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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Academic editor: Silke Schweiger  •  Received 30 December 2020  •  Accepted 24 February 2021  •  Published 15 March 2021

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...
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 NDH dehydrogenase subunit 2 (ND2) and its flanking tRNAs was amplified and sequenced by using the primers L3705 (5'-ATT AGG GTG TCG TAC ACA AGC AGT TGG-3') and H5162 (5'-GTT TGA RAG TAR TCA TCG AGA TTA GAA CGC-3') (Huang et al. 2013). PCR was conducted as in Huang et al. (2013): an initial denaturing step at 95 ℃ for 4 min; 35 cycles of denaturing at 94 ℃ for 35 s, annealing at 65 ℃ for 45 s, and extending at 72 ℃ for 90 s; and a final extending step of 72 ℃ 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.

**Table 1. Sequences used for phylogenetic analysis.**

| Taxon          | Voucher no. | Locality                          | GenBank no. |
|----------------|-------------|-----------------------------------|-------------|
| Calotes calotes | WHT1679     | Nanima, Gale, Sri Lanka            | MW591520    |
| Calotes cyrtomenis | WHT1624  | Yodagumawa, Gale, Sri Lanka        | MW591522    |
| Calotes cinctilineum | CAS220582 | Vung Hai, Soc Trang, Vietnam        | MW591495    |
| Calotes chalcoglossus | CAS215505 | Sogaing, Myanmar                   | MW591447    |
| Calotes emma | MVZ224102    | Jam Dao NIP, Vinh Phuc, Vietnam    | MW591489    |
| Calotes hanunii | USNM 524044 | Chatham WS, Saigon, Vietnam         | MW591461    |
| Calotes irawadi | CAS204851 | Mandalay, Myanmar                  | MW591462    |
| Calotes jerdoni | USNM-GZ 36709 | Mandalay, Myanmar                 | MW591467    |
| Calotes liolepis | KIZ 059191 | Yingjiang, Yunnan, China           | MW591517    |
| Calotes irawadi | KIZ 02180905 | Yingjiang, Dehong, China           | MW591513    |
| Calotes irawadi | KIZ HHB20020913 | Yingjiang, Dehong, China          | MW591515    |
| Calotes irawadi | KIZ HHB20020914 | Yingjiang, Dehong, China         | MW591518    |
| Calotes jarori | CAS 219992 | Nat Ma Itang NF, Ch.in, Myanmar    | MG502781    |
| Calotes locephalus | WHT1632 | Krimetiyakandia, Knuckles, Sri Lanka | AS214848    |
| Calotes liolepis | WHT1808 | Puwakot, Knuckles, Sri Lanka       | AS214837    |
| Calotes minor | NCBS AQ035 | Chottila, Saarashtra, India        | KJ592396    |
| Calotes minor | CASGU 162  | Gujarat, Kutch, India              | KJ592397    |
| Calotes mystaceus | CAS204884 | Mandalay, Myanmar                  | AE592849    |
| Calotes nigricrista | WHT 1680 | Sita Eliya, Gale, Sri Lanka        | AS214848    |
| Calotes paulus | NCBSAQ- AC96 | Meghalaya, Cherrapunjee, India     | MK795773    |
| Calotes cf. versicolor | CAS 220508 | Mwe Hauk, Ayeyarwadi, Myanmar      | AS214848    |
| Calotes cf. versicolor | CAS 220606 | Mwe Hauk, Ayeyarwadi, Myanmar      | AS214847    |
| Calotes cf. versicolor | CAS 236841 | Ywa-Ngan, Shan, Myanmar            | AS214847    |
| Calotes zolakting | NCBS-AU1155 | Meghalaya, Dimrantang, India       | AS214847    |
| Draco blanfordii | WHT 222156 | Glax Lao, Vietnam                  | AS214847    |
| Gonocephalus grandis | TNHC 56500 | Ulu Gombak, Selangor, Malaysia     | AS214846    |
| Pseudoallocationis kakhkienensis | CAS 207492 | Quahi, Brachan, Yunnan, China      | GG502578    |

(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
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 Yun-nan, China, clustered with Calotes irawadi. Other abbreviations are: NR: Nature Reserve, NP: National Park, WS: Wildlife Sanctuary.

