

Taxonomic Paper

First record of *Calotes vindumbarbatus* Wagner, Ihlow, Hartmann, Flecks, Schmitz & Böhme, 2021 (Squamata: Agamidae) from China, with revised diagnosis of this species

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Abstract

Background

Three new species were recently described from the *Calotes mystaceus* Duméril & Bibron, 1837 complex. Of the three new species, *C. vindumbarbatus* Wagner, Ihlow, Hartmann, Flecks, Schmitz & Böhme, 2021 was known only from northern Myanmar.

New information

Seven specimens of lizard were collected from Tongbiguan Nature Reserve, western Yunnan, China. Phylogenetically, these specimens clustered with the type specimens of *Calotes vindumbarbatus* from Myanmar with strong support and showed inappreciable genetic divergence from the type specimens of *C. vindumbarbatus*. We report the first country record of *C. vindumbarbatus* from China. In addition, a supplementary description,

based on the newly-collected specimens and revised diagnosis of this species, was provided.

Keywords

Agamidae, lizard, mtDNA, Tongbiguan Nature Reserve, western Yunnan

Introduction

The spectacularly coloured Blue Forest Lizard *Calotes mystaceus*, originally described from "Pays de Birmans" (= Myanmar), was previously considered to be widely distributed from India, Myanmar, China, Laos, Cambodia, Thailand and Vietnam (Bain and Hurley 2011, Hartmann et al. 2013, Chan-Ard et al. 2015, Das 2015, Pham et al. 2018). Hartmann et al. (2013) described the population in Vietnam as a distinct species *C. bachae* Hartmann, Geissler, Poyarkov, Ihlow, Galoyan, Rödder & Böhme, 2013. Wagner et al. (2021) restricted *C. mystaceus* to southern coastal Myanmar and described three new species occurring in Cambodia, China, Laos, Myanmar, Thailand and India. *Calotes geissleri* Wagner, Ihlow, Hartmann, Flecks, Schmitz & Böhme, 2021 was known from Myanmar and India; *C. goetzi* Wagner, Ihlow, Hartmann, Flecks, Schmitz & Böhme, 2021 was known from Myanmar and India; China, Laos, Myanmar and Thailand; and *C. vindumbarbatus* was known only from northern Myanmar.

During our field surveys in western Yunnan, China, from 2018 to 2020, seven specimens of lizard, previously confused with *Calotes mystaceus*, were collected from Tongbiguan Nature Reserve. Detailed morphological comparisons and molecular analysis indicated these specimens to be *C. vindumbarbatus*. Herein, we report this new record for China in detail.

Materials and methods

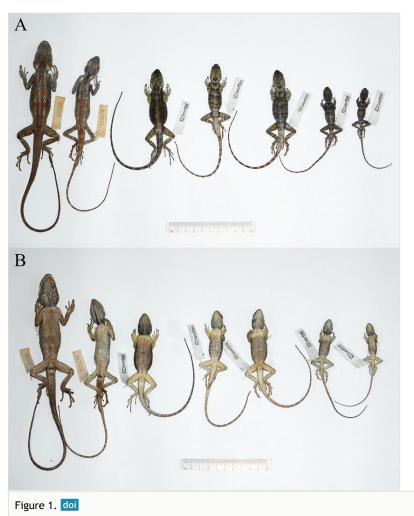
Sampling

Field surveys were conducted in Tongbiguan Nature Reserve, Yingjiang County, Dehong Prefecture, Yunnan Province, China, under the permit from the Tongbiguan Provincial Natural Reserve Management and Protection Bureau. Lizards were collected, euthanised and then fixed in 75% ethanol for storage. Liver tissue samples were preserved in 99% ethanol for molecular analysis. The specimens (Fig. 1) were deposited at Kunming Natural History Museum of Zoology, Kunming Institute of Zoology, Chinese Academy of Sciences (KIZ).

Morphological characteristics

Measurements were taken with a digital caliper to the nearest 0.1 mm, except tail length (TL) which was measured using a string and a ruler. Morphological terminology followed

