REPTILES & AMPHIBIANS

On the Occurrence of the Myanmar Brown Leaf Turtle, *Cyclemys fusca* (Testudines: Geoemydidae), from Manipur, India

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Species in the genus *Cyclemys* (Bell 1834) (Geoemydidae) are small to medium-sized semi-aquatic turtles (Iverson 1992; Fritz et. al. 2008) that form a morphologically distinct group (Praschag et al. 2009). These species are distributed throughout southern and southeastern Asia (Fritz et al. 2008) and, to date, *C. gemeli* and *C. dentata* are the only species documented from India (Aengals et al. 2018).

The holotype of the Myanmar Brown Leaf Turtle (*Cyclemys fusca*) was obtained by M. Reimann in 2000 via the international pet trade. The species was described by Fritz et al. (2008) from the adult female specimen deposited in the Museum of Zoology Dresden (MTD D 42578). Based on five specimens, *C. fusca* was subsequently reported from the vicinity of Kyin Dwe Village, Kachin, Myanmar (Piatt et al. 2012), but knowledge of the species' distribution is limited; they have been thought to occur only in northern Myanmar but were suspected to occur in Bangladesh and adjacent India (Fritz et al. 2008; Uetz et al. 2024). They inhabit freshwater systems, primarily rivers and swamps (Uetz et al. 2024), and at present the species is listed as Least Concern (LC) on the IUCN Red List of Threatened Species (Platt et al. 2021).

At about 2015 h on 6 November 2022, a subadult female *Cyclemys* sp. (Fig. 1; Table 1) was collected in the downstream portion of the Chakpi River, Chandel District, Manipur, India (24.296226, 93.974557; elev. 876 m asl), where it was on the muddy bank of a backwater. This turtle had a deformed left hindlimb that had developed only as far as the pelvic region (Fig. 2). The animal was euthanized, preserved in 70% ethanol, and deposited in the Departmental Museum of Zoology, Mizoram University (MZMU 3048).

The collection site is heavily utilized for homestead gardening and paddy fields by the inhabitants of Modi and Lamphou Pasna, two nearby villages. Nonetheless, this site is an important part of the Indo-Burma biodiversity hotspot that supports a diverse flora and fauna. Mean annual rainfall is 1,138–1,548 mm (Directorate of Environment and Climate Change, Government of Manipur 2023). Based on the classification of Champion and Seth (1968), vegetation

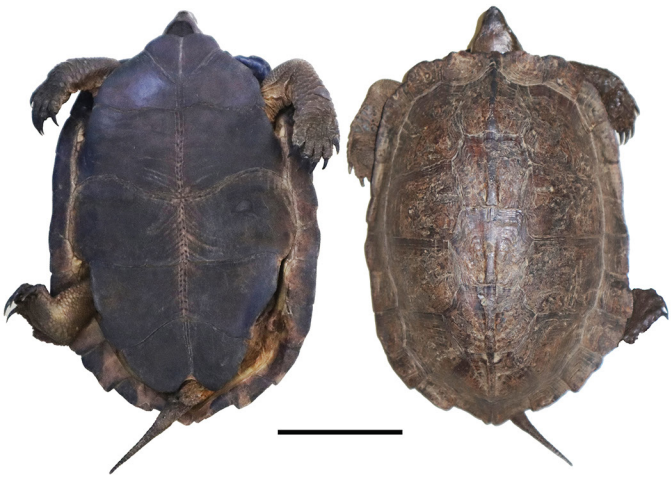

Figure 1. Ventral and dorsal views of a subadult Myanmar Brown Leaf Turtle (*Cyclemys fusca*) collected in Chandel District, Manipur, India. Scale bar = 130 mm. Photographs by Gospel Zothanmawia Hmar.

Figure 2. Abnormal left hindlimb of a subadult Myanmar Brown Leaf Turtle (*Cyclemys fusca*) from Chandel District, Manipur, India. Photograph by Gospel Zothanmawia Hmar.

Reptiles & Amphibians ISSN 2332-4961
https://doi.org/10.17161/randa.v31i1.20816

Table 1. Morphometrics of a Myanmar Brown Leaf Turtle (*Cyclemys fusca*) from Manipur, India. Measurements in mm, weight in g.

