

Letter to the editor

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A new species of the genus *Kurixalus* (Anura: Rhacophoridae) from Sichuan Province, southwestern China

Combining morphological and molecular data, we describe a new amphibian species of the genus *Kurixalus* Ye, Fei, & Dubois, 1999 from the Qionglai Mountain within the western margin of the Sichuan Basin in China. *Kurixalus qionglaiensis* **sp. nov.** can be diagnosed based on a combination of the following morphological characters: medium-sized within genus (snout-vent length (SVL) in males 28.9–33.3 mm); tympanum distinct, subequal to half of eye diameter; snout pointed, prominence on tip; iris golden with brown spots; slight nuptial pad on first finger in males; background coloration of dorsal surface brown, lateral body and femoral yellow; white, dark brown edged triangular markings on cheek; chin shaded dark brown; pair of large symmetrical dark patches on chest; belly clouded brown and scattered black spots; toes moderately webbed, formula I 2–2 II 1.5–2.5 III 1.5–2.5 IV 2.5–1.5 V; tibia length slightly shorter than half of SVL, tibiotarsal articulation reaching posterior border of eye; single external vocal sac present. Based on the 16S rRNA gene, the genetic distance between the new species and its sister taxon *K. idiootocus* (Kuramoto & Wang, 1987) was 4%. At present, the new species is known only from a single location at an elevation of ~600 m in Pingle Town, Qionglai City, Sichuan, southwestern China. This location can represent a new northernmost geographical limit of the genus *Kurixalus*.

Seven tree frogs were collected in the Qionglai Mountains of Sichuan Province in southwestern China. Although these frogs were morphologically similar to frilled tree frogs (genus *Kurixalus* Ye, Fei, & Dubois, 1999) from the mountains of southern China, northeast India, Indochina, and Indonesia (Figure 1A), no species in the genus has been recorded north of N30°. Based on a combination of morphological characters and molecular data (see detailed comparison below), these specimens are described as a new species herein, named

Kurixalus qionglaiensis **sp. nov.** This species represents a sister taxon to *K. idiootocus* (Kuramoto & Wang, 1987), with a mean 16S rRNA genetic distance of 4%, which is sufficient to indicate species-level divergence in Anura (Vieites et al., 2009).

The genus *Kurixalus* is widely distributed in northeastern India, southern mainland China and adjacent islands, including Taiwan and Hainan, Ryukyu archipelago, Indochina, Peninsular Malaysia, and Indonesia (Yu et al., 2020). The genus currently contains 20 known species (Frost, 2021), 10 of which are recorded in China (i.e., *K. berylliniris* Wu, Huang, Tsai, Li, Jhang & Wu, 2016, *K. eiffingeri* (Boettger, 1895), *K. hainanus* (Zhao, Wang & Shi, 2005), *K. idiootocus*, *K. lenquanensis* Yu, Wang, Hou, Rao & Yang, 2017, *K. naso* (Annandale, 1912), *K. odontotarsus* (Ye & Fei, 1993), *K. raoi* Zeng, Wang, Yu & Du, 2021, *K. wangi* Wu, Huang, Tsai, Li, Jhang & Wu, 2016, and *K. yangi* Yu, Hui, Rao & Yang, 2018 (Zeng et al., 2021)).

In June 2021, seven tree frog specimens were collected and photographed from bamboo and scrub forests on the western margin of the Sichuan Basin in China. Tissue samples were taken for genetic analyses, and deposited with voucher specimens in the Herpetological Museum, Chengdu Institute of Biology (CIB), Chinese Academy of Sciences (CAS). Measurements were taken using a digital caliper to the nearest 0.1 mm, with morphometrics summarized in Supplementary Table S1. Morphological terminology followed Fei et al. (2009) and Yu et al. (2018).

To reconstruct the phylogeny of *Kurixalus*, we extracted genomic DNA and amplified and sequenced a 550 bp fragment of the mitochondrial 16S rRNA gene. DNA extraction and amplification protocols followed Cheng et al. (2021). The GenBank accession Nos. of the 16S rRNA gene sequences for the 20 species are listed in Supplementary Table S2.