Wagner et al. (2021). Morphometric characters included: 4th finger length (4thFingL), distance from juncture of 3rd and 4th digits to distalmost extent (outer/distalmost surface of claw) of 4th finger; 4th toe length (4thToeL), distance from juncture of 3rd and 4th digits to distal end of 4th digit on hind-foot; Crus length (CrusL), length of tibia from knee to heel; Eye-ear length (EyeEar), distance from anterior edge of tympanum to posterior of orbit (not pupil opening); Forefoot length (ForefL), distance from proximal end of forefoot to tip of fourth digit; Head height (HeadH), dorsoventral distance from top of head to underside of jaw at transverse plane intersecting angle of jaws; Head length (HeadL), distance from anterior edge of tympanum to tip of snout; Head width (HeadW), distance from left to right outer edge of temporal or jaw muscles at their widest point without compression of soft tissue; Hind-foot length (HindfL), distance from proximal end (heel) of hind-foot to distalmost surface of fourth toe; Interorbital width (Interorb), transverse distance between anterodorsal corners of left and right orbits; Jaw width (JawW), distance from left to right outer edge of jaw angles, this measurement excludes jaw musculature broadening of head; Lower arm length (LoArmL), distance from elbow to distal end of wrist or just before underside of forefoot; Naris-eye length (NarEye), distance from anterior edge of orbit to posterior edge of naris; Snout-eye length (SnEye), distance from anterior edge of orbit to tip of snout (rostral scale); Snout-forelimb length (SnForeL), distance from anterior of forelimb, or shoulder, to tip of snout; Snout width (SnW), internasal or internarial distance, transverse distance between left and right nares; Snout-vent length (SVL); tail height (TailH), distance from dorsal to ventral surface of tail base measured just posterior to vent; Tail length (TailL), distance from vent to distal end of tail, noting completeness or regeneration of tail; Tail width (TailW), distance from left to right side of tail base just posterior to vent; Trunk length (TrunkL), body length or axilla-groin length of others, distance between posterior edge of forelimb insertion (axilla) to anterior edge of hindlimb insertion (inguen); Upper arm length (UpArmL), distance from anterior insertion of forelimb, or shoulder, to elbow; Upper leg length (UpLegL), distance from anterior edge of hindlimb insertion to knee. Meristic characters included: Forefoot lamellae (4FingLm), number of 4th digit lamellae, from 1st lamella at digits' cleft that is wider than deep and touches dorsal digital scale (on at least one side) to most distal lamella, fragmented proximal scales are excluded; Hindfoot lamellae (4ToeLm), analogous to 4FingLm at 4th toe; Canthus rostralis (CanthR), number of elongate scales along 'dorsolateral snout ridge' from above posterodorsal corner of nasal scale to and including posteriormost supraciliary scale; Dorsal eyelid scales (Eyelid), number of scales found along dorsal edge of eyelid; Dorsal head scales (HeadSLn), number of scales longitudinally on mid-line between interparietal and rostral scale; Head scales (HeadSTr), number of scales in transverse line between posteriormost left and right supraciliary scales, just anterior of interparietal; Infralabials (Inflab), posterior end defined by posteriormost enlarged scales that touches with Suplab at rear corner of mouth; Mid-body scale rows (MidbS), number of scale rows around trunk at mid-body; Snout scales (SnS), number of scales on line transversally between left and right nasal scales (single scale surrounding naris); Supralabials (Suplab), posterior end defined by posteriormost enlarged scales that touches Inflab at rear corner of mouth; Vertebral scales or spines (VertS), number of mid-dorsal scales (spines or not), beginning with first enlarged spine-like scale on nape to above vent.



The specimens of *Calotes vindumbarbatus* from Tongbiguan Nature Reserve, western Yunnan, China, in preservative: **A** dorsal view; **B** ventral view.

Molecular analysis

Total genomic DNA was extracted from liver tissues with the universal protocol of DNA extraction (Aljanabi and Martinez 1997). A region of mitochondrial genes 12S rRNA was amplified and sequenced by using the primers L1091 (5'–AAAAAGCTTCAAAC TGGGATTAGATACCCCACTAT–3') and H1478 (5'–TGACTGCAGAGGGTGACGG GCGGTGTGT–3') (Kocher et al. 1989). PCR cycling conditions follow Schmitz et al. (2005) and Nazarov et al. (2012). The products were purified and sequenced by Tsingke Biotechnology (Beijing) Co. Ltd., using the same primers as in PCR. All new sequences were deposited in GenBank. Homologous and outgroup sequences were obtained from GenBank (Table 1).

Table 1.

Sequences (12S rRNA) used for phylogenetic analysis.