Carapace length	198
Carapace width between 7th and 8th marginals	164
Shell height	60
Plastron length (maximum)	163
Plastron length (mid-seam)	158
Gular mid-seam length	25
Humeral mid-seam length	16
Pectoral mid-seam length	40
Abdominal mid-seam length	30
Femoral mid-seam length	27
Anal mid-seam length	28
Weight	650

is sub-tropical semi-evergreen and sub-tropical hill forests, mostly dominated by *Lantana camara*, *Mikania micrantha*, *Quercus* sp., *Macaranga denticulata*, *Schima wallichii*, *Pinus* sp., *Salix tetrasperma*, bamboo, small grasses, and ferns.

Whole DNA was extracted from tissue samples (MZMU3048_OQ473649) using QIAamp DNA Mini Kit (Cat No. ID: 51306; Qiagen, Valencia, California, USA) in accordance with the manufacturer's instructions. The mitochondrial cytochrome b (cytb) gene was amplified and sequenced using forward primers (L14910-5'-GAC CTG TGA TMT GAA AAA CCA YCG TTG T-3') and reverse primer (H16064-5'-CTT TGG TTT ACA AGA ACA ATG CTT TA-3') (Burbrink et al. 2000). The PCR protocol for amplification consisted of an initial 3 min at 94 °C followed by 35 cycles of 30 sec at 94 °C for denaturation, 30 sec for annealing at 50.3 °C, elongation for 1 min at 72 °C, and a final elongation for 5 min at 72 °C. The amplicons were observed in 1.5% agarose gel containing ethidium bromide under UV light. To ascertain the identity of our sample through DNA-barcoding, we retrieved the cytb sequences of congeners from NCBI Genbank, using *Heosemys spinosa* (JN797982) as the outgroup. The sequences were aligned using the MUSCLE algorithm and uncorrected pairwise genetic distances were calculated in MEGA-X (Kumar et al. 2018). A maximum-likelihood phylogenetic tree was constructed by executing 1,000 rapid bootstrap replicates with ML search using the GTR+GAMMA model in RAxML 7.3.0 (Stamatakis et al. 2008) as implemented in raxmlGUI 2 (Silvestro and Michalak 2012). The Bayesian Inference (BI) phylogenetic tree was generated in MrBayes 3.2.5 with the GTR+G model. The MCMC (one cold and three hot chains) was run for 1,000,000 generations, with sampling every 500 generations and a burn-in of 25%. The analysis terminated when the standard deviation of split frequencies was less than 0.001. The percentage

Table 2. Uncorrected k2p- distance of *Cyclemys* spp. using partial cytb gene sequences.

No.	Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	OQ473649_*C. fusca*	—																			
2	AM931651_*C. fusca*	0.00																			
3	JX218031_*C. fusca*	0.00	0.00																		
4	JQ266014_*C. fusca*	0.00	0.00	0.00																	
5	FM877769_*C. gemeli*	0.06	0.06	0.06	0.06																
6	FM877768_*C. gemeli*	0.06	0.06	0.06	0.06	0.00															
7	AM931646_*C. enigmatica*	0.11	0.11	0.11	0.11	0.11	0.11														
8	AM931645_*C. enigmatica*	0.11	0.11	0.11	0.11	0.11	0.11	0.00													
9	JN860660_*C. dentata*	0.11	0.11	0.11	0.11	0.11	0.11	0.00	0.00												
10	JN582334_*C. dentata*	0.12	0.12	0.12	0.12	0.12	0.12	0.01	0.01	0.01											
11	AJ604513_*C. oldhamii*	0.11	0.11	0.11	0.11	0.10	0.10	0.07	0.07	0.07	0.07										
12	JQ277464_*C. oldhamii*	0.12	0.11	0.11	0.11	0.11	0.11	0.07	0.07	0.07	0.08	0.00									
13	NC023221_*C. oldhamii*	0.12	0.11	0.11	0.11	0.11	0.11	0.07	0.07	0.07	0.08	0.00	0.00								
14	AM931692_*C. pulchristriata*	0.12	0.12	0.12	0.12	0.11	0.11	0.05	0.05	0.04	0.05	0.08	0.08	0.08							
15	AM931693_*C. pulchristriata*	0.12	0.12	0.12	0.12	0.11	0.11	0.05	0.05	0.04	0.05	0.08	0.08	0.08	0.01						
16	MT366592_*C. atripons*	0.12	0.12	0.12	0.12	0.13	0.13	0.03	0.03	0.03	0.03	0.08	0.08	0.08	0.05	0.05					
17	MT366590_*C. atripons*	0.12	0.12	0.12	0.12	0.13	0.13	0.03	0.03	0.03	0.03	0.08	0.08	0.08	0.05	0.05	0.00				
18	AM931678_*C. oldhamii*	0.11	0.11	0.11	0.11	0.10	0.11	0.07	0.07	0.07	0.07	0.00	0.01	0.01	0.08	0.08	0.08	0.08			
19	AM931679_*C. oldhamii*	0.11	0.11	0.11	0.11	0.10	0.10	0.07	0.07	0.07	0.07	0.00	0.00	0.00	0.08	0.08	0.08	0.08	0.00		
20	JN797982_*H. spinosa*	0.14	0.14	0.14	0.14	0.13	0.14	0.14	0.14	0.15	0.15	0.14	0.14	0.14	0.15	0.14	0.15	0.15	0.14	0.14	—

