Anatoli Togridou, Richard Southworth, Jasmine Torrez, Sankha Suvra Nandy, Shyamal Kumar Ghosh and Anweshan Patra for volunteering their help during fieldwork in 2018.

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DOI
Date submitted 15/02/2021
Date accepted 10/06/2021
Available online 22/07/2022

Hamadryad Vol. 39 (1&2), pp. 88–94, 2022.

Confirmation of the occurrence of *Calotes irawadi* Zug, Brown, Schulte & Vindum 2006 (Squamata: Agamidae) in Mizoram, Northeast India

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

At present, the agamid genus *Calotes* (Cuvier, 1817) consists of 29 species, out of which

Table 1. 16S rRNA sequences of *Calotes* species used in the present study.

Sl. No.	Species	Specimen vouchers	NCBI Accession Nos.	Grid references	Locality	References
1	*Calotes irawadi*	MZMU2385	OL691118	23.73772°N, 92.66773°E	Mizoram, India	This study
2	*C. irawadi*	AG 855	MW901300	23.5743°N, 95.7376°E	Tripura, India	Gowande et al. 2021
3	*C. irawadi*	CAS 204862	MW901301	23.5743°N, 95.7376°E	Sagaing, Myanmar	Gowande et al. 2021
4	*C. irawadi*	CESL 1089	MW901302	23.5743°N, 95.7376°E	Arunachal Pradesh, India	Gowande et al. 2021
5	*C. versicolor*	L036C	MH844713	8.62448°N,77.1364°E	Kerala, India	Gowande et al. 2016
6	*C. versicolor*	L182C	MH844729	9.97136°N,77.2328°E	Kerala, India	Gowande et al. 2016
7	*C. versicolor*	L190C	MH844730	11.9664°N,79.7633°E,	Tamil Nadu, India	Gowande et al. 2016
8	*C. versicolor*	NCBS AT 102	MW901307	12.02909°N, 79.8503°E;	Puducherry, India	Gowande et al. 2016
9	*C. emma*	NCBSAQ	MH844707	-	-	Pal et al. 2018
10	*C. emma*	NCBS	MK789847	-	Meghalaya, India	Pal et al. 2018
11	*C. emma*	USNM:Herp:587023	MG935747	12.4359°N, 98.5941°E	Tanintharyi,Myanma	Mulcahy et al. 2018
12	*C. farooqi*	DJ 10247	MW901314	-	Pakhtunkhwa, Pakistan	Gowande et al. 2021
13	*C. farooqi*	DJ 10228	MW901313	-	Pakhtunkhwa, Pakistan	Gowande et al. 2021
14	*C. grandisquamis*	L120C	MH844722	-	Kerala, India	Pal et al. 2018
15	*C. grandisquamis*	L035C	MH844712	-	Kerala, India	Pal et al. 2018
16	*C. mystaceus*	NCBSAQ	MH844750	-	Nagaland, India	Giri et al. 2019
17	*C. mystaceus*	WII	MK789848	-	Nagaland, India	Giri et al. 2019
18	*C. nemoricola*	L038C	MH844715	-	Tamil Nadu, India	Pal et al. 2018
19	*C. nemoricola*	L555C	MH844745	-	Tamil Nadu, India	Pal et al. 2018
20	*C. calotes*	L037C	MH844714	-	Tamil Nadu, India	Pal et al. 2018
21	*C. calotes*	L374C	MH844738	-	Tamil Nadu, India	Pal et al. 2018
22	*Psammophilus dorsalis*	L326P	MH844734	-	India	Pal et al. 2018

12 species have been recorded in India and six species are known to occur in Mizoram, namely, *Calotes emma* Gray, 1845*, C. jerdoni* Günther, 1870*, C. maria* Gray, 1845*, C. geissleri* Wagner, Ihlow, Hartmann, Flecks, Schmitz & Böhme, 2021*, C. versicolor* (Daudin, 1802) and *C. zolaiking* Giri, Chaitanya, Mahony, Lalronunga, Lalrinchhana, Das, Sarkar, Karanth & Deepak, 2019 (Gowande et al. 2021; Uetz et al. 2021; Wagner et al. 2021). In the present note, we report the occurrence of the Ayeyarwady crested lizard, *Calotes irawadi* in Mizoram. Prior to this communication, the species was known from its type locality in Sagaing Division, Myanmar (Zug et al. 2006), Western Yunnan in China (Liu et al. 2021), and Arunachal Pradesh and Tripu-

Table 2. Morphometric and meristic measurements of the collected specimens from Mizoram.