Species	Voucher no.	Locality	GenBank no.	Reference	
Calotes bachae	ZFMK 88935 (holotype)	Vietnam: Dong Nai: Cat Tien National Park	<u>MW817231</u>	Wagner et al. (2021)	
Calotes bachae	ZFMK 92028	Vietnam: Cao Bang: Trung Khanh	<u>MW817232</u>	Wagner et al. (2021)	
Calotes bachae	ZFMK 96231	Vietnam	<u>MW817236</u>	Wagner et al. (2021)	
Calotes bachae	MW817236	Cambodia: Ratanakiri: Banlung	<u>MW817233</u>	Wagner et al. (2021)	
Calotes geissleri	CAS 215539 (holotype)	Myanmar: Sagaing: Alaungdaw Kathapa National Park	<u>MW817189</u>	Wagner et al. (2021)	
Calotes geissleri	CAS 210270 (paratype)	Myanmar: Sagaing: Alaungdaw Kathapa National Park	<u>MW817187</u>	Wagner et al. (2021)	
Calotes geissleri	ZFMK 97991 (paratype)	Myanmar: Chin: Falam: Simggial Village	<u>MW817238</u>	Wagner et al. (2021)	
Calotes geissleri	CAS 243028 (paratype)	Myanmar: Magway: Gangaw: Gangaw: Mauk Village	<u>MW817211</u>	Wagner et al. (2021)	
Calotes goetzi	CAS 242463	China: Yunnan: Baoshan: Longling	<u>MW817210</u>	Wagner et al. (2021)	
Calotes goetzi	CAS 242457	China: Yunnan: Baoshan: Longyang	<u>MW817209</u>	Wagner et al. (2021)	
Calotes goetzi	CAS 228144	China: Yunnan: Nujiang	<u>MW817191</u>	Wagner et al. (2021)	
Calotes goetzi	CAS 207489	China: Yunnan: Nujiang	<u>MW817186</u>	Wagner et al. (2021)	
Calotes goetzi	CAS 204849	Myanmar: Mandalay	<u>MW817184</u>	Wagner et al. (2021)	
Calotes goetzi	NME R 0584/09a	Thailand: Chiang Mai	<u>MW817216</u>	Wagner et al. (2021)	
Calotes mystaceus	CAS 240296	Myanmar: Mon: Kyaikhto	<u>MW817206</u>	Wagner et al. (2021)	
Calotes mystaceus	CAS 206548	Myanmar: Yangon: Letpein Village	<u>MW817185</u>	Wagner et al. (2021)	
Calotes mystaceus	CAS 240287	Myanmar: Mon: Kyaikhto	<u>MW817205</u>	Wagner et al. (2021)	
Calotes mystaceus	CAS 213300	Myanmar: Yangon: Hlawga National Park: Mingalardon	<u>MW817188</u>	Wagner et al. (2021)	
Calotes vindumbarbatus	CAS 232388 (holotype)	Myanmar: Kachin: Myitkyina: Gat Shang Yang Village	<u>MW817198</u>	Wagner et al. (2021)	
Calotes vindumbarbatus	CAS 232387 (paratype)	Myanmar: Kachin: Myitkyina: Gat Shang Yang Village	<u>MW817197</u>	Wagner et al. (2021)	

Species	Voucher no.	Locality	GenBank no.	Reference
Calotes vindumbarbatus	ZFMK 97990 (paratype)	Myanmar: Kachin: Myitkyina: Gat Shang Yang Village	<u>MW817237</u>	Wagner et al. (2021)
Calotes vindumbarbatus	CAS 239206 (paratype)	Myanmar: Sagaing: Hkanti: Hkanti: Linpha Village	<u>MW817202</u>	Wagner et al. (2021)
Calotes vindumbarbatus	CAS 232247 (paratype)	Myanmar: Sagaing: Homalin: N of Swekawngaw	<u>MW817196</u>	Wagner et al. (2021)
Calotes vindumbarbatus	KIZ20209131	China: Yunnan: Dehong: Yingjiang: Taiping Town	<u>OM418450</u>	This study
Calotes vindumbarbatus	KIZ20209132	China: Yunnan: Dehong: Yingjiang: Taiping Town	<u>OM418451</u>	This study
Calotes vindumbarbatus	KIZ20209133	China: Yunnan: Dehong: Yingjiang: Taiping Town	<u>OM418452</u>	This study
Calotes vindumbarbatus	KIZ20209134	China: Yunnan: Dehong: Yingjiang: Taiping Town	<u>OM418453</u>	This study
Calotes vindumbarbatus	KIZ20209135	China: Yunnan: Dehong: Yingjiang: Taiping Town	<u>OM418454</u>	This study
Calotes vindumbarbatus	KIZ 059176	China: Yunnan: Dehong: Yingjiang: Nabang Town	<u>OM418455</u>	This study
Calotes vindumbarbatus	KIZ 059299	China: Yunnan: Dehong: Yingjiang: Nabang Town	<u>OM418456</u>	This study
Calotes emma	NME R 0590/09	Laos: Phongsali	<u>MW817218</u>	Wagner et al. (2021)
Calotes cf. versicolor	35570	Thailand: Ko Chang	<u>AB031964</u>	Honda et al. (2000)

Phylogenetic analyses

Sequences were aligned using ClustalW (Thompson et al. 2003) with default parameters in Mega X (Kumar et al. 2018). The genetic distance (uncorrected p-distance) between species was calculated in Mega X (Kumar et al. 2018). The best substitution model GTR+F+I+G4 was selected using the Akaike Information Criterion (AIC) in ModelFinder (Kalyaanamoorthy et al. 2017). Bayesian Inference (BI) was performed in MrBayes 3.2.6 (Ronquist et al. 2012). Two runs were performed simultaneously with four Markov chains starting from the random tree. The chains were run for 1,000,000 generations and sampled every 100 generations. The first 25% of the sampled trees were discarded as burn-in after the standard deviation of split frequencies of the two runs was less than 0.01. The remaining trees were then used to create a consensus tree and to estimate Bayesian posterior probabilities. Maximum Likelihood (ML) analysis was performed in raxmIGUI 2.0 (Silvestro and Michalak 2011) and nodal support values were estimated by 1,000 rapid bootstrap replicates.