of trees with associated taxa was displayed next to the branches (Ronquist and Huelsenbeck 2003) and the phylogenetic tree was illustrated with Figtree v1.4.4 software.

The final alignment of the cytb gene contained 970 aligned nucleotides, of which 758 sites were conserved and 212 sites were variable, and of which 161 were parsimony-informative. The estimated Transition/Transversion bias (R) was 7.94. Substitution pattern and rates were estimated under the Kimura (1980) 2-parameter model (+G). Nucleotide frequencies were 29.00% (A), 27.30% (T), 31.00% (C), and 12.70% (G). *Cyclemys fusca* nucleotide divergences and congener sequences are summarized in Table 2. The sample from Manipur, India, and that of the holotype from Kachin, Myanmar, differed 0.001% from other known populations of the species. Interspecific divergence in species of *Cyclemys* ranged from 0.00% to 0.12% depending on distribution (Table 2). The ML phylogeny (Fig. 3) and the Bayesian Inference (BI) tree yielded similar topologies, providing strong support for the clustering of all populations of *C. fusca* and for *C. fusca* and *C. gemeli* forming a monophyletic group.

These results confirm the occurrence of *Cyclemys fusca* in Manipur, India, which represents the first record for the state and the country (Fig. 4). The closest known locality, Elephant Camp, Alaungdaw Kathapa National Park, Sagaing Division, Myanmar, is approximately 226 km to the south, and Kyin Dwe Village, the farthest site, is ~368 km to the south. Few museum specimens are available and the geographic distribution is poorly understood and remains to be fully resolved (Piatt et al. 2012). Complicating matters, Kundu et al. (2016) had suggested that multiple individuals of *C. fusca* kept as pets in public houses in Mizoram represented the importation of a non-native species. However, as studies within the last decade have revealed new records of reptiles and amphibians in the state (e.g., Decemson et al. 2021a, 2021b, 2021c, 2021d, 2022, 2023; Hmar et al. 2022; Lalremsanga et al. 2017, 2021, 2022; Siammawii et al. 2022; Tariang et al. 2022), additional surveys along with systematics research are needed for adequate herpetological knowledge of the region.

Acknowledgements

We thank the Principal Chief Conservator of Forests, Government of Manipur, for issuing permit No. 3/22/2018-WL-Vol. II to collect herpetofauna in Manipur; and the Defence Research Development Organisation (DRDO), Ministry of Defence (No. DFTM/07/3606/NESTC/ABR/M/P-01), Department of Science and Technology (No. DST-SERB/EEQ/2021/000243); and