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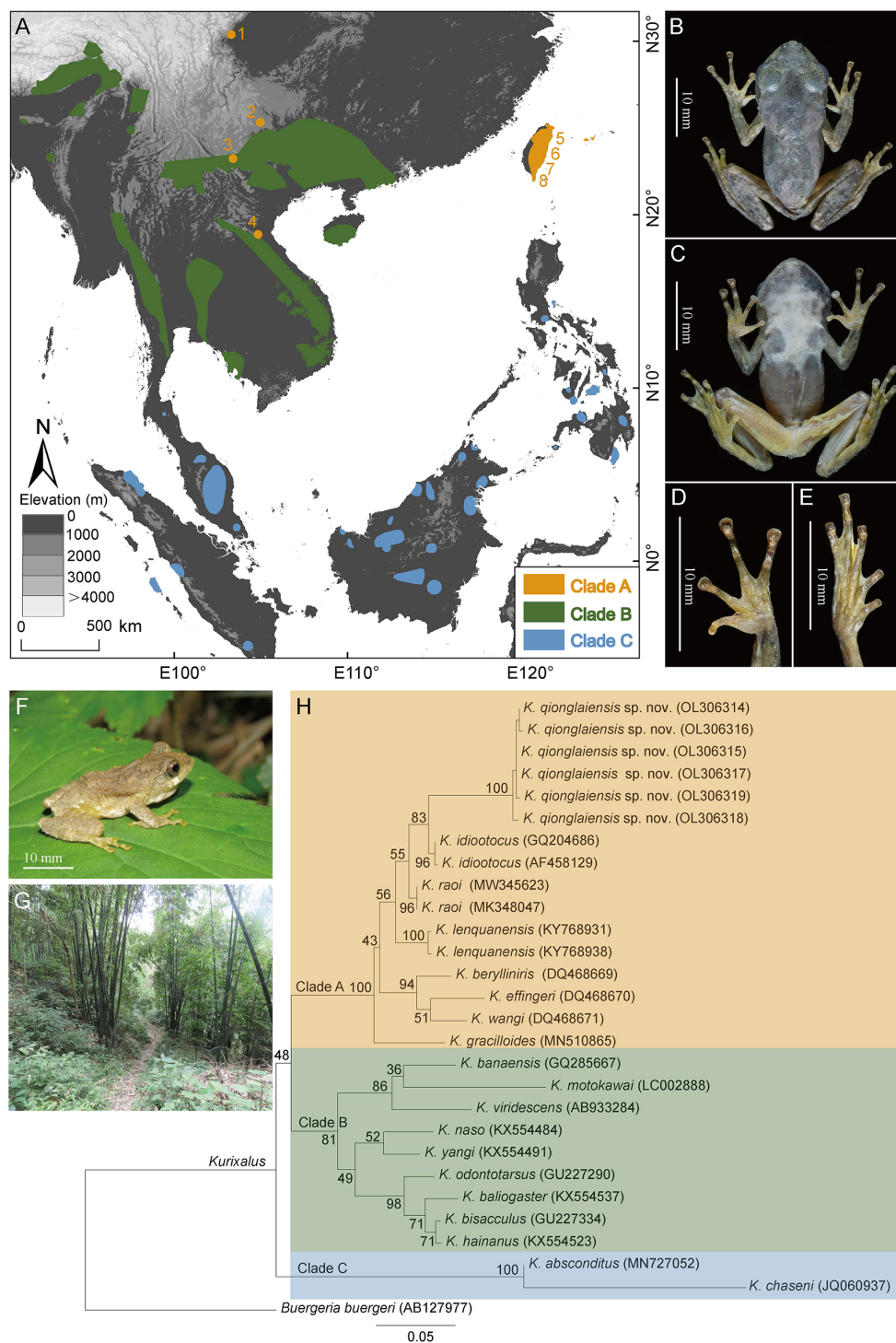


Figure 1 Geographic distribution and phylogenetic relationships of the genus *Kurixalus* and photos of *Kurixalus qionglaiensis* sp. nov. and habitat at type locality