Taxon treatment

Calotes vindumbarbatus Wagner, Ihlow, Hartmann, Flecks, Schmitz & Böhme, 2021

Materials

- country: China; stateProvince: Yunnan; locality: Nabang Town, Yingjiang County, Dehong Prefecture; verbatimElevation: 320 m; verbatimCoordinates: 24°45′47″N 97°34′15″E; eventRemarks: collected by Shuo Liu on 5 September 2018; individualCount: 1; sex: male; lifeStage: adult; catalogNumber: KIZ 059299; basisOfRecord: preserved specime
- country: China; stateProvince: Yunnan; locality: Nabang Town, Yingjiang County, Dehong Prefecture; verbatimElevation: 320 m; verbatimCoordinates: 24°45'47"N 97°34'15"E; eventRemarks: collected by Shuo Liu on 5 September 2018; individualCount: 1; sex: female; lifeStage: adult; catalogNumber: KIZ 059176; basisOfRecord: preserved specime
- country: China; stateProvince: Yunnan; locality: Xueli Village, Taiping Town, Yingjiang County, Dehong Prefecture; verbatimElevation: 350 m; verbatimCoordinates: 24°26'32"N, 97°33'4"E; eventRemarks: collected by Shuo Liu on 13 September 2020; individualCount: 1; lifeStage: juvenile; catalogNumber: KIZ20209131; basisOfRecord: preserved specime
- country: China; stateProvince: Yunnan; locality: Xueli Village, Taiping Town, Yingjiang County, Dehong Prefecture; verbatimElevation: 350 m; verbatimCoordinates: 24°26'32"N, 97°33'4"E; eventRemarks: collected by Shuo Liu on 13 September 2020; individualCount: 1; lifeStage: juvenile; catalogNumber: KIZ20209132; basisOfRecord: preserved specime
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 County, Dehong Prefecture; verbatimElevation: 350 m; verbatimCoordinates: 24°26'32"N,
 97°33'4"E; eventRemarks: collected by Shuo Liu on 13 September 2020; individualCount:
 1; lifeStage: juvenile; catalogNumber: KIZ20209135; basisOfRecord: preserved specime

Description of the two adult specimens (KIZ 059299 and KIZ 059167): Morphometric and meristic data are presented in Table 2. Male body large (SVL 116.4 mm), female body relatively small (SVL 97.2 mm). Tail relatively short (TaiL/SVL 2.10 in male and 1.95 in female), extremities relatively short and robust. Head large, distinct from the neck and lateral sides flat. Posterior parts of jaw angle slightly swollen in male, not swollen in female. Snout-tip blunt. Nostril in a single scale, separated from the labial scale by one or two scales. Rostral and mental scales small. Canthus rostralis sharp and straight from the nostril to the posterior part of the eye, including 6–7 scales between the nostril and the eye and 8–9 supraciliary scales. Ten supralabial scales, 9 infralabial scales. Tympanum distinct. Two short separated spines on each side of the upper head above the tympanum and two shorter spines beside each of them. Scales on chin and throat keeled. Nuchal crest with 10–11 scales, dorsal crest with 35–39 scales. Nuchal and dorsal crest well developed and high in male, composed of erected compressed scales, directed posteriorly, highest above the insertion of the front limbs and gradually decreasing towards the tail. Nuchal crest developed in female, composed of erected compressed scales, dorsal crest poorly developed in female. Oblique fold of skin in front of forelimb insertion distinct, covered with small granular dark scales. Dorsal scales feebly keeled, pointing upwards and backwards. Ventral scales parallel and strongly keeled. Caudal scales keeled, directed backwards. Subcaudal scales parallel and strongly keeled.

Table 2.

Measurements (in mm) and scalation data for the specimens of *Calotes vindumbarbatus* collected from China. For character abbreviations, see material and methods. Paired meristic characters were made on the left side.