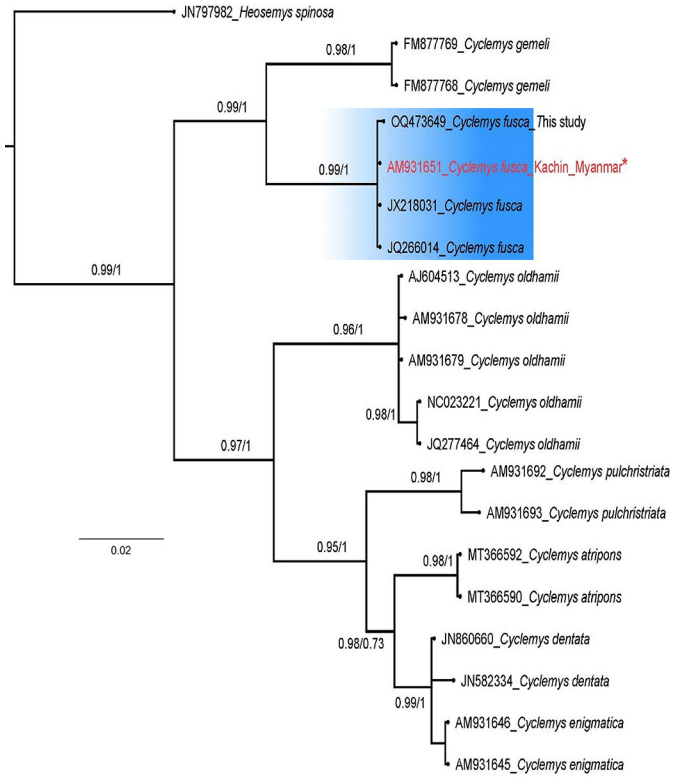

Figure 3. Maximum-likelihood phylogenetic tree based on partial mitochondrial cytochrome b sequences. Numbers at node indicates Bayesian posterior probability (BPP)/bootstrap support values. The asterisk (*) denotes material from the type locality.

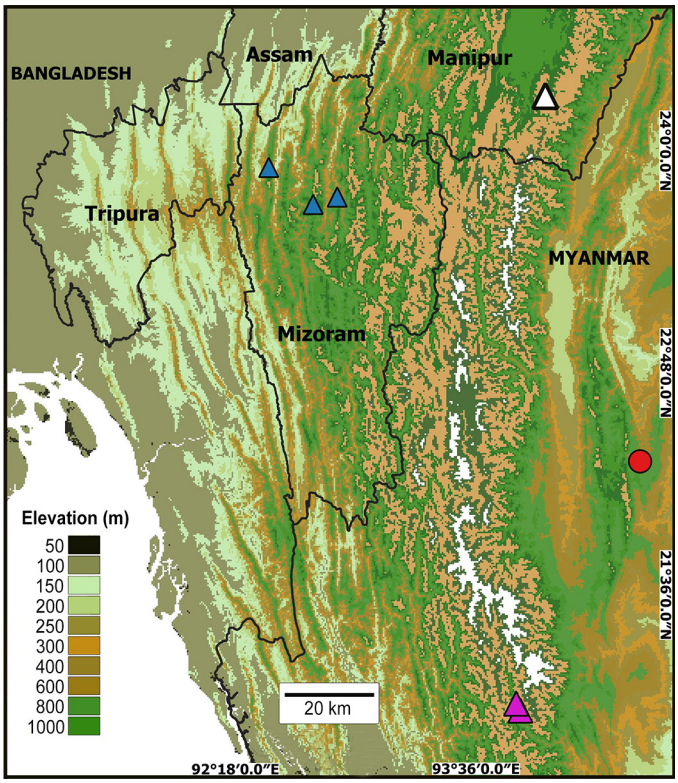

Figure 4. Map of northeastern India showing records of Myanmar Brown Leaf Turtles (*Cyclemys fusca*): Paratype from Alaungdaw Kathapa National Park, Sagaing Division, Myanmar (Fritz et al. 2008) (red dot); records from Mon River and Kyin Dwe Village, Myanmar (Piatt et al. 2012) (purple triangles); pets kept in public houses (Kundu et al. 2016) (blue triangles); new record in Chakpi River, Chandel District, Manipur, India (white triangle).

the Department of Biotechnology (DBT), Ministry of Science and Technology (No. DBT-NER/AAB/64/2017), Government of India, New Delhi, and The Habitats Trust, Noida, Uttar Pradesh, for financial support.