A: Current distribution map of the genus *Kurixalus*, retrieved from the International Union for Conservation of Nature (IUCN; <http://www.iucn.org/>). Localities of species classified as Clade A in this study: (1) *Kurixalus qionglaiensis* sp. nov.; (2) *K. raoi*; (3) *K. lenquanensis*; (4) *K. gracilloides*; (5) *K. eiffingeri*; (6) *K. idiootocus*; (7) *K. berylliniris*; and (8) *K. wangi*. Adult male *Kurixalus qionglaiensis* sp. nov. (holotype CIB 118031) in preservative. B: Dorsal view. C: Ventral view. D: Left hand. E: Right foot. F: Dorsolateral view of *Kurixalus qionglaiensis* sp. nov. (paratype CIB 118032) in life. G: Bamboo and scrub forests near stream at type locality. H: Bayesian inference tree based on concatenated sequences of 16S rRNA gene. Node support is presented as Bayesian posterior probability (PP). Specimens are indicated by the GenBank accession Nos. Photos by Ka Wah Leung, Mao-Jun Zhong, and Chun-Peng Guo.

Details on maximum-likelihood (ML) analysis are provided in the Supplementary Materials and Methods. Pairwise sequence divergence (uncorrected *P*-distances) was calculated using MEGA X (Kumar et al., 2018). Without available molecular data, *K. verrucosus* (Boulenger, 1983) and *K. appendiculatus* (Günther, 1858) were not included in our phylogenetic analyses. These two species are similar to those classified in Clades B & C, respectively (Zeng et al., 2021). Therefore, their absence had no impact on the phylogenetic position of the new species.

Based on ML analysis, *Kurixalus qionglaiensis* sp. nov., which is distributed in the lowlands of the Qionglai Mountains, is the fourth mainland member of Clade A (Figure 1A), which also includes *K. raoi* and *K. lenquanensis* from the Yunnan-Guizhou Plateau, and *K. gracilloides* Nguyen, Duong, Luu, & Poyarkov, 2020, from the Annamese Mountains (Van Nguyen et al., 2020; Yu et al., 2017; Zeng et al., 2021).

Average uncorrected pairwise distances between *Kurixalus qionglaiensis* sp. nov. and all other congeners ranged from 4% (*K. idiotocous*) to 21.6% (*K. chaseni* (Smith, 1924)) (Supplementary Table S3). Based on morphological examination, the new species could be clearly distinguished from all other congeners. Thus, we formally described the *Kurixalus* population from the Qionglai Mountain, Sichuan Province, as a new species, representing the 21st known species in the genus *Kurixalus*.

Taxonomic account

***Kurixalus qionglaiensis* sp. nov.** Guo, Zhong, Leung, Wang & Hu (Figure 1B–E; Supplementary Table S1).

Qionglai frilled tree frog/Qióng Láí Yuán Zhī Shù Wā (邛崃原指树蛙).

Holotype: CIB 118031 (Figure 1B–E), adult male, collected at 2300 h on 5 June 2021 by Chun-Peng Guo and Jian Chen from Lugou Bamboo Sea (芦沟竹海), Pingle Town, Qionglai City, Sichuan Province, southwestern China (N30°21'51", E103°18'30"; elevation 615 m a.s.l.).

Paratypes: Six adult males (CIB 118032–118037) collected at 2110 h on 5 June 2021 by Mao-Jun Zhong and Xiao-Yi Wang from the same location as the holotype.

Etymology: The specific epithet “*qionglaiensis*” refers to the locality of the holotype in the Qionglai Mountain, Sichuan Province, southwestern China.

Diagnosis: The new species is assigned to the *Kurixalus* genus based on the following morphological characters: small body size; vomerine teeth present; inner and outer fingers not opposed; serrated dermal fringes along outer edge of forearm and tarsus; rough dorsal and lateral surface with tubercles; white tipped dermal tubercles on posterior thigh (Fei et al., 2009; Zeng et al., 2021).

Kurixalus qionglaiensis sp. nov. can be distinguished from all known congeners by a combination of the following morphological characters: medium-sized within genus (SVL: 28.9–33.3 mm in adult males; *n*=7); snout pointed and projecting; tympanum subequal to half of eye diameter; iris golden with brown spots; weak nuptial pad present on first finger in males; background coloration of dorsal surface brown, lateral body and femoral yellow; white, dark brown

edged triangular markings on cheek; chin shaded dark brown; pair of large symmetrical dark patches present on chest; ventral surface shaded brown; toes moderately webbed, formula I 2–2 II 1.5–2.5 III 1.5–2.5 IV 2.5–1.5 V; tibia length slightly shorter than half of SVL, tibiotarsal articulation reaching posterior border of eye; single external vocal sac present.

