

## 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

Ht. Decemson<sup>1</sup>, Mathipi Vabeiryureilai<sup>1</sup>, Lal Biakzuala<sup>1</sup> and Hmar Tlawmte Lalremsanga<sup>1\*</sup>

Developmental Biology and Herpetology Laboratory, Department of Zoology, Mizoram University, Aizawl-796004, Mizoram, India

\*Corresponding author ✉: [htlrsa@yahoo.co.in](mailto:htlrsa@yahoo.co.in)

### Abstract

We report *Calotes geissleri* from Chandel district in Manipur, India. Till recently allocated to *Calotes mystaceus*, this complex was revised and *C. geissleri* described from northeast India and Myanmar. We here report its occurrence in Chandel district, Manipur, adjacent to other northeastern states from where it was known previously. Our Manipur specimens have 0.003% genetic distance from its published type sequences, thereby confirming the identification and the range extension.

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**Key words:** *Calotes mystaceus* complex, *C. geissleri*, first state report, N.E. India

The newly discovered agamid lizard species *Calotes geissleri* was originally described from Sagaing Division, Mon Ywa District, Myanmar by Wagner et al. (2021). Earlier, *C. mystaceus* and now, *C. geissleri* was documented from Myanmar and northeastern India, including Nagaland and Mizoram (Lalremsanga et al., 2010). *Calotes geissleri* can be distinguished from *C. mystaceus* in having a whitish stripe from snout-tip to hind limb insertion that continuous between orange to light brown blotches (vs. from snout-tip to front limb insertion, posterior to tympanum becoming brownish beige and fusing with faint brownish dorsolateral blotches); body robust with relatively short limbs (vs. body slender with relatively long limbs)

There is little information on the natural history of *C. geissleri*. It is diurnal and semi-arboreal and but habitat information is not known. The midbody scales individuals of adult male and subadult male from Chandel, Manipur were 51 and 48, respectively.

Herein, we present a confirmation of the occurrence of *C. geissleri* from Chandel district headquarter in Manipur supported by the phylogenetic position.

Morphometric data were taken using dial caliper (Mitutoyo™ 505–730), to nearest 0.1 mm. Morphometric (in mm) and meristic characters (Table 1) largely follow those of Zug et al. (2006). Photographs were taken using a

digital camera (Sony DSC-HX400V–50x optical zoom). Locality altitudes with the geolocations were recorded using a portable unit global positioning system (GPS) navigator device (Garmin Montana 650–GPS). For measurement of the temperature and relative humidity, a digital thermo hygrometer (Kusam–Meco KM 918) was used. Individuals of *C. geissleri* were preserved in 70% ethyl alcohol and catalogued in the Departmental Museum of Zoology, Mizoram University (MZMU).

Genomic DNA was extracted from the 95% ethanol preserved of adult *Calotes* according to manufacturer protocols (QIAamp DNA Mini Kit, Cat No.ID: 51306). PCR reaction was prepared for 20 µL reaction mixture containing 1X amplification buffer, 2.5 mM MgCl<sub>2</sub>, 0.25 mM dNTPs, 0.2 pM each forward and reverse primer, 1µL genomic DNA, and 1U Taq DNA polymerase with a pair of partial 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 s 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 visualised by 1.5% agarose gel electrophoresis containing ethidium bromide. Samples were sequenced using

Sanger's dideoxy method (Sanger, 1974), and sequencing reactions were carried out in both directions (Barcode Bioscience, Bangalore, India). All the sequences were checked using BLAST (NCBI). A total of 15 individuals including study and outgroup sequences were used in the analysis. The newly generated partial 16S rRNA gene sequences of *Calotes geissleri* were deposited in GenBank (MZ026484).

In this analysis, we included 13 congeneric sequences and *Iguana iguana* (AB028756) as an outgroup, retrieved from NCBI database along with newly generated sequence. The sequences were aligned by MEGA-7 Software and the K2P distances were

estimated using the Maximum Composite Likelihood (MCL) approach (Kumar et al., 2016). From the estimated K2P genetic distances, we diagnosed that genetic distance between the present study (Chandel, Manipur), *C. geissleri* (MZ026484) and *C. geissleri* (MK789848) from Nagaland were found to be 0.003% (Table 2). The phylogenetic analysis was inferred by using the MrBayes based on the General Time Reversible model as per the lowest BIC (Bayesian Information Criterion). The analysis involved 15 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 379 positions in the final dataset.

**Table 1:** Morphometric and meristic data from *Calotes geissleri* collected from Hnatham and Abungnikhu village, respectively in Chandel, Manipur, India.

