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Distribution extension of *Calotes irawadi* Zug, Brown, Schulte & Vindum, 2006, previously confused with *C. versicolor* (Daudin, 1802): first record from China

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Abstract

We report the first country record of *Calotes irawadi*, identified previously as *C. versicolor*, from China based on four specimens collected from Tongbiguan Nature Reserve, Western Yunnan, China. Morphologically, the specimens show good agreement with the original description of *C. irawadi*, and phylogenetically clustered with specimens (including holotype) of *C. irawadi* from Myanmar with strong support. This is also the first record of *C. irawadi* from outside Myanmar.

Key Words

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

Introduction

The agamid genus *Calotes* Cuvier, 1816 currently consists of 25 species (Uetz et al. 2021). Six of them are recorded in China, namely: *C. emma* Gray, 1845; *C. jerdoni* Günther, 1870; *C. medogensis* Zhao & Li, 1984; *C. mystaceus* Duméril & Bibron, 1837; *C. paulus* (Smith, 1935) and *C. versicolor* (Daudin, 1802) [Wang et al. 2020]. The Garden Fence Crested Lizard *C. versicolor*, an agamid lizard found commonly across the Indian subcontinent and the Indo-Chinese region, has a complicated taxonomic history because it was described without a locality (Matyot 2004; Gowande et al. 2016; Chaitanya et al. 2017). In China, *C. versicolor* is recorded from Guangdong, Hainan, and Yunnan provinces, Hong Kong Special Administrative Region, and Guangxi Autonomous Region (Zhao et al. 1999; Yang and Rao 2008; Uetz et al. 2021). The Ayeyarwady Crested Lizard *C. irawadi* (Zug, Brown, Schulte & Vindum, 2006), a species which was separated from the *C. versicolor* complex, was previously only known from Myanmar (Zug et al. 2006; Uetz et al. 2021).

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

Materials and methods

Field surveys were conducted in Tongbiguan Nature Reserve, Yingjiang County, Dehong Prefecture, Yunnan Province, China, under the permit of Tongbiguan Provincial Natural Reserve Management and Protection Bureau. The specimens that were collected, by the authors, were

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euthanized with ethyl acetate and then fixed in 75% ethanol for storage after taking photographs. Liver tissue samples were preserved in 99% ethanol for molecular analysis. The specimen was deposited at Kunming Natural History Museum of Zoology, Kunming Institute of Zoology, Chinese Academy of Sciences (KIZ), Yunnan, China.

Total genomic DNA was extracted from liver tissues with a universal protocol of DNA extraction (Aljanabi and Martinez 1997). A region of the mitochondrial gene NADH dehydrogenase subunit 2 (ND2) and its flanking tRNAs was amplified and sequenced by using the primers L3705 (5'-ATT AGG GTC TGC TAC ACA AGC AGT TGG-3') and H5162 (5'-GGT TGA RAG TAR TCA TCG AGT TAA GAA CGAC-3') (Huang et al. 2013). PCR was conducted as in Huang et al. (2013): an initial denaturing step at 95 °C for 4 min; 35 cycles of denaturing at 94 °C for 35 s, annealing at 65 °C for 45 s, and extending at 72 °C for 90 s; and a final extending step of 72 °C for 8 min. PCR products were electrophoresed in 0.8% agarose gels, visualized with ethidium bromide. The products were purified and sequenced by Invitrogen Trading (Shanghai) Co., Ltd, using the same primers as in PCR. All new sequences were deposited in GenBank. *Draco blanfordii* Boulenger, 1885; *Pseudocalotes kakhienensis* (Anderson, 1879) and *Gonocephalus grandis* (Gray, 1845) were chosen as outgroups based on Zug et al. (2006) and Giri et al. (2019). Homologous and outgroup sequences were obtained from GenBank (Table 1).

Sequences were aligned using ClustalW (Thompson et al. 2002) 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) with pairwise deletions of missing data and gaps. The substitution model GTR+G was used according to Giri et al. (2019). Bayesian Inference (BI) was performed in MrBayes v3.2.6 (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 RaxmlGUI 1.5 (Silvestro and Michalak 2012) and nodal support values were estimated by 1,000 rapid bootstrap replicates.

