greatest length of inner metacarpal tubercle); OMC length of outer metacarpal tubercle (greatest length of outer metacarpal tubercle); HLL, hind limb length (from groin to tip of 4th toe); TBL, tibia length (distance between surface of knee and surface of heel, with both tibia and tarsus flexed); TBW, tibia width (maximum width of tibia along its length); T1 to T5, length of 1st to 5th toes (from the base of the outer metatarsal tubercle to the tip of the respective toe); IMT, length of inner metatarsal tubercle (greatest length of inner metatarsal tubercle); OMT, length of outer metatarsal tubercle (greatest length of outer metatarsal tubercle). All measurements are in mm.

MZMU	**884**	**885**	**886**	**887**	**1499**
SEX	F	F	M	M	F
SVL	70.4	75.3	59.8	55.2	48.1
SL	8.2	6.7	6.5	6.4	6.1
EN	4.2	4.3	3.6	4.3	3.8
INS	4.7	4.4	4.2	3.3	3.5
IOS	8.4	8.4	6.6	6.3	4.7
ED	8.5	8.6	7.0	8.2	5.1
HL	21.7	22.3	18.4	19.1	13.1
HWAJ	28.1	28.7	20.0	22.1	16.6
HDN	5.8	6.4	5.4	5.2	4.3
FLL	50.5	52.4	38.8	39.8	28.0
F1	16.2	14.4	9.8	9.7	7.8
F2	14.4	14.2	9.6	9.6	7.7
F3	17.2	20.3	15.8	14.1	12.1
F4	16.5	18.2	13.0	13.2	9.9
IMC	3.1	3.2	2.9	2.4	2.2
OMC	4.5	4.8	3.4	3.2	2.9
HLL	93.2	82.6	71.8	68.7	50.2
TBL	30.6	28.6	22.5	21.8	16.9
TBW	9.5	8.2	7.9	6.0	3.7
T1	12.2	11.5	11.3	8.2	6.6
T2	17.3	16.7	12.4	11.4	8.7
T3	23.0	22.9	16.7	16.4	12.1
T4	31.6	30.8	22.4	21.6	17.5
T5	21.8	21.7	15.2	16.2	11.4
IMT	3.3	3.7	3.6	3.2	2.7
OMT	3.2	3.1	2.3	2.1	1.7
PGL	20.9	19.6	14.5	14.4	11.8
PGW	6.6	5.9	5.4	5.4	3.8
P-P	10.8	10.6	7.9	7.8	6.7

92°75'22.51"E; 1,481 m asl) on 14 April 2015, MZMU 887 from a drain near the lodge buildings (23°45'48.02"N; 92°75'12.82"E; 1,483 m asl) on 20 April 2016, and MZMU 1499 from beneath the log in a forested area adjacent to a stream (23°45'61.28"N; 92°75'39.86"E; 1,431 m asl) on 12 September 2019. These first records of the species from Mizoram extend the range of the species by ca. 285 airline km southwestward of the type locality of the species at Khonoma, Nagaland, India.

In the original description (Das et al. 2013), *Duttaphrynus chandai* was described as a large toad (SVL 61.2–88.1 mm) with a middorsal line and a head much wider than long; tympanum hidden or indistinct; paratoid gland broad and elongated; finger 1<2; and preorbital, postorbital, and parietal cephalic ridges present (Das et al. 2013). Morphometric data from our specimens are provided in Table 1. These are large toads (SVL 48.1–75.3 mm) with large heads (HL/SVL 0.33–0.40; mean 0.37); heads wider than long (HL/HW 0.77–0.92; 0.82); snouts more than one third of head length (SL:HL 0.30–0.46; 0.37); nostrils closer to snout than eye (EN/SL 0.51–0.67; 0.60); internarial distances more than half the interorbital distances (INS/IOS 0.52–0.74; 0.59); eyes large (ED/HL0.38–0.43; 0.40); paratoid glands large, about one fourth SVL (PGL/SVL 0.24–0.30; 0.26) and one third as long as wide (PGW/PGL 0.30–0.38; 0.34); forelimbs long (FLL/SVL 0.58–0.72; 0.67); relative finger length II<I<IV<III; both inner and outer metacarpal tubercles present; tibiae long (TBL/SVL 0.35–0.43; 0.39); tibia-tarsus articulations reach two thirds of the posterior paratoid glands; heels do not meet when folded at right angles; relative toe length 1<2<5<3<4; toes webbed; dorsum and dorsal surfaces of limbs with spiny and large blunt warts; limbs with warts and tubercles of various sizes decreasing distally; and pupil horizontally elliptical.