Morphometric measurements (in mm)	This study		Wagner et al. 2021	
	MZMU 2228 Adult male	MZMU 2229 Sub-adult male	Female	Male
Snout-vent length (SVL)	71.03	56.09	92-114	106-122
Tail length (TL)	123.02	103.80	189-223	197-270
Head length (HL)	17.91	16.66	21-28	24-31
Head-width (HW)	12.86	11.05	17-21	20-30
Snout length (SNL)	8.12	7.12	10-13	11-13
Inter-orbital distance (IOD)	13.4	12.26	12-15	9-14
Mid-body scale rows (MSR)	51	48	50-54	50-62
Weight of individuals (in g)	19.04 g	7.15 g	*	*

**Table 2:** Estimated uncorrected K2P distance, using 16S rRNA partial gene of *Calotes* along with congener sequences, as retrieved from NCBI database.

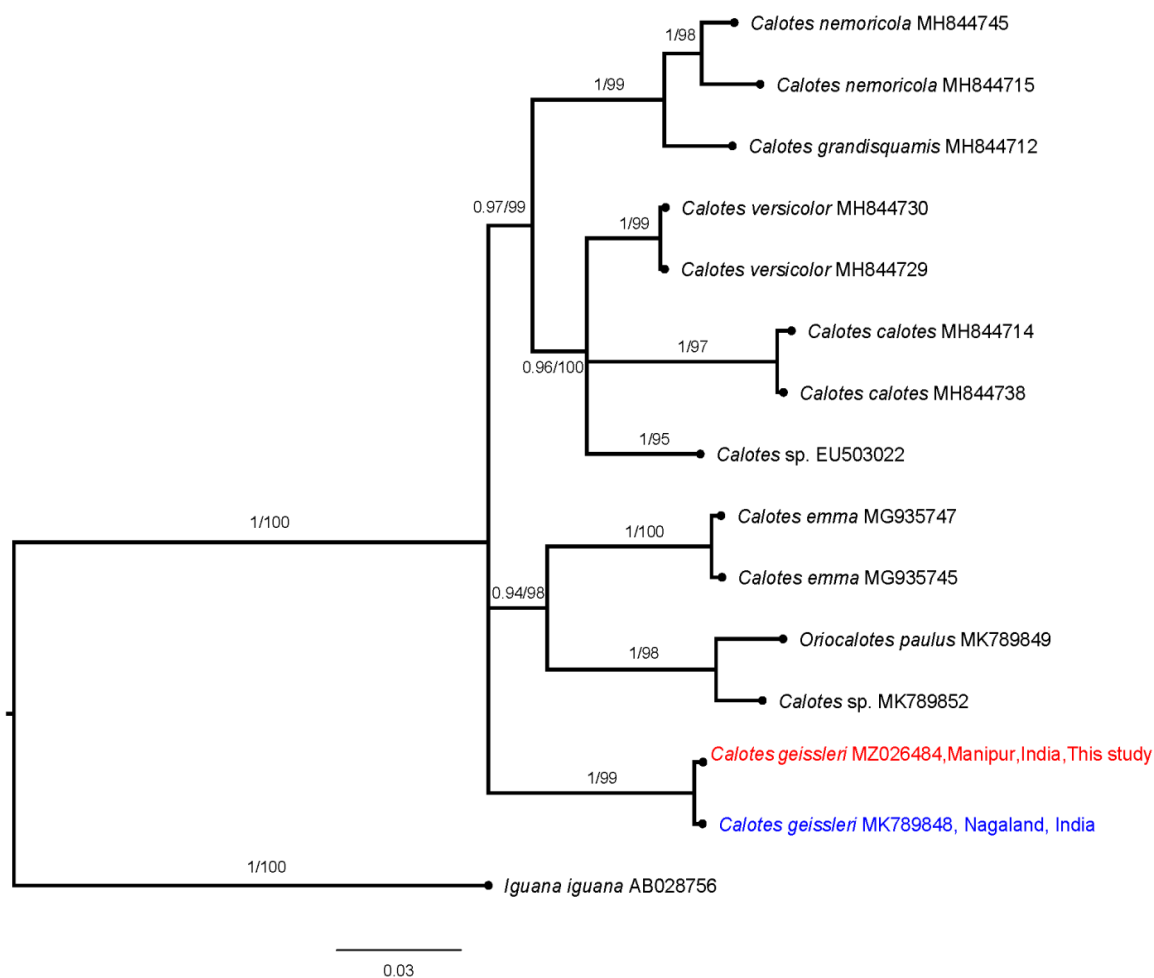
Sl. No.	Species	K2P distance														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	<i>Calotes geissleri</i> (MZ026484) This study															
2	<i>Calotes geissleri</i> (MK789848) Nagaland	0.003														
3	<i>Calotes calotes</i> (MH844738)	0.108	0.108													
4	<i>Calotes calotes</i> (MH844714)	0.111	0.111	0.003												
5	<i>Calotes emma</i> (MG935747)	0.106	0.103	0.108	0.111											
6	<i>Calotes emma</i> (MG935745)	0.103	0.100	0.108	0.111	0.003										
7	<i>Calotes grandisquamis</i> (MH844712)	0.079	0.079	0.084	0.087	0.100	0.100									
8	<i>Calotes nemoricola</i> (MH844745)	0.092	0.092	0.087	0.090	0.095	0.098	0.032								
9	<i>Calotes nemoricola</i> (MH844715)	0.095	0.095	0.092	0.095	0.108	0.108	0.037	0.026							
10	<i>Calotes paulus</i> (MK789849)	0.103	0.103	0.108	0.111	0.084	0.084	0.103	0.106	0.111						
11	<i>Calotes versicolor</i> (MH844730)	0.095	0.095	0.058	0.058	0.079	0.082	0.071	0.058	0.077	0.100					
12	<i>Calotes versicolor</i> (MH844729)	0.095	0.095	0.058	0.058	0.079	0.082	0.071	0.058	0.077	0.100	0.000				
13	<i>Calotes</i> sp. (MK789852)	0.095	0.095	0.103	0.106	0.082	0.082	0.092	0.095	0.100	0.024	0.098	0.098			
14	<i>Calotes</i> sp. (EU503022)	0.092	0.092	0.066	0.069	0.079	0.082	0.074	0.055	0.071	0.100	0.047	0.047	0.095		
15	<i>Iguana iguana</i> (AB028756)	0.264	0.266	0.248	0.248	0.248	0.248	0.245	0.251	0.253	0.259	0.237	0.237	0.253	0.261	