Museum No.	MZMU0166	MZMU1110	MZMU2611	MZMU2385	MZMU2596
Sex	Male	Female	Male	Female	Female
Locality	Tamdil	Hmuifang	Durlui	Mizoram University campus	Sihphir
GPS location	23.74112°N, 92.95005°E, 818 m a.s.l.	23.45536°N, 92.75234°E, 1459 m a.s.l.	23.89684°N, 92.65187°E, 106 m a.s.l.	23.73772°N, 92.66773°E, 849 m a.s.l.	23.83348°N, 92.74365°E, 1327 m a.s.l.
Date	14 April 2007	4 August 2011	24 March 2013	11 October 2021	23 October 2021
Morphometric measurements (in mm)					
Eye-ear length	7.4	4.3	4.6	6.0	6.2
Head Height	19.5	10.9	12.6	13.5	16.7
Head Length	24.0	16.3	19.1	21.8	21.8
Head Width	21.4	12.0	14.2	15.9	16.7
Interorbital	13.5	9.8	12.5	11.7	12.9
Snout vent length	98.0	72.5	83.5	88.9	90.3
Snout to Forelimb	36.4	28.6	29.7	30.6	33.9
Tail Length	251.0	223.0	255.2	241.5	246.3
Tail Width	9.9	5.4	5.8	10.7	10.2
Trunk Length	43.4	39.8	35.8	45.4	43.5
Meristic features					
Head scales	12	12	11	12	12
Infralabial	10	9	10	11	10
Supralabial	11	10	11	11	10
4th Finger Lamellae	20	19	19	21	22
4th Toe Lamellae	24	26	26	25	27
Dorsal scales	50	51	57	50	53
Midbody scales	44	48	45	47	45

ra in northeastern India (Gowande et al. 2021). Although earlier studies like Pawar & Birand (2001), Mathew (2007), Lalrinchhana et al. (2011) and Decemson (2021) reported *Calotes versicolor* from various parts of Mizoram, there is no proper taxonomic confirmation on the identity of this species in the state. Thus, to test the specific status of *C. versicolor* in Mizoram, the present work was carried out based on morphology and molecular analysis.

Five specimens of adult *Calotes cf. versicolor* from different localities of Mizoram catalogued in the Departmental Museum of Zoology, Mizoram University (MZMU) were morphologically examined following Zug et al. (2006). The sex of each individual was determined by checking the presence or absence of a hemipenis, and later confirmed through dissection. Details of specimens with their morphometric and meristic measurements are shown in Table 1. Our specimens agreed with the diagnostic features of *Calotes irawadi* provided in Zug et al. (2006), and the adults' morphological features in Liu et al. (2021) (in parentheses). For example, snout-vent length 72.5–98.0 mm (64.3–106.8 mm); head scales 11–12 (10–15); 4th finger lamellae 19–22 (17–24); 4th toe lamellae 24–27 (22–29); dorsal scales 50–57 (36–59) and midbody scales 44–48 (40–51). Prior to the preservation of the specimen (MZMU2385), a liver tissue sample was excised and stored in PCR grade absolute EtOH at -4 °C for molecular analyses. Genomic