In life (Fig. 2), the dorsum is dark brown with black blotches, darker between the paratoid glands, and lighter laterally; canthus yellow with black ridges; paratoids with dark blotches and tiny yellow dots; pale yellow middorsal line extends from snout to vent; upper surfaces of fore- and hindlimbs pale brown with dark brown patches; phalanges pale brown; tips of digits dark brown; venter dirty white with variegated grayish patches, chin and throat darker with dusty white specks; and iris golden brown.

Specimens were fixed in 4% formalin and preserved in 70% ethanol. Liver tissues were used for genetic analysis. Partial sequences (to 3882 nucleotides) for the 16s rRNA gene were generated using the primers (forward primer [L02510]–CGCCTGTTTATCAAAAACAT; reverse primer [H03063]–CTCCGGTTTGAACTCAGATC; Palumbi 1996) and compared with congeners using published 16s rRNA sequence data (Ao et al. 2003; Bocxlaer et al. 2009; Meegaskumbura et al. 2015; Portik and Papenfuss 2015; Table 2). Sequences were aligned with Mega 7 using the muscle algorithm with default parameter settings (Kumar et al. 2016). Phylogenetic relationships were reconstructed using Maximum Likelihood (ML) and IQ TREE (Nguyen et al. 2015) with 1,000 ultrafast bootstrap (Hoang et al. 2017) using TIM2+F+G4 (Kalyaanamoorthy et al. 2017). Uncorrected p-distance was calculated in MEGA 7 (Kumar et al. 2016).

Table 2. List of 16s rRNA sequence data used in this study.

Species	Gene Bank Accession No.	Reference
Duttaphrynus atukoralei	KM921792	Meegaskumbura et al. 2015
Duttaphrynus brevirostris	FJ882786	Bocxlaer et al. 2009
Duttaphrynus chandai	MT705015	This study
Duttaphrynus crocus	FJ882789	Bocxlaer et al. 2009
Duttaphrynus dhufarensis	KT031498	Portik and Papenfuss 2015
Duttaphrynus himalayanus	FJ882790	Bocxlaer et al. 2009
Duttaphrynus hololius	FJ882781	Bocxlaer et al. 2009
Duttaphrynus melanostictus	KU589218	Ao et al. 2003
Duttaphrynus olivaceus	KT031509	Portik and Papenfuss 2015
Duttaphrynus parietalis	FJ882784	Bocxlaer et al. 2009
Duttaphrynus scaber	FJ882785	Bocxlaer et al. 2009
Duttaphrynus stomaticus	FJ882787	Bocxlaer et al. 2009
Duttaphrynus stuarti	FJ882788	Bocxlaer et al. 2009
Ingerophrynus divergens	FJ882802	Bocxlaer et al. 2009