The present study confirmed the presence of *C. geissleri* based on the phylogenetic relationship along with *C. geissleri* (MK789848) from Nagaland (Fig. 1) indicates the formation of a monophyletic clade.

During fieldwork on 29 January 2021 at 1342 hr. (ambience temperature and relative humidity being 26 °C and 72.39%, respectively), we surveyed a site located ca. 3.5 km west of Chandel town from Hnatham, Chandel district, within a secondary forest (24.20304°N, 93.59153°E, 924 m a.s.l.). The location is surrounded by subtropical secondary forests, represented predominantly by *Pinus* sp., *Quercus* sp., *Schima wallichii*, *Albizia* sp. and *Macaranga denticulata*. The open forest in the moist valleys is lofty, while the slopes are covered with closed canopy (Champion and Seth, 1968; Singh et al., 2000; Forest Survey of India, 2019). Attention was drawn due to rustling in the dry leaf litter made by an agamid lizard that we later identified as adult male *Calotes geissleri* (Fig. 2A). This lizard was sheltering near a hut, close to an abandoned jhum cultivation

land (Fig. 2B). It sprinted for short distance well and perched on a dry branch nearby. Photographs were taken *in situ*. The lizard remained grayish-brown under shade. However, it changed the head and anterior portion of the trunk to a light blue color under exposure to sun light.