Literature Cited

Aengals, R., V.S. Kumar, M.J. Palot, and S.R. Ganesh. 2018. *A Checklist of Reptiles of India*. Version 3.0. Zoological Survey of India, Kolkata, India.

Burbrink, F.T., R. Lawson, and J.B. Slowinski. 2000. Mitochondrial DNA phylogeography of the polytypic North American rat snake (*Elaphe obsoleta*): a critique of the subspecies concept. *Evolution* 54: 2107–2118. https://doi.org/10.1554/0014-3820(2000)054[2107: MDPOTP]2.0.CO;2.

Champion, S.H.G., and S.K. Seth. 1968. *A Revised Survey of the Forest Types of India*. The Manager of Publication, Government of India, New Delhi, India.

Decemson, Ht., Vanlalsiammawii, L. Biakzuala, M. Vabeiryureilai, F. Malsawmdawngliana, and H.T. Lalremsanga. 2021a. Occurrence of Tamdil Leaf-litter Frog *Leptobrachella tamdil* (Sengupta et al., 2010) (Amphibia: Megophryidae) from Manipur, India and its phylogenetic position. *Journal of Threatened Taxa* 13: 18624–18630. https://doi.org/10.11609/jott.7250.13.6.18624-18630.

Decemson, Ht., M. Vabeiryureilai, Vanlalsiammawii., L. Biakzuala, S. Saipari, and H.T. Lalremsanga. 2021b. A new record of the Bangladeshi Cricket Frog, *Minervarya asmati* (Howlader, 2011), from Manipur State, with comments on the occurrence of the Paddy Frog, *Fejervarya multistriata* (Hallowell 1861) (Anura: Dicroglossidae), in Mizoram, India. *Reptiles & Amphibians* 28: 250–254. https://doi.org/10.17161/randa.v28i2.15533.

Decemson, Ht., V. Siammawii, M. Vabeiryureilai, L. Biakzuala, and H.T. Lalremsanga. 2021c. First record of *Duttaphrynus chandai* (Anura: Bufonidae) from Manipur State, northeastern India, with updated information on its distribution and natural history. *Herpetology Notes* 14: 1219–1223.

Decemson, Ht., M. Vabeiryureilai, L. Biakzuala, and H.T. Lalremsanga. 2021d. Confirmation on the occurrence of *Calotes geissleri* Wagner, Ihlow, Hartman, Flecks, Schmitz, and Böhme 2021 (Sauria: Agamidae) in Manipur, India, with comments on its phylogenetic position. *Journal of Animal Diversity* 3: 14–19. http://dx.doi.org/10.52547/JAD.2021.3.4.2.

Decemson, Ht., V. Siammawii, M. Vabeiryureilai, L. Biakzuala, and H.T. Lalremsanga. 2022. Geographic distribution. *Amolops indoburmanensis* (Indo-Burma Torrent Frog). *Herpetological Review* 53: 69.

Decemson, Ht., R.R. Sinate, L., Biakzuala, M., Vabeiryureilai, P. Lalnuntluanga, J. Malsawma and H.T. Lalremsanga. 2023. Confirmation of Irawadi Forest Lizard, *Calotes irawadi* Zug, Brown, Schulte and Vindum 2006 (Squamata: Agamidae), from Manipur, India. *Reptiles & Amphibians* 30: e17043. https://doi.org/10.17161/randa.v30i1.17043.

Directorate of Environment and Climate Change, Government of Manipur. 2023. Directorate of Environment and Climate Change, Government of Manipur, Porompat, Imphal East Manipur-795005, India. <//ccmanipur.mn.gov.in/en/>.

Fritz, U., D. Guickling, M. Auer, R.S. Sommer, M. Wink, and A.K. Hunsdo. 2008. Diversity of the Southeast Asian leaf turtle genus *Cyclemys*: How many leaves on its tree of life? *Zoologica Scripta* 37: 367–390. https://doi.org/10.1111/j.1463-6409.2008.00332.x.