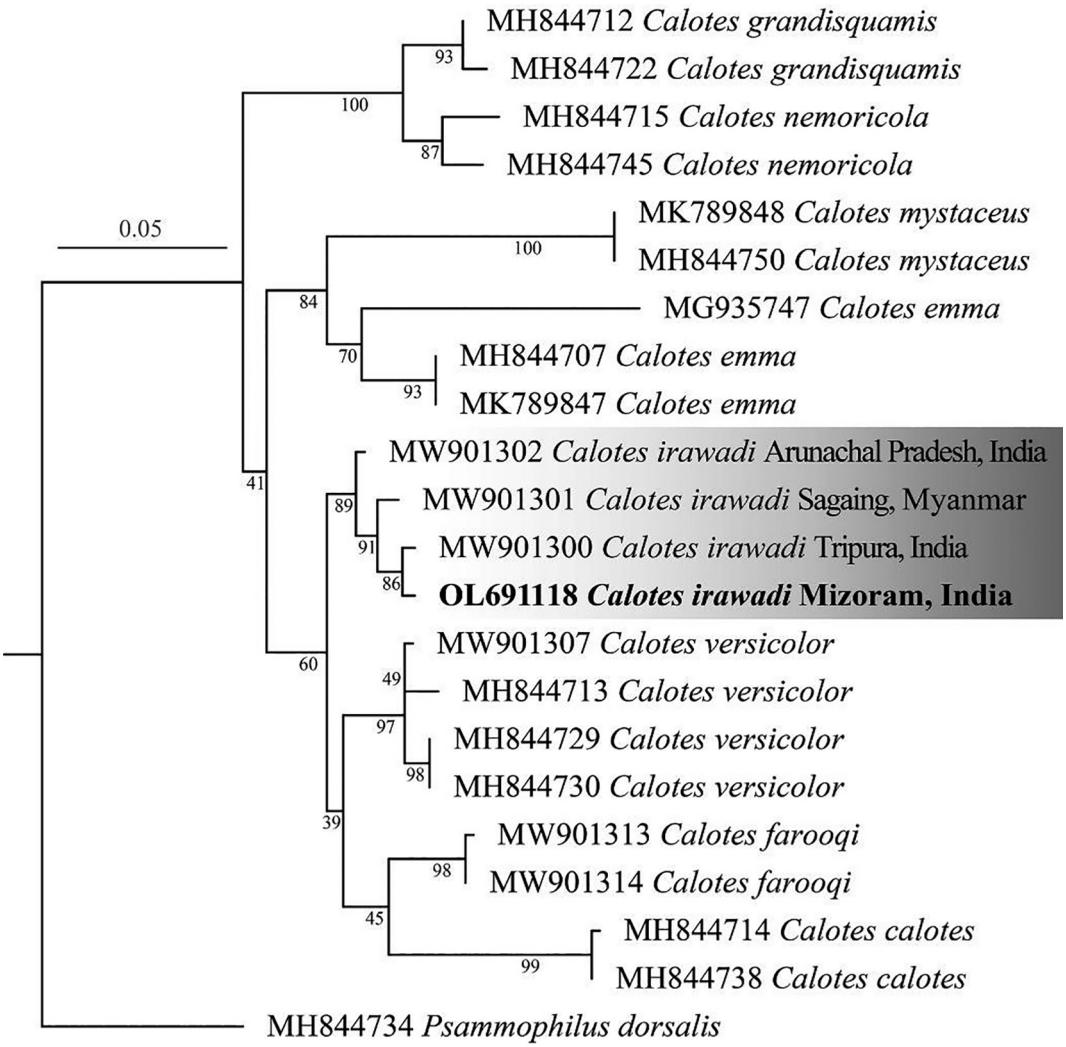

Figure 1. Maximum Likelihood phylogeny of the nominate *Calotes* species inferred from 16S rRNA gene sequence. Numbers at each node represent bootstrap support values. The sequence of *C. irawadi* from Mizoram, Northeast India is given in bold.