	KIZ 059299	KIZ 059176	KIZ20209131 Juvenile	KIZ20209132 Juvenile	KIZ20209133 Juvenile	KIZ20209134 Juvenile	KIZ20209135 Juvenile
	Adult male	Adult female					
SVL	116.4	97.2	79.5	67.4	72.5	52.6	45.0
EyeEar	8.6	6.1	5.2	4.3	4.9	3.7	3.3
HeadH	18.7	13.9	13.3	11.4	12.1	9.3	8.4
HeadL	29.8	23.5	19.7	17.3	18.7	14.1	11.9
HeadW	23.8	18.2	16.7	14.1	15.1	11.6	9.9
Interorb	14.8	12.2	10.4	9.0	9.6	7.2	5.8
JawW	22.0	18.2	16.7	14.1	15.1	11.6	9.9
NarEye	8.0	5.6	5.7	4.7	4.7	3.3	2.8
SnEye	13.0	10.1	9.6	7.7	8.6	6.1	5.1
SnW	7.4	6.7	5.9	4.9	5.5	3.7	3.7
4FingL	13.6	10.9	10.7	9.5	9.9	6.8	6.4
4ToeL	17.4	15.9	16.9	13.9	11.3	9.7	8.6
CrusL	24.1	20.3	18.1	15.6	16.9	12.7	10.1
ForefL	20.2	16.9	16.3	14.7	14.8	11.0	9.8
HindfL	32.3	28.4	28.9	24.3	22.7	17.7	15.4
LoArmL	18.9	16.3	13.8	12.2	13.1	9.2	7.4
SnForeL	43.9	35.1	29.6	25.3	27.2	20.7	17.0
TailH	14.4	9.4	9.8	7.4	9.1	5.6	4.8
TailL	244.0	189.5	179.5	144.0	154.5	106.0	88.5
TailW	12.7	9.4	8.8	7.2	7.7	5.6	4.6

	KIZ 059299 Adult male	KIZ 059176 Adult female	KIZ20209131 Juvenile	KIZ20209132 Juvenile	KIZ20209133 Juvenile	KIZ20209134 Juvenile	KIZ20209135 Juvenile
TrunkL	52.9	45.0	36.6	30.3	32.4	22.1	20.2
UparmL	23.6	19.1	16.4	14.7	14.8	10.6	9.0
UplegL	23.9	20.8	18.7	15.4	17.7	12.4	9.9
CanthR	8	9	10	9	9	9	10
Eyelid	13	12	11	13	11	11	11
HeadSLn	15	16	16	17	17	18	17
HeadSTr	14	13	15	17	14	14	16
Inflab	9	9	10	10	10	11	11
Sns	7	6	7	8	7	7	7
Suplab	10	10	9	10	9	11	11
4FingLm	19	19	20	20	18	21	22
4ToeLm	24	23	24	27	22	25	24
VertS	46	49	48	47	46	49	48
MidbS	53	53	56	52	50	53	52

Colouration: This species has a very strong ability to change the body colouration (Fig. 2). Usually, the head, forelimbs and anterior half of the body of adult males are blue, a white stripe, as high as the tympanum, is present from between nostril and orbit along the upper lip and the tympanum to the insertion of the front limb. The stripe is followed by 4–5 white blotches and there are thinner white stripes between each blotch. Gradually increasing brown blotches are present just on each white blotch, the first two are smaller than the white blotches and the rest almost covered the whole of the white blotches. The posterior half of the body, hind limbs and tail almost uniform brown. When they were disturbed or the environment changed etc., they can change their body colouration. The head, forelimbs and anterior half of the body may become brownish-grey, the white stripe become brownish-yellow, the posterior half of the body, hindlimbs and tail are still brown, but darker.

The colourations of adult females are relatively dim. Usually, the ground colour of females is purple brown, there are dark longitudinal stripes on the chin region and dark reticulate stripes on the back and there are radial dark stripes around the eyes, the white stripe along the upper lip and the dorsolateral blotches are more indistinct. However, the females can also change their body colouration. The stripes on the back, chin region and around the eyes may become indistinct. The head, forelimbs and anterior half of the body may become bluish, the white stripe and dorsolateral blotches may become more distinct. However, the colourations of females are still not as bright as those of males.

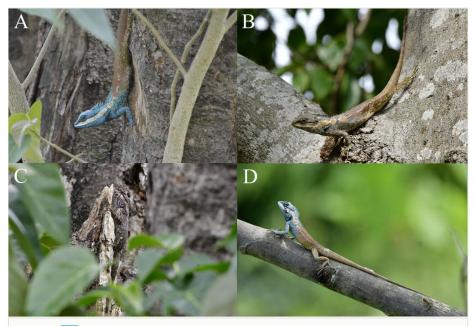


Figure 2. doi Calotes vindur

Calotes vindumbarbatus from Tongbiguan Nature Reserve, western Yunnan, China, in life. **A** an adult male in bright colouration; **B** an adult male in sombre colouration; **C** an adult female; **D** a subadult male with smaller body size and low nuchal and dorsal crest.

Revised diagnosis: A medium-sized *Calotes* species of the complex, males with a known maximum SVL of 116.4 mm, females with a SVL of 97.2 mm. Tail length short, approximately twice the length of SVL. It can be distinguished from the other species of the complex by the combination of the following characters: 1) head and body robust, posterior parts of jaw angle slightly swollen in adult males, not swollen in female; 2) dorsal scales large, feebly keeled, pointing upwards and backwards, ventral scales small, parallel and strongly keeled; 3) body scales arranged in 50-56 rows around midbody; 4) 18-22 lamellae on the fourth finger and 22-27 lamellae on the fourth toe; 5) 40-49 vertebral scales, nuchal crest developed in adults, dorsal crest developed in adult males, but undeveloped in females; 6) two short separated spines on each side above the tympanum and two shorter spines beside each of them; 7) oblique fold of skin in front of forelimb insertion distinct, covered with small granular dark scales; 8) the head, forelimbs and anterior half of the body blue in adult males, white stripe along the upper lip present, the posterior half of the body, hindlimbs and tail almost uniform brown; 9) 4-5 white blotches on each side of lateral body, gradually increasing brown blotches are present just on each white blotch, the first two are smaller than the white blotches and the rest almost covering the whole of the white blotches; 10) the colourations of adult females similar to those of males, but relatively dim; 11) nuchal and dorsal crest undeveloped in juveniles, the white stripe along the upper lip distinct,

but the dorsolateral blotches indistinct and dark reticulate pattern present on the back in juveniles.