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 Zug et al. (2006). Morphometric characters included: Eye-ear length (EyeEar, distance from anterior edge of tympanum to posterior of orbit); Head height (HeadH, dorsoventral distance from top of head to underside of jay 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); 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); 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); Snout width (SnW, transverse distance between left and right nares); 4th finger (4FingLng, distance from juncture of 3rd and 4th digits to distalmost extent of 4th finger); 4th toe (4ToeLng, distance from juncture of 3rd and 4th digits to distal end of 4th digit on hindfoot); Crus length (CrusL, length of crus from knee to heel); Forefoot length (ForefL, distance from proximal end of forefoot to tip of fourth digit); Hindfoot length (HindfL, distance from proximal end of hindfoot to distalmost surface of fourth toe); Lower arm length (LoArmL, distance from elbow to distal end of wrist); Pectoral width (PectW, distance between left and right axilla, posterior to forelimb insertions, measured on ventral side); Pelvic width (PelvW, distance between left and right inguen, posterior to

Table 1. Sequences used for phylogenetic analysis.

Taxon	Voucher no.	Locality	GenBank no.
Calotes calotes	WHT 1679	Navinna, Galle, Sri Lanka	AF128482
Calotes ceylonensis	WHT 1624	Yodaganawa, Galle, Sri Lanka	AF128483
Calotes chincollium	CAS 220582	Kan Pelet, Min Dat, Chin, Myanmar	DQ289458
Calotes chincollium	CAS 215505	Sagaing, Myanmar	DQ289459
Calotes emma	MVZ 224102	Tam Dao NP, Vinh Phuc, Vietnam	AF128489
Calotes htunwini	USNM 524044	Chatthin WS, Sagaing, Myanmar	DQ289461
Calotes htunwini	CAS 204851	Mandalay, Myanmar	DQ289462
Calotes htunwini	USNM-GZ 36408	Magwe, Myanmar	DQ289464
Calotes irawadi	USNM 520543	Chatthin WS, Sagaing, Myanmar	DQ289465
Calotes irawadi	USNM-GZ 36709	Mandalay, Myanmar	DQ289467
Calotes irawadi	CAS 204862	Sagaing, Myanmar	DQ289468
Calotes irawadi	KIZ 059191	Yingjiang, Dehong, Yunnan, China	MW591520
Calotes irawadi	KIZ NB20180905	Yingjiang, Dehong, Yunnan, China	MW591517
Calotes irawadi	KIZ HBH20200913	Yingjiang, Dehong, Yunnan, China	MW591519
Calotes irawadi	KIZ HBH20200914	Yingjiang, Dehong, Yunnan, China	MW591518
Calotes jerdoni	CAS 219992	Nat Ma Taung NP, Chin, Myanmar	GQ502783
Calotes liocephalus	WHT1632	Kirimetiyakanda, Knuckles, Sri Lanka	AF128484
Calotes liolepis	WHT1808	Puwakpitiya, Knuckles, Sri Lanka	AF128485
Calotes minor	NCBS AQ035	Chotila, Saurashtra, India	KT952396
Calotes minor	CESG 162	Gujarat, Kutch, India	KT952397
Calotes mystaceus	CAS204848	Mandalay, Myanmar	AF128488
Calotes nigrilabris	WHT 1680	Sita Eliya, Galle, Sri Lanka	AF128486
Calotes paulus	NCBSAQ-AC696	Meghalaya, Cherrapunjee, India	MK795773
Calotes cf. *versicolor*	CAS 205008	Mwe Hauk, Ayeyarwadi, Myanmar	DQ289469
Calotes cf. *versicolor*	CAS 222606	Mudon, Mawlamyine, Mon, Myanmar	DQ289472
Calotes cf. *versicolor*	CAS 230481	Ywa-Ngan, Shan, Myanmar	DQ289477
Calotes zolaiking	NCBS-AU155	Mizoram, Hmuifang, India	MK795774
Draco blanfordii	MVZ 222156	Gia Lai, Vietnam	AF128477
Gonocephalus grandis	TNHC 56500	Ulu Gombak, Selangor, Malaysia	AF128496
Pseudocalotes kakhienensis	CAS 207492	Qushi, Baoshan, Yunnan, China	GQ502784