Description of holotype: Small body size (SVL 28.9 mm); head width longer than length (HW/HL=1.10); canthus rostralis curved and blunt, snout shape in dorsal and ventral view with pointed tip; snout length longer than eye diameter (SL/ED=1.13); loreal region oblique, slightly concave; nostril oval, slightly protuberant, closer to tip of snout than eye (SL=4.4 mm; DNE=3.2 mm); distance between nares slightly shorter than distance between upper eyelids and between nostril and eye (IND=2.7 mm; IOD=3.4 mm; UEW=2.9 mm); nostril to eye distance shorter than eye diameter (DNE/ED=0.86); pineal spot absent; pupil oval, horizontal; tympanum distinct, rounded, subequal to half of eye diameter (TD/ED=0.49); supratympanic fold distinct, curving from posterior edge of eye to insertion of arm; tongue large, forked, and shallowly emarginated; vomerine teeth in two short oblique series, anterior edges in line with centers of choanae; single external vocal sac and paired vocal slits present.

Forearm and hand length slightly longer than half of SVL (FLL/SVL=0.52). Tips of all four fingers enlarged to discs with distinct transverse circum-marginal grooves; relative width of discs I < II < IV < III, disc of finger I small, same width as phalanx width, disc of finger III about two thirds tympanum diameter (TD=1.8 mm; TDW 3=1.2 mm); relative length of fingers I < II < IV < III; fingers weakly webbed at base; slight nuptial pad present on first finger; subarticular tubercles prominent, rounded, formula 1, 2, 2, 1; two metacarpal tubercles present, subequal in size; several white tipped tubercles present on lateral lower arm in ventral view; outer margin of fourth finger with longitudinal flat tubercles connected into weak flap.

Heels just meeting when flexed hindlimbs held at right angles to body axis; hindlimb moderate in length, tibiotarsal articulation reaching posterior border of eye when appressed; shank shorter than thigh and longer than foot (THL=13.9 mm; TL=13.4 mm; FL=13.3 mm); tips of toes expanded into discs with distinct circum-marginal and transverse grooves; relative length of toes I < II < III < V < IV; relative size of discs I < II < III < IV < V; disc on toe I same width as phalanx width; toes dorsoventrally compressed, narrow lateral fringes on outer edge of all toes. Webbing moderate on all toes, webbing formula I 2–2 II 1.5–2.5 III 1.5–2.5 IV 2.5–1.5 V; subarticular tubercles prominent, rounded, formula 1, 1, 2, 3, 2; inner metatarsal tubercle distinct, oval; outer metatarsal tubercle absent, supernumerary tubercles absent; small, white-tipped tubercle present on heel; outer margin of fifth toe with longitudinal flat tubercles connected into weak flap, series of large tubercles scattered along outer edge of tarsus.

Upper eyelid, head, dorsum, flanks, and dorsal surface of limbs scattered with numerous large tubercles, several white tubercles below anal opening; white conical tubercle present on tibiotarsal articulation; chin, throat, breast, belly, and femoral with flat tubercles, more so on throat.

Coloration of holotype in life: Iris background coloration golden, upper one fourth golden yellow with small brown spots, lower part dark golden yellow with brown spots. Background coloration of dorsal surface brown, lateral body and femoral yellow; pair of dark brown bands on dorsum behind eye; brown saddle-shaped mark on dorsum; chin shaded dark brown, pair of large symmetrical dark patches on chest; ventral surface posteriorly shaded brown and scattered black spots; dorsal surface of forelimbs and hindlimbs with irregular dark colored crossbars.

Coloration of holotype in preservative: Dorsal surface grayish brown, dark brown marking pattern as in life. White, dark brown edged triangular markings on cheek in life and preservative, connected to white area on throat through mandible. Chest white with pair of large symmetrical dark patches; chin and abdomen shaded dark brown; ventral view of lateral body, hands, and legs yellow.