Ecological notes: The specimens were found on the trunks beside roads during the day (Fig. 3) and were found on the branches at night. Two other species of *Calotes, C. irawadi* Zug, Brown, Schulte & Vindum, 2006 and *C. emma* Gray, 1845 were observed to be sympatric with this species.



Figure 3. doi

Habitat of *Calotes vindumbarbatus* at Xueli Village, Taiping Town, Yingjiang County, Dehong Prefecture, Yunnan Province, China.

Analysis

BI and ML analyses showed consistent topology (Fig. 4). The seven specimens collected from Tongbiguan Natural Reserve, western Yunnan, China, were homogeneous and clustered with the type specimens of *Calotes vindumbarbatus* from Myanmar with strong support. The genetic distance (uncorrected p-distance) between the specimens from China and the type specimens of *C. vindumbarbatus* from Myanmar was only 0.9% (Table 3). Therefore, we considered these specimens from China belong to *C. vindumbarbatus*.

According to Wagner et al. (2021), *Calotes vindumbarbatus* has a small body size (maximum SVL 77 mm), low nuchal and dorsal crest, no brownish dorsolateral blotches.

Some of the newly-collected specimens of *C. vindumbarbatus* from western Yunnan agree well with these diagnoses; however, some of the newly-collected specimens of *C. vindumbarbatus* from western Yunnan have much larger body sizes (maximum SVL 116.4 mm), much longer nuchal and dorsal crest and obvious brown dorsolateral blotches. Therefore, we consider that the specimens of *C. vindumbarbatus* in Wagner et al. (2021) are juveniles rather than adults and the description and diagnosis of this species in Wagner et al. (2021) are only based on juveniles. Herein, we provide supplementary description of adults, based on the newly-collected specimens and revised diagnosis of this species.

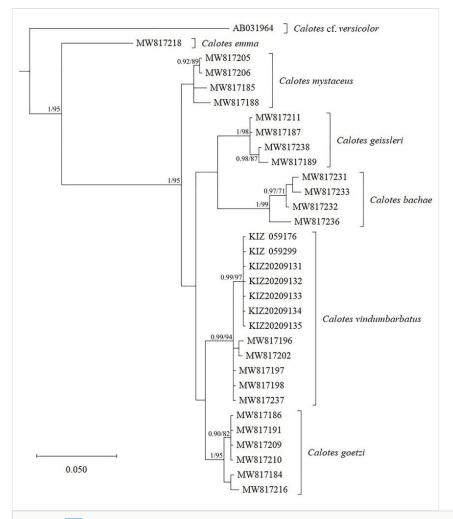


Figure 4. doi

Bayesian Inference tree, based on mitochondrial 12S rRNA sequences. Numbers before slashes indicate Bayesian posterior probabilities (values below 0.90 are not shown) and numbers after slashes indicate bootstrap support for Maximum Likelihood analyses (values below 70 are not shown).

Table 3.							
Mean uncorrected p-distances (%),	based or	12S rRI	NA seque	nces.			
	1	2	3	4	5	6	7
1 Calotes bachae							
2 Calotes geissleri	6.3						
3 Calotes goetzi	6.7	4.9					
4 Calotes mystaceus	6.3	4.6	3.2				
5 Calotes vindumbarbatus (China)	6.8	5.2	3.7	4.5			
6 Calotes vindumbarbatus (Myanmar)	6.5	5.0	3.5	4.2	0.9		
7 Calotes emma	13.5	13.0	11.8	10.8	11.6	12.2	
8 Calotes cf. versicolor	19.2	19.6	18.0	19.2	19.0	18.4	16.7

Discussion

Calotes vindumbarbatus was known previously only from northern Myanmar (Wagner et al. 2021). This is the first record of *C. vindumbarbatus* from China and from outside of Myanmar. The new localities in China are approximately 75–100 km away from the type locality in Myanmar (Fig. 5).

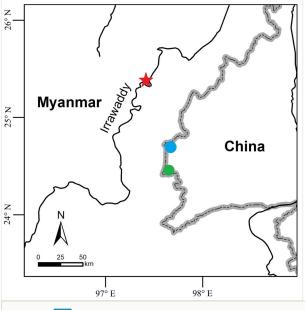


Figure 5. doi

Collection sites of *Calotes vindumbarbatus* in China (green dot and blue dot) and the type locality (red star) of *C. vindumbarbatus* in Myanmar.