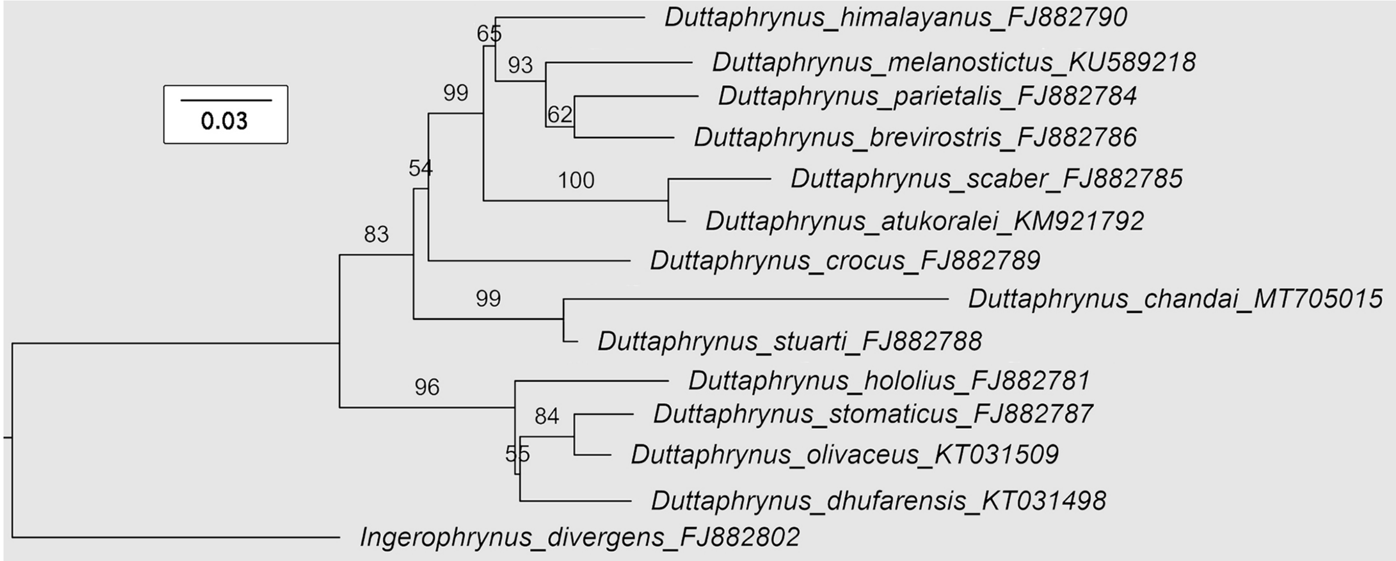

Fig. 3. Maximum-likelihood phylogeny of selected species of toads in the genus *Duttaphrynus* with *Ingerophynus divergenes* as the outgroup. Numbers at nodes represent bootstrap support.

Table 3. Pairwise uncorrected 16s rRNA sequence divergence (%) between selected species of toads in the genus *Duttaphrynus* with *Ingerophynus divergens* as the outgroup.

D. chandai													
D. stuarti	6.54												
D. himalayanus	10.29	8.61											
D. melanostictus	10.46	7.20	5.3										
D. crocus	10.82	8.33	9.14	6.67									
D. scaber	12.42	10.71	9.46	7.53	10.64								
D. hololius	12.93	11.06	11.36	9.08	10.83	11.42							
D. parietalis	11.53	8.83	8.04	5.14	9.63	9.47	11.73						
D. brevirostris	11.18	9.16	7.71	4.29	9.12	9.80	11.65	5.77					
I. divergens	15.64	13.96	13.52	11.67	13.92	13.81	14.13	14.38	14.42				
D. stomaticus	12.39	10.5	11.16	9.08	10.57	11.31	6.74	11.1	11.44	13.66			
D. olivaceus	9.89	4.29	4.29	5.45	5.27	6.05	3.89	5.45	4.48	6.81	1.56		
D. dhufarensis	10.08	4.48	5.07	5.45	5.66	6.83	4.67	5.26	4.87	6.43	3.12	2.53	
D. atukoralei	10.47	6.79	5.61	5.23	6.39	2.52	7.76	5.24	5.04	12.24	7.95	5.42	5.20

Maximum-likelihood (ML) phylogenetic reconstruction (Fig. 3) revealed *D. chandai* to be a well-supported sister taxon to *D. stuarti*, differing from other sampled species in the genus *Duttaphrynus* by an uncorrected pairwise sequence divergence of 6.54–12.93%, and with *D. stuarti* and *D. hololius* being the least and the most divergent species, respectively (Table 3).