Hmar, G.Z., Ht. Decemson, M. Vabeiryureilai, H.T. Lalremsanga, and K.C. Das. 2022. Geographic distribution. *Amyda ornata jongli* (Asian Softshell Turtle). *Herpetological Review* 53: 440.

Iverson, J.B. 1992. *A Revised Checklist with Distribution Maps of the Turtles of the World*. Privately published, Richmond, Indiana, USA.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16: 111–120.

Kumar, S., G. Stecher, M. Li, C. Knyaz, and K. Tamura. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* 35: 1547–1549. https://doi.org/10.1093/molbev/msy096.

Kundu, S., V. Kumar, B.A. Laskar, K. Chandra, and K. Tyagi. 2016. Mitochondrial DNA effectively detects non-native Testudines: Invisible wildlife trade in northeast India. *Gene Reports* 4: 10–15. https://doi.org/10.1016/j.genrep.2016.02.002.

Lalremsanga, H.T., Lalrinsanga, and Lalbiakzuala. 2017. Geographic distribution. *Protobothrops mucrosquamatus* (Brown Spotted Pitviper). *Herpetological Review* 48: 131.

Lalremsanga, H.T., J. Purkayastha, L. Biakzuala, M. Vabeiryureilai, L. Muansanga, and G.Z. Hmar. 2021. A new striped species of *Ichthyophis* Fitzinger, 1826 (Amphibia: Gymnophiona: Ichthyophiidae) from Mizoram, northeast India. *Amphibian and Reptile Conservation* 15: 198–209.

Lalremsanga, H.T., Ht. Decemson, M. Vabeiryureilai, and L. Biakzuala. 2022. An updated distribution map and a new elevational record of the Coastal Bullfrog (*Hoplobatrachus litoralis*). *Reptiles & Amphibians* 29: 237–240. https://doi.org/10.17161/randa.v29i1.16510.

Piatt, S.G., W.K. Ko, K. Platt, K.M. Myo, M.M. Soe, and W. Rainwater. 2012. Species inventory and conservation status of chelonians in Natma taung National Park, Myanmar. *Hamadryad* 36: 1–11.

Platt, K., P. Praschag, and B.D. Horne. 2021. *Cyclemys fusca*. *The IUCN Red List of Threatened Species* 2021: e.T170506A1315427. https://dx.doi.org/10.2305/IUCN.UK.20211.RLTS.T170506A1315427.en.

Praschag, P., A.K. Hundsdörfer, and U. Fritz. 2009. Further specimens and phylogenetic position of the recently described leaf turtle species *Cyclemys gemeli* (Testudines: Geoemydidae). *Zootaxa* 2008: 29–37. https://doi.org/10.11646/zootaxa.2008.1.3.

Ronquist, F. and J.P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574. https://doi.org/10.1093/bioinformatics/btg180.

Siammawii, V., Ht. Decemson, F. Malsawmdawngliana, L. Biakzuala, L. Muansanga, V. Mathipi, and H.T. Lalremsanga. 2022. DNA barcoding elucidates the range-extension of the Bangladesh Skittering Frog, *Euphlyctis kalasgramensis* (Dicroglossidae), in northeast India. *Reptiles & Amphibians* 29: 321–325. https://doi.org/10.17161/randa.v29i1.17029.

Silvestro, D. and I. Michalak. 2012. raxmlGUI: a graphical front-end for RAxML. *Organisms Diversity and Evolution* 12: 335–337. https://doi.org/10.1007/s13127-011-0056-0.

Stamatakis, A., P. Hoover, and J. Rougemont. 2008. A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology* 57: 758–771. https://doi.org/10.1080/10635150802429642.

Tariang, A.D., F. Malsawmdawngliana, L. Biakzuala, Ht. Decemson, L. Muansanga, L. Rinsanga, M. Vabeiryureilai, and H.T. Lalremsanga. 2022. Confirmation on the occurrence of *Calotes irawadi* Zug, Brown, Schulte & Vindum 2006 (Squamata: Agamidae) in Mizoram, Northeast India. *Hamadryad* 39: 88–94.

Uetz, P., P. Freed, R. Aguilar, F. Reyes, J. Kudera, and J. Hošek (eds.). 2024. *The Reptile Database*. <http://www.reptiledatabase.org>.