DNA was extracted using QIAamp DNA Mini Kit (Cat No. ID: 51306) following the manufacturer's instructions. The PCR was performed in a 20 µL reaction mixture containing 2 µL of 1X amplification buffer, 14.3 µL of water, 0.5 µL of 2.5 mM MgCl2, 1 µL of 0.25 mM dNTPs, 0.5 µL of 0.2 pM each of forward and reverse primers, 1µL of extracted DNA (approximately 50 ng/µL), and 0.2 µL of 1U Taq DNA polymerase. Sequences were generated using the 16S rRNA primers: forward (L02510 — CGC CTG TTT ATC AAA AAC AT, Palumbi 1996) and reverse (H03063 — CTC CGG TTT GAA CTC AGA TC, Rassmann 1997). The PCR thermal regime for amplification was 5 minutes at 95 °C for initial denaturation, followed by 35 cycles of 1 minute at 95 °C for denaturation, 30 seconds for annealing at 50.3 °C, elongation for 1 minute at 72 °C, and a final elongation for 5 minutes at 72 °C. PCR products were sequenced using Sanger's dideoxy method at Barcode Bioscience, Bangalore, India. To form a dataset, the newly generated sequences were combined with GenBank (Benson et al. 2017) sequences of *Calotes* species along with *Psammophilus dorsalis* as the outgroup (Table 2). A sequence alignment (458 bp) was generated using the MUSCLE algorithm (Edgar 2004) and p-distances estimation was performed in MEGA X (Kumar et al. 2018) using default