Previously, *Calotes mystaceus* was considered to be widely distributed from India, Myanmar, China, Laos, Cambodia, Thailand and Vietnam (Bain and Hurley 2011, Hartmann et al. 2013, Chan-Ard et al. 2015, Das 2015, Pham et al. 2018). Wagner et al. (2021) restricted *C. mystaceus* to southern coastal Myanmar. Therefore, *C. mystaceus* is not distributed in China. According to Wagner et al. (2021) and this study, the species, previously confused with *C. mystaceus* in China, actually refer to *C. goetzi* and *C. vindumbarbatus*.

Gowande et al. (2021) restricted *Calotes versicolor* (Daudin, 1802) to parts of southern and eastern India. Therefore, *C. versicolor* is also not distributed in China. Liu et al. (2021) recorded *C. irawadi* in China. In conclusion, there are seven species of *Calotes* distributed in China to date, namely: *C. emma* Gray, 1845; *C. goetzi* Wagner, Ihlow, Hartmann, Flecks, Schmitz & Böhme, 2021; *C. irawadi* Zug, Brown, Schulte & Vindum, 2006; *C. jerdoni* Günther, 1870; *C. medogensis* Zhao & Li, 1984; *C. paulus* (Smith, 1935); and *C. vindumbarbatus* Wagner, Ihlow, Hartmann, Flecks, Schmitz & Böhme, 2021.

The fauna of the agamids in China was analysed 10 years ago (Ananjeva et al. 2011). Thereafter, the research on the cryptic diversity of agamids in China has made continuous progress. Wang et al. (2020b) compiled the checklists of amphibians and reptiles of China as at the end of 2019. Compared with ten years ago, Wang et al. (2020b) recorded 66 species of the agamids in China, 17 species more than Ananjeva et al. (2011). There are minor changes at the genus level, *Oriocalotes* Günther, 1864 was abolished and *Diploderma* Hallowell, 1861 was resurrected; therefore, the agamids in China still consist of 12 genera (Wang et al. 2020b). In the past two years, some more new species and new records of the agamids from China have been described (e.g. Liu et al. 2020a, Liu et al. 2020b, Wang et al. 2020a, Liu et al. 2021, Wang et al. 2021). However, the diversity of the agamids in China is far from clear and more research in this field is needed.

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References

 Aljanabi S, Martinez I (1997) Universal and rapid salt-extraction of high quality genomic DNA for PCR- based techniques. Nucleic Acids Research 25 (22): 4692-4693. <u>https://doi.org/10.1093/nar/25.22.4692</u>

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- Ananjeva N, Guo X, Wang Y (2011) Taxonomic Diversity of Agamid Lizards (Reptilia, Sauria, Acrodonta, Agamidae) from China: A Comparative Analysis. Asian Herpetological Research 2 (3): 117-128. <u>https://doi.org/10.3724/sp.j.1245.2011.00117</u>
- Bain R, Hurley M (2011) A biogeographic synthesis of the amphibians and reptiles of Indochina. Bulletin of the American Museum of Natural History 360: 1-138. <u>https://doi.org/10.1206/360.1</u>
- Chan-Ard T, Parr J, Nabhitabhata J (2015) A field guide to the reptiles of Thailand. Oxford University Press, New York, 352 pp. [ISBN 9780199736492]
- Das I (2015) A field guide to the reptiles of south-east Asia, Myanmar, Thailand, Laos, Cambodia, Vietnam, peninsular Malaysia, Singapore, Sumatra, Borneo, Java, Bali. Bloomsbury Natural History, London, 376 pp. [ISBN 9781472920577]
- Gowande G, Pal S, Jablonski D, Masroor R, Phansalkar P, Dsouza P, Jayarajan A, Shanker K (2021) Molecular phylogenetics and taxonomic reassessment of the widespread agamid lizard *Calotes versicolor* (Daudin, 1802) (Squamata, Agamidae) across south Asia. Vertebrate Zoology 71: 669-696. <u>https://doi.org/10.3897/vz.</u> 71.e62787
- Hartmann T, Ihlow F, Edwards S, Sovath S, Handschuh M, Böhme W (2013) A preliminary annotated checklist of the amphibians and reptiles of the Kulen Promtep Wildlife Sanctuary in northern Cambodia. Asian Herpetological Research 4 (1): 36-55. <u>https://doi.org/10.3724/sp.j.1245.2013.00036</u>
- Honda M, Ota H, Kobayashi M, Nabhitabhata J, Yong H, Sengoku S, Hikida T (2000) Phylogenetic relationships of the family Agamidae (Reptilia: Iguania) inferred from mitochondrial DNA sequences. Zoological Science 17 (4): 527-537. <u>https://doi.org/ 10.2108/0289-0003(2000)17[527:protfa]2.0.co;2</u>
- Kalyaanamoorthy S, Minh B, Wong T, von Haeseler A, Jermiin L (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. Nature Methods 14 (6): 587-589. <u>https://doi.org/10.1038/nmeth.4285</u>
- Kocher T, Thomas W, Meyer A, Edwards S, Paabo S, Villablanca F, Wilson A (1989) Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proceedings of the National Academy of Sciences 86 (16): 6196-6200. <u>https://doi.org/10.1073/pnas.86.16.6196</u>
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution 35 (6): 1547-1549. <u>https://doi.org/10.1093/molbev/msy096</u>
- Liu S, Hou M, Mo M, Rao D (2020a) A new species of the genus Acanthosaura (Squamata, Agamidae) from Yunnan, China, with comments on its conservation status. Zookeys 959: 113-135. <u>https://doi.org/10.3897/zookeys.959.54601</u>
- Liu S, Hou M, Wang J, Ananjeva N, Rao D (2020b) A New Species of *Diploderma* (Squamata: Sauria: Agamidae) from Yunnan Province, China. Russian Journal of Herpetology 27 (3): 127-148. <u>https://doi.org/10.30906/1026-2296-2020-27-3-127-148</u>
- Liu S, Zuo C, Rao D (2021) Distribution extension of *Calotes irawadi* Zug, Brown, Schulte & Vindum, 2006, previously confused with *C. versicolor* (Daudin, 1802): first record from China. Herpetozoa 34: 83-88. <u>https://doi.org/10.3897/herpetozoa.</u> <u>34.e62596</u>
- Nazarov R, Poyarkov N, Orlov N, Phung T, Nguyen T, Hoang D, Ziegler T (2012) Two new cryptic species of the *Cyrtodactylus irregularis* complex (Squamata: Gekkonidae) from southern Vietnam. Zootaxa 3302: 1-24. <u>https://doi.org/10.11646/zootaxa.3302.1.1</u>