hindlimb insertions); Snout-vent length (SVL); Snout-forelimb length (SnForel, distance from anterior of forelimb to tip of snout); 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); Tail width (TailW, distance from left to right side of tail base just posterior to vent); Trunk length (TrunkL, distance between posterior edge of forelimb insertion to anterior edge of hindlimb insertion); Upper arm length (UparmL, distance from anterior insertion of forelimb to elbow); Upper leg length (UplegL, distance from anterior edge of hindlimb insertion to knee). Meristic characters included: Canthus rostralis (CanthR, number of elongate scales along dorsolateral snout ridge from above posterodorsal corner of nasal scale to and including posterior most supraciliary scale); Intralabials (Inflab, posterior end defined by posteriormost enlarged scales that touches with Supralabials at rear corner of mouth); Snout scales (Sns, number of scales on line transversally between left and right nasal scales); Supralabials (Suplab, posterior end defined by posteriormost enlarged scales that touches Intralabials at rear corner of mouth); Forefoot lamellae (4FingLm, number of 4th digit lamellae, from 1st lamella at digits' cleft that is wider than deep and touches dorsal digital scale to most distal lamella, the fragmented proximal scales are excluded); Hindfoot lamellae (4ToeLm, as for 4FingLm); Dorsal scales or spines (Dorsal, number of middorsal scales, beginning with first enlarged spine-like scale on nape to above vent); Midbody scale rows (Midbody, number of scale rows around trunk at midbody). Other abbreviations are: NR: Nature Reserve, NP: National Park, WS: Wildlife Sanctuary.

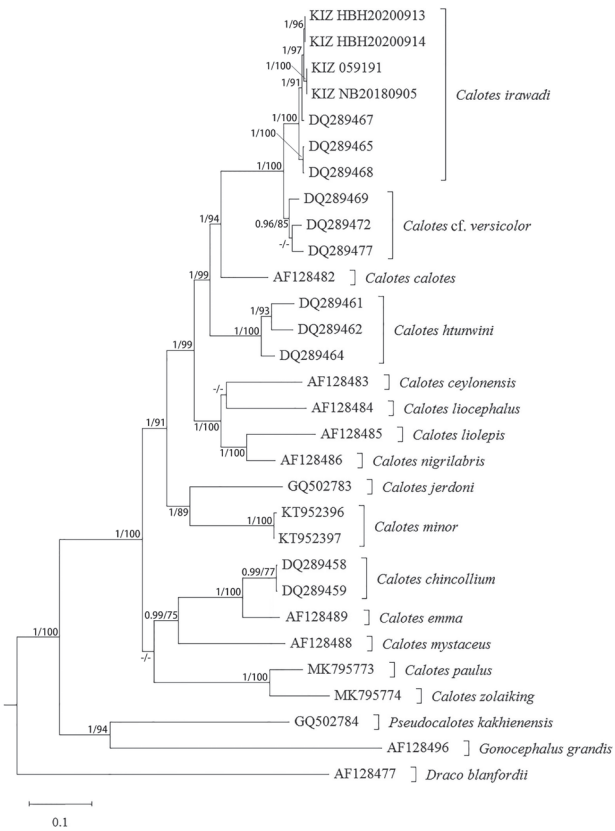

Figure 1. Bayesian Inference tree based on mtDNA sequences of ND2 and its flanking tRNAs. Numbers before slashes indicate Bayesian posterior probabilities (> 0.9) and numbers after slashes indicate bootstrap support for Maximum Likelihood analyses (> 70).

Results

BI and ML analyses shows the same topology, consistent with that of Zug et al. (2006) and Giri et al. (2019). The specimens collected from Tongbiguan NR, Western Yunnan, China, clustered with *Calotes irawadi* from Myanmar (including holotype); this was strongly supported by both BI and ML (Fig. 1). The genetic distance (uncorrected p-distance) between species within the genus ranged from 5.33% to 38.02% and the genetic distance (uncorrected p-distance) between the specimens from China and *C. irawadi* from Myanmar (including holotype) was 1.58% (Table 2). Furthermore, morphological characters of the specimens from China agreed with the original description of *C. irawadi* by Zug et al. (2006). Therefore, we considered that the specimens from Western Yunnan, China belong to *C. irawadi*.