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 |
|-------------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| Calotes calotes   | 19.81 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Calotes cyclomenis | 25.65 | 27.94 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Calotes chincollium | 27.44 | 29.54 | 10.43 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Calotes emma      | 14.93 | 21.45 | 24.54 | 26.30 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Calotes htunwini  | 16.53 | 22.71 | 28.24 | 28.63 | 18.30 |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Calotes irawadi (China) | 16.34 | 20.94 | 27.16 | 27.27 | 18.02 | 1.58 |    |    |    |    |    |    |    |    |    |    |    |    |
| Calotes irawadi (Myanmar) | 20.75 | 23.76 | 27.00 | 25.41 | 23.65 | 23.58 | 22.65 |    |    |    |    |    |    |    |    |    |    |    |
| Calotes jerdoni   | 19.66 | 20.07 | 26.97 | 28.53 | 21.85 | 21.94 | 21.31 | 23.27 |    |    |    |    |    |    |    |    |    |    |
| Calotes leiolepis | 20.53 | 20.26 | 26.92 | 29.92 | 22.99 | 23.36 | 22.17 | 25.40 | 19.82 |    |    |    |    |    |    |    |    |    |
| Calotes minor     | 25.64 | 31.16 | 31.80 | 32.12 | 27.95 | 24.99 | 25.23 | 25.34 | 26.19 | 28.98 |    |    |    |    |    |    |    |    |
| Calotes mystaceus | 26.73 | 27.99 | 22.67 | 24.03 | 26.01 | 30.59 | 29.10 | 29.41 | 27.57 | 26.88 | 31.42 |    |    |    |    |    |    |    |
| 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 |    |    |    |    |    |    |
| Calotes palpis    | 31.24 | 33.31 | 33.19 | 33.06 | 34.88 | 31.83 | 35.71 | 29.31 | 32.97 | 32.20 | 35.55 | 31.61 | 32.02 |    |    |    |    |
| 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 |    |    |    |
| Calotes zolaikling | 33.29 | 37.38 | 35.31 | 35.42 | 38.02 | 37.85 | 37.62 | 32.82 | 34.39 | 34.98 | 34.36 | 34.77 | 36.90 | 13.81 | 36.07 |    |    |
| Draco blanfordi   | 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 |    |
| Gonocephalus grandis | 38.74 | 46.58 | 44.05 | 45.14 | 41.25 | 44.50 | 41.92 | 42.77 | 44.90 | 45.84 | 49.07 | 44.17 | 44.03 | 54.79 | 42.59 | 58.46 | 51.64 |
| Pseudocalotes kakhienensis | 36.21 | 40.62 | 37.77 | 37.70 | 38.81 | 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 |

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).
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, Xueili 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 corer 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 supratympanic 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.

| Specimen | KIZ 059191  | KIZ NB20180905 | KIZ HBH20200913 | KIZ HBH20200914 |
|----------|-------------|----------------|-----------------|-----------------|
|          | Juvenile    | Male           | Female          | 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             |
| 4FingLm  | 7.3         | 11.5           | 12.0            | 7.5             |
| 4ToeLm   | 11.1        | 17.2           | 16.1            | 11.3            |
| CrusL    | 11.7        | 19.9           | 18.4            | 11.8            |
| ForeL    | 9.8         | 16.6           | 14.5            | 10.3            |
| HindL    | 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            |
| TailL    | 4.8         | 10.5           | 8.8             | 4.6             |
| TailW    | 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   | 9           | 9              | 8               | 9               |
| Inflab   | 9           | 10             | 10              | 10              |
| Sus      | 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              | 42              |
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 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 HBB20200913) 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.]

![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.]

Discussion

In Yunnan Province, China, *Calotes versicolor* was recorded from Wenshan Prefecture, Dehong Prefecture, Nuijiang 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 record the species as *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*.

*Calotes irawadi* was known previously only from the Central Dry Zone in Sagaing, Magwe, Mandalay division 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 approximate 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.

Acknowledgements

We would like to thank Decai Ouyang and Lei Ouyang for assistance in the field. Thanks to Hong Hui for providing photos. Thanks also to our colleagues for their help and advice. We also thank the reviewers for their valuable comments on the manuscript. This work was supported by Science-Technology Basic Condition Platform from the Ministry of Science and Technology of the People’s Republic of China (Grant No. 2005DKA21402).

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