- Pham A, Hoang V, Nguyen T, Ziegler T, Nguyen T (2018) New records and an updated list of lizards from Son La Province, Vietnam. Herpetology Notes 1: 209-216. URL: https://www.biotaxa.org/hn/article/view/32165
- Ronquist F, Teslenko M, van der Mark P, Ayres D, Darling A, Höhna S, Larget B, Liu L, Suchard M, Huelsenbeck J (2012) MrBayes 3.2: efficient bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61 (3): 539-542. <u>https://doi.org/10.1093/sysbio/sys029</u>
- Schmitz A, Ineich I, Chirio L (2005) Molecular review of the genus *Panaspis* sensu lato (Reptilia: Scincidae) in Cameroon, with special reference to the status of the proposed subgenera. Zootaxa 863: 1-28. <u>https://doi.org/10.11646/zootaxa.863.1.1</u>
- Silvestro D, Michalak I (2011) raxmlGUI: a graphical front-end for RAxML. Organisms
 Diversity & Evolution 12 (4): 335-337. <u>https://doi.org/10.1007/s13127-011-0056-0</u>
- Thompson J, Gibson T, Higgins D (2003) Multiple sequence alignment using ClustalW and ClustalX. Current Protocols in Bioinformatics 00 (1): 2.3.1-2.3.22. <u>https://doi.org/10.1002/0471250953.bi0203s00</u>
- Wagner P, Ihlow F, Hartmann T, Flecks M, Schmitz A, Böhme W (2021) Integrative approach to resolve the *Calotes mystaceus* Duméril & Bibron, 1837 species complex (Squamata: Agamidae). Bonn Zoological Bulletin 70 (1): 141-171. <u>https://doi.org/ 10.20363/BZB-2021.70.1.141</u>
- Wang K, Ren J, Wu J, Jiang K, Jin J, Hou S, Zheng P, Xie F, Siler C, Che J (2020a) Systematic revision of mountain dragons (Reptilia: Agamidae:Diploderma) in China, with descriptions of six new species and discussion on their conservation. Journal of Zoological Systematics and Evolutionary Research 59 (1): 222-263. <u>https://doi.org/ 10.1111/jzs.12414</u>
- Wang K, Ren J, Chen H, Lyu Z, Guo X, Jiang K, Chen J, Li J, Guo P, Wang Y, Che J (2020b) The updated checklists of amphibians and reptiles of China. Biodiversity Science 28 (2): 189-218. <u>https://doi.org/10.17520/biods.2019238</u>
- Wang K, Gao W, Wu J, Dong W, Feng X, Shen W, Jin J, Shi X, Qi Y, Siler C, Che J (2021) Two New Species of *Diploderma* Hallowell, 1861 (Reptilia: Squamata: Agamidae) from the Hengduan Mountain Region in China and Rediscovery of *D. brevicaudum* (Manthey, Wolfgang, Hou, Wang, 2012). Zootaxa 4941 (1): 1-32. https://doi.org/10.11646/zootaxa.4941.1.1