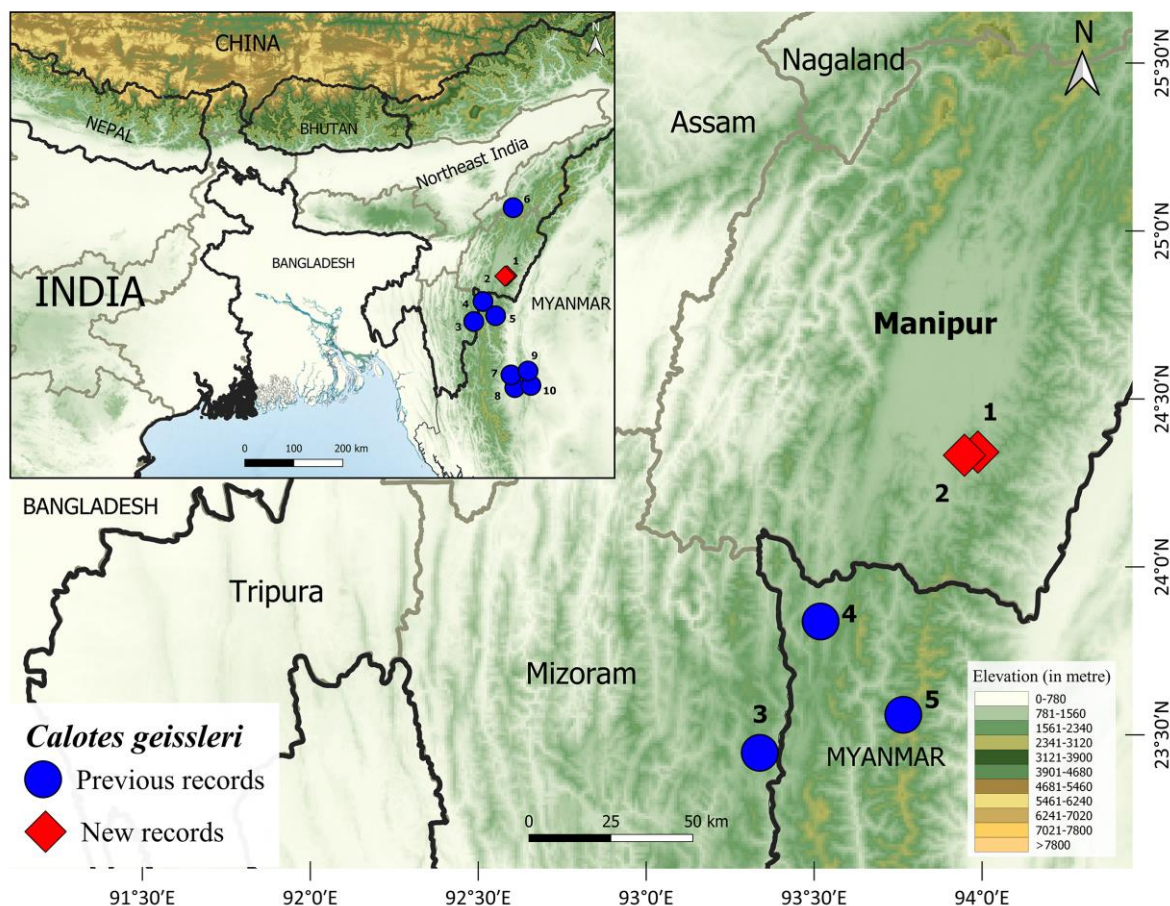
We found a male road-killed fresh specimen in a rather urbanized township area at Abungnikhu, Chandel (24.19564°N, 93.56512° E, 894 m a.s.l) on 26 December 2020 at 1114 hr. Both specimens were identified as *C. geissleri* on the basis of morphometric diagnostic features provided by Wagner et al. (2021) and later confirmed with the help of molecular analysis. The morphometric data is provided in Table 1. Like other *Calotes* species, *C. geissleri* feeds on arthropods, such as members of Coleoptera and Formicidae, as in other species of *Calotes*. The collection sites in aerial distance between Chandel town and type locality, Alaungdaw Kathapa National Park, Myanmar is approximately 272 km apart (Fig. 3).



**Figure 1:** Bayesian phylogram of *Calotes* inferred from 16S rRNA sequences. Numbers at tree nodes indicated to BI/BS support values, respectively; value above >90 considered well-supported and below <90 considered moderate-support. Colours denote this study, red font indicate study species and blue font represents type material, with GenBank accession numbers followed by their distribution.



**Figure 2A:** Adult male *Calotes geissleri*; **2B:** microhabitat at abandoned jhum cultivation in Chandel, Manipur, India.



**Figure 3:** Distribution map of *Calotes geissleri* showing new state records from Manipur, India: 1. Hnatham village and 2. Abungnikhu village in Chandel district headquarter (red tilted squares); Previous records (blue circles), 3. Zotlang, Champhai, Mizoram, India; 4. Simggial village, Myanmar; 5. Natzang village, Myanmar; 6. Kohima, Nagaland, India; 7 and 8. Mauk village, Myanmar; 9 and 10. Alaungdaw Kathapa National Park, Myanmar (see Lalremsanga et al., 2010, Wagner et al., 2021).

In the present study, individuals collected from between Hnatham and Abungnikhu were aerial distance 1.78 km apart in Chandel with the altitudinal range difference of 30 m. The occurrence of the lizard links the distribution range in between Mizoram and Nagaland.

This study provides a range of extension for *C. geissleri* from the type locality in Myanmar, northwest towards the Nagaland, Mizoram and Manipur states in northeast India. In Northeastern India, the species occurs in sympatry with *Calotes jerdoni* and *C. versicolor* (see Lalremsanga et al., 2010).

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### Conflict of interest

The authors declare that there are no conflicting issues related to this short communication.

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