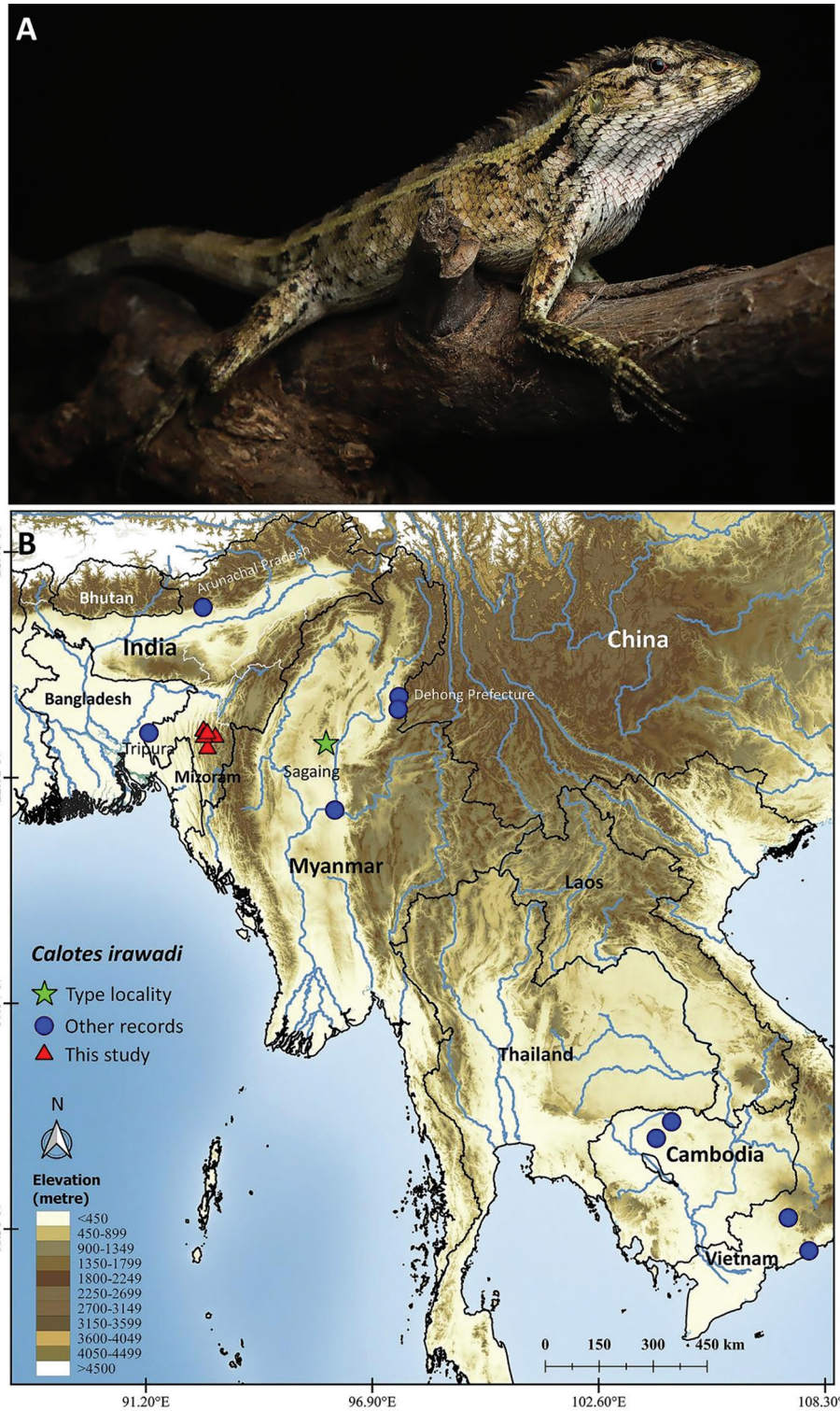

Figure 2. (A) Live individual of female *Calotes irawadi* (MZMU2385), (B) Map showing distributional records of *Calotes irawadi*: type locality in green star (Zug et al. 2006), other records in blue circles (Gowande et al. 2021; Liu et al. 2021), and new records from this study in red triangles.

parameter settings. The Maximum Likelihood (ML) phylogeny was reconstructed in IQ-TREE (Nguyen et al. 2015) with 10,000 Ultrafast bootstrap replicates using the best nucleotide substitution model (TIM2+F+1+G4) selected by ModelFinder (Kalyaanamoorthy et al. 2017) based on Bayesian Information Criterion. The ML phylogeny showed that the sequence from Mizoram (Voucher MZMU2385; GenBank OL691118) is grouped with the sequences of *C. irawadi* from Tripura, India (MW901300; Bootstrap value 86; p-distance 0.7%), Sagaing, Myanmar (MW901301; 1.4%), and Arunachal Pradesh, India (MW901302; 1.9%) (Fig. 1). Gowande et al. (2021) reported the intraspecific 16S divergence among *C. irawadi* was up to 2.4%. Interspecific genetic divergences of 3.3 – 4.1% are also seen between the Mizoram sequence and *C. versicolor* sequences from Puducherry (Neotype, MW901307, 3.3%), and Kerala, India (MH844713, 3.6%; MH844729 & MH844730, 4.1%).

Our results confirm the occurrence of *Calotes irawadi* in Mizoram (Figure 2A). Earlier reports on *Calotes versicolor* in Mizoram might be due to misidentification. The aerial distance from the present record at Durlui to the nearest locality at Unakoti, Tripura is approximately 70 km to the west. The present records bridge the other records from Northeast India and the type locality at Sagaing in Myanmar (Figure 2B). We suggest more extensive surveys for a taxonomic reassessment of species earlier identified as *Calotes versicolor* from the other states of northeastern India, which will provide a more accurate distributional range of *C. irawadi.*

Acknowledgements

We acknowledge the Chief Wildlife Warden of Environment, Forests and Climate change Department, for the herpetofaunal collection permit within Mizoram state (permit no. A.33011/2/99-CWLW/225). We are grateful to DBT-NERB (DBT-NER/AAB/64/2017) and DST-SERB (No. EEQ/2021/000243), New Delhi for the financial support. We appreciate Gospel Zothanmawia Hmar, Lalengzuala Tochhawng and Lalhunthara Chawngthu for their field assistance. We thank Gaurang Gowande and Rachunliu G. Kamei for valuable comments and suggestions that have helped improve the manuscript.

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DOI
Date submitted 7/12/2021
Date accepted 26/4/2022
Available online 22/07/2022

Hamadryad Vol. 39 (1&2), pp. 94–96, 2022.

A new locality record of the king cobra *Ophiophagus hannah* (Cantor, 1836) from Western Himalayas, Himachal Pradesh, India

CITATION. Kapoor, R., Bhardwaj, V. K. & Sharma, V. (2022). A new locality record of the king cobra *Ophiophagus hannah* (Cantor, 1836) from