Acknowledgements

We thank the Chief Wildlife Warden, Environment, Forest and Climate Change Department, Government of Mizoram, India, for issuing collection permit No. A.33011/2/99-CWLW/225. We also express our sincere thanks to the Department of Biotechnology (DBT), Government of India, New Delhi, for providing financial assistance for laboratory facilities under NER-BPMC (North Eastern Region - Biotechnology Programme Management Cell) number DBT-NER/AAB/64/2017. We also appreciated Gospel Zothanmawia Hmar, Lalruatthara, H. Laltlanchhuaha and Zothangliana for their assistance in the fieldwork.

Literature Cited

Ao, J.M., S. Bordoloi, and A. Ohler. 2003. Amphibian fauna of Nagaland with nineteen new records from the state including five new records for India. *Zoos' Print Journal* 18: 1117–1125.

Bocxlaer, I.V., S. Biju, S.P. Loader, and F. Bossuyt. 2009. Toad radiation reveals into-India dispersal as a source of endemism in the Western Ghats-Sri Lanka biodiversity hotspot. *BMC Evolutionary Biology* 9: 131. https://doi.org/10.1186/1471-2148-9-131.

Das, A., M. Chetia, S.K. Dutta, and S. Sengupta. 2013. A new species of *Duttaphrynus* (Anura: Bufonidae) from Northeast India. *Zootaxa* 3646: 336–348. https://doi.org/10.11646/zootaxa.3646.4.2.

Frost, D.R. 2020. Amphibian Species of the World: An Online Reference. Version 6.1. American Museum of Natural History, New York, New York, USA. https://doi.org/10.5531/db.vz.0001. <https://amphibiansoftheworld.amnh.org/index.php>.

Hoang, D.T., O. Chernomor, A.V. Haeseler, B.Q. Minh, and L.S. Vinh. 2017. UFBoot2: Improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution* 35: 518–522. https://doi.org/10.1093/molbev/msx281.

Kalyaanamoorthy, S., B.Q. Minh, T.K.F. Wong, A.V. Haeseler, and L.S. Jermiin. 2017. ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nature Methods* 14: 587–589. https://doi.org/10.1038/nmeth.4285.

Kumar, S., G. Stecher, and K. Tamura. 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for bigger datasets. Molecular Biology and Evolution 33: 1870–1874. https://doi.org/10.1093/molbev/msw054.

Meegaskumbura, M., G. Senevirathne, N. Wijayathilaka, B. Jayawardena, C. Bandara, K. Manamendra-Arachchi, and R. Pethiyagoda. 2015. The Sri Lankan torrent toads (Bufonidae: Adenominae: Adenomus): Species boundaries assessed using multiple criteria. *Zootaxa* 3911: 245–261. https://doi.org/10.11646/zootaxa.3911.2.6.

Nguyen, L.T., H.A. Schmidt, A.V. Haeseler, and B.Q. Minh. 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. *Molecular Biology and Evolution* 32: 268–274. https://doi.org/10.1093/molbev/msu300.

Palumbi, S.R. 1996. Nucleic acids II: the polymerase chain reaction, pp. 205–247. In: D.M. Hillis, C. Moritz, and B.K. Mable (eds.), *Molecular Systematics*. Sinauer Associates, Sunderland, Massachusetts, USA.

Portik, D.M. and T.J. Papenfuss. 2015. Historical biogeography resolves the origins of endemic Arabian toad lineages (Anura: Bufonidae): Evidence for ancient vicariance and dispersal events with the Horn of Africa and South Asia. *BMC Evolutionary Biology* 15: 152. https://doi.org/10.1186/s12862-015-0417-y.