Table 2. Genetic uncorrected p-distances (%) based on the mtDNA ND2 sequences and its flanking tRNAs.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1 *Calotes calotes*																		
2 *Calotes ceylonensis*	19.81																	
3 *Calotes chincollium*	25.65	27.94																
4 *Calotes emma*	27.44	28.54	10.43															
5 *Calotes htunwini*	14.93	21.45	24.54	26.30														
6 *Calotes irawadi* (China)	16.53	22.71	28.24	28.63	18.30													
7 *Calotes irawadi* (Myanmar)	16.34	20.94	27.16	27.27	18.02	1.58												
8 *Calotes jerdoni*	20.75	23.76	27.00	25.41	23.65	23.58	22.65											
9 *Calotes liocephalus*	19.66	19.07	26.97	28.53	21.85	21.94	21.31	23.27										
10 *Calotes liolepis*	20.53	20.26	29.12	29.92	22.99	23.36	22.17	25.40	19.82									
11 *Calotes minor*	25.64	31.16	31.80	32.51	27.95	24.99	25.23	24.54	26.19	28.98								
12 *Calotes mystaceus*	26.73	27.99	22.67	24.03	26.01	30.59	29.10	29.41	27.57	26.88	31.42							
13 *Calotes nigrilabris*	17.77	15.77	25.15	26.27	19.44	19.90	18.90	24.20	17.11	12.42	26.47	25.04						
14 *Calotes paulus*	31.24	33.31	33.19	33.06	34.88	35.83	35.71	29.31	32.97	32.20	32.55	31.61	32.02					
15 *Calotes* cf. *versicolor*	15.02	21.62	26.63	28.25	17.83	5.72	5.33	22.38	21.29	23.17	24.57	29.03	19.96	34.55				
16 *Calotes zolaiking*	33.29	37.38	35.51	35.42	38.02	37.85	37.62	32.82	34.39	35.98	34.36	34.77	36.90	13.81	36.07			
17 *Draco blanfordii*	44.95	47.25	45.42	46.00	45.83	46.77	43.63	45.47	46.09	50.20	52.90	48.20	45.23	55.96	43.28	57.59		
18 *Gonocephalus grandis*	38.74	46.58	44.05	45.14	41.25	44.50	41.92	42.77	44.90	44.85	49.07	44.17	44.03	54.79	42.59	58.46	51.64	
19 *Pseudocalotes kakhienensis*	36.21	40.62	37.77	37.70	38.11	43.70	39.31	36.96	39.82	41.21	44.27	37.50	39.40	45.62	39.13	49.11	45.19	38.82

Taxonomic account

Calotes irawadi Zug, Brown, Schulte & Vindum, 2006

Figs 2, 3A, B

Suggested Chinese name: 实皆树蜥 (Wei et al. 2017)

Specimens examined. KIZ 059191 (juvenile) and KIZ NB20180905 (adult male) collected by Shuo Liu on 5 September 2018 from Tongbiguan NR, Nabang Town, Yingjiang County, Dehong Prefecture, Yunnan Province, China (24°45'47"N, 97°34'15"E; at an elevation of 320 m); KIZ HBH20200913 (adult female) and KIZ HBH20200914 (juvenile) collected by Shuo Liu on 13 and 14 September 2020 (respectively) from Tongbiguan NR, Xueli Village, Taiping Town, Yingjiang County, Dehong Prefecture, Yunnan Province, China (24°26'32"N, 97°33'4"E; at an elevation of 350 m).

Morphological description. Morphometric and meristic data are presented in Table 3. Head is triangular and distinct from neck; snout-tip blunt; head behind eyes with edges slightly bowed outward by jaw muscles but edges largely parallel; sides of head flat; dorsally head scales are variable in size and smooth surfaced, most equivalent in size to dorsal trunk scales; 6–7 scales on line transversally between left and right nasal scales; 8–9 elongate and sharply folded scales along dorsolateral snout ridge from above postero-dorsal corner of nasal scale to and including the posterior most supraciliary scale; rostral equivalent to the supralabials in height; supralabials 11; laterally head with single large nasal scale on each side abutting rostral; loreal and preocular area with small scales. The tympanum is large and naked with a pair of spines or clusters in supratympanum area; medially the chin throat scales triangular and smooth to lightly keeled; mental triangular; intralabials 9–10.

Trunk scalation generally keeled dorsally and laterally; middorsal crest of elongate scales; the dorsal spines scales are blade-like and laterally compressed; 44–53 middorsal scales, 38–46 scale rows around trunk at midbody, all trunk scales are keeled, weakly so on ventrolateral half of

Table 3. Measurements (in mm) and scalation data for the specimens of *Calotes irawadi* collected from China. For character abbreviations see Materials and methods. Paired meristic characters were made on the left side.

	KIZ 059191 Juvenile	KIZ NB20180905 Male	KIZ HBH20200913 Female	KIZ HBH20200914 Juvenile
EyeEar	3.1	5.2	4.5	2.5
HeadH	8.6	13.8	12.8	8.2
HeadL	12.3	20.6	18.1	12.1
HeadW	8.8	14.2	13.7	9.0
Interorb	6.3	9.4	8.7	5.7
JawW	8.3	14.7	14.1	8.2
NarEye	2.2	4.7	4.5	2.3
SnEye	5.4	8.6	8.8	5.3
SnW	3.5	5.4	5.2	3.6
4FingLng	7.3	11.5	12.0	7.5
4ToeLng	11.1	17.2	16.1	11.3
CrusL	11.7	19.9	18.4	11.8
ForefL	9.8	16.6	14.5	10.3
HindfL	18.6	28.5	25.8	18.8
LoArmL	9.2	15.7	13.4	8.8
PectW	6.4	11.5	11.4	6.9
PelvW	4.2	8.5	6.9	4.1
SVL	49.3	86.3	80.5	47.8
SnForel	17.9	32.3	28.3	17.2
TailH	4.8	10.5	8.8	4.6
TailL	135.3	260.0	161.5+	148.9
TailW	4.7	9.4	7.8	4.5
TrunkL	30.6	51.4	49.0	29.5
UparmL	10.5	18.2	16.4	10.3
UplegL	12.4	22.1	17.3	11.9
CanthR	8	9	8	9
Inflab	9	10	10	10
Sns	7	6	7	6
Suplab	11	11	11	11
4FingLm	21	19	23	22
4ToeLm	24	23	25	25
Dorsal	44	46	53	47
Midbody	38	41	46	43

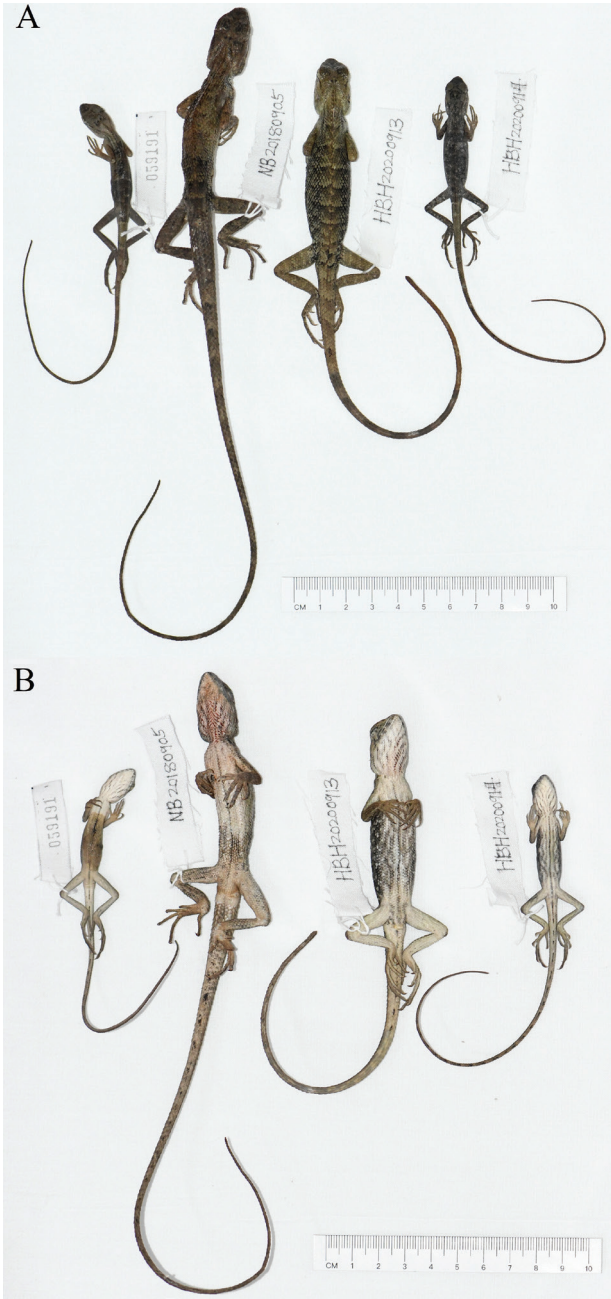

Figure 2. The specimens of *Calotes irawadi* from Tongbiguan NR, Western Yunnan, China in preservative. **A.** Dorsal view; **B.** Ventral view.

Figure 3. *Calotes irawadi* in life and its habitats. **A.** Adult male (KIZ NB20180905) from Nabang Town, Yingjiang County, Dehong Prefecture, Yunnan Province, China; **B.** Adult female (KIZ HBH20200913) from Xueli Village, Taiping Town, Yingjiang County, Dehong Prefecture, Yunnan Province, China; **C.** Habitat at Nabang Town, Yingjiang County, Dehong Prefecture, Yunnan Province, China; **D.** Habitat at Xueli Village, Taiping Town, Yingjiang County, Dehong Prefecture, Yunnan Province, China.

neck and trunk. Keel and scale orientation are diagonally upward from neck and supra-axillary area to base of tail; preaxillary scales mostly smooth; ventral scales large and uniform in size from throat to vent and strongly keeled.

Limbs have modest to large scales, all keeled; 19–23 lamellae on fourth finger and 23–25 lamellae on fourth toe; each finger and toe with strongly bicarinate lamellae ventrally, whilst claws are long, thin and sharply pointed on all digits.

Tail length is 2.7–3.1 times of SVL; tail scalation similar to trunk although more strongly keeled with progressive loss of scale rows distally.

Coloration. These lizards have a very clear ability to change their body colors. In life, body color varies from yellowish white to almost wholly black with or without dark or light stripes (Fig. 3A, B). In preservative, the dorsal color is dark brown with some indistinct black or white stripes; the ventral color is white with some black stripes (Fig. 2B).

Sexual dimorphism. The width of the base of tail in adult males is significantly thicker than that in adult females, except for this, there is little difference in morphological characters between females and males, and there is little difference in body color between males and females during the nonbreeding season. It is difficult to distinguish the gender of the juveniles in appearance.

Ecological notes. The specimens from Nabang Town and from Hongbenghe were found on the sides of a small road (Fig. 3C) near a village and on the sides of a big road

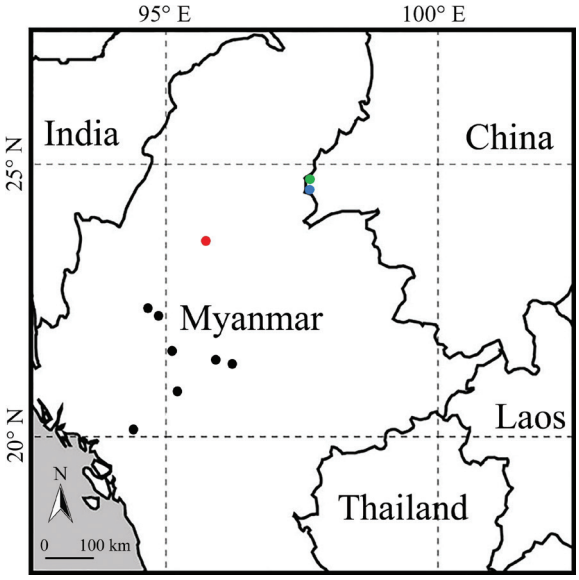

Figure 4. Collection sites of *Calotes irawadi* in China (green dot and blue dot), the type locality (red dot), and other locations (black dots) of *C. irawadi* recorded in Myanmar.

(Fig. 3D), respectively. Specimens were collected during both the day and night. They were active on the trunks near the ground during the day, and asleep on the higher branches at night. Reproductive behavior was not observed.

Discussion

In Yunnan Province, China, *Calotes versicolor* was recorded from Wenshan Prefecture, Dehong Prefecture, Nujiang Prefecture, Baoshan City, Dali Prefecture, Pu'er City, and Xishuangbanna Prefecture (Zhao et al. 1999; Yang and Rao 2008). After many years of field surveys in Yunnan and examinations of related specimens, we have never found *C. versicolor* in Xishuangbanna and the specimens that were identified as *C. versicolor* collected from Xishuangbanna are actually *C. emma*. Therefore, we consider the record of *C. versicolor* from Xishuangbanna as probably incorrect. Additionally, during surveys, we found that the lizards previously identified as *C. versicolor* from Eastern Yunnan (Wenshan Prefecture) and Western Yunnan (Dehong Prefecture), belong to two different species. Based on morphological comparisons and molecular analyses, we found that the species previously identified as *C. versicolor* from Dehong Prefecture is actually *C. irawadi*. As for the species distributed in Nujiang Prefecture, Baoshan City, Dali Prefecture, and Pu'er City, because we have not collected specimens from these places yet, whether they also belong to *C. irawadi* remains unknown.

Calotes irawadi was known previously only from the Central Dry Zone in Sagaing, Magwe, Mandalay divisions of Myanmar (Zug et al. 2006). This is the first record of *C. irawadi* from China and from outside of Myanmar. The new localities in China are approximately 210–240 km away from the type locality in Myanmar (Fig. 4). Our work brings the total species number of the genus *Calotes* in China to seven.

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