Variation: Head width longer than length in holotype and most paratypes, but head length slightly longer than head width in paratype CIB 118033 (HL=10.6; HW=10.5). Pattern of dark brown markings on dorsum varied across specimens. Holotype and most paratypes showed saddle-shaped dorsal marking but marking indistinct in paratype CIB 118036. Color pattern of paratype CIB 118032 differed from other specimens, with chin showing numerous light brown patches (Supplementary Figure S1). Number of small black spots across ventral surface also varied among individuals (Supplementary Figure S1). In preservative, external subgular skin pouch of holotype and two paratypes (CIB 118035 and CIB 118036) was distinctly expanded compared to other paratypes.

Ecological and natural history notes: Loud calls from adult males were heard during the field survey. Four individuals were found calling on shrub leaves ~0.5 m above the ground in a bamboo forest (Figure 1F). Three other individuals were found sitting on bamboo plants ~1 m above the ground. *Bufo gargarizans* Cantor, 1842 and *Rana omeimontis* Ye & Fei, 1993 were also encountered at the type locality.

There were no still water pools or other small water bodies in the surrounding area, but there were many bamboo plants and tree holes in the biotope. Rainfall accumulation in small depressions, tree holes, and bamboo stems could provide favorable conditions for reproductive activity of the new species. However, due to the scarcity of females and eggs discovered during the field survey, other details about reproductive behavior remain unknown.

Distribution: We formally report a new northernmost geographical limit of the genus *Kurixalus* (Figure 1A). *Kurixalus qionglaiensis* sp. nov. appears to be restricted to bamboo and scrub forests (Figure 1G) at elevations of ~600 m a.s.l., and likely inhabits similar habitats in the Sichuan Basin.

Comparisons: *Kurixalus qionglaiensis* sp. nov. is morphologically similar to *K. idiootocus*, but differs by presence of nuptial pad on first finger (vs. presence of nuptial pads on first and second fingers); relatively larger size (28.9 to 33.3 mm SVL in males (average of 30.8 mm) vs. 26.3–30.8 mm SVL in males (average of 28.2 mm)); tibiotarsal articulation reaching posterior border of eye (vs. reaching center of eye); forelimb length slightly longer than half of SVL,

average FLL/SVL 0.51 in males (vs. average FLL/SVL 0.48 in males).

Kurixalus qionglaiensis sp. nov. differs from *K. absconditus* Mediyansyah, Hamidy, Munir, and Matsui, 2019, *K. berylliniris*, *K. bisacculus* (Taylor, 1962), *K. chaseni*, *K. gracilloides*, *K. hainanus*, *K. lenquanensis*, *K. motokawai* Nguyen, Matsui, and Eto, 2014, *K. odontotarsus*, *K. raoi*, *K. verrucosus*, and *K. viridescens* (Rafinesque, 1820) by relatively shorter hindlimb (tibiotarsal articulation reaching posterior border of eye when appressed vs. beyond posterior border of eye), and differs from *K. appendiculatus*, *K. baliogaster* (Inger, Orlov, and Darevsky, 1999), *K. banaensis* (Bourret, 1939), *K. eiffingeri*, *K. lenquanensis*, *K. naso*, *K. wangi*, and *K. yangi* by pair of large symmetrical dark patches present on chest (vs. absent). In addition, *Kurixalus qionglaiensis* sp. nov. can be distinguished from *K. eiffingeri* and *K. berylliniris* by finely granular throat skin (vs. smooth), from *K. lenquanensis* and *K. raoi* by snout pointed and projecting (vs. no projection on tip), from *K. gracilloides* and *K. lenquanensis* by SL longer than ED (vs. SL shorter than ED), and from *K. wangi* by brown color pattern on dorsal surface (vs. dark brownish green) and relatively rough flank (vs. smooth).

NOMENCLATURAL ACTS REGISTRATION

The electronic version of this article in portable document format will represent a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new name contained in the electronic version is effectively published under that Code from the electronic edition alone (see Articles 8.5–8.6 of the Code). This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information can be viewed through any standard web browser by appending the LSID to the prefix <http://zoobank.org/>.

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SCIENTIFIC FIELD SURVEY PERMISSION INFORMATION

The collection of all animals used for this study obeyed the Wildlife Protection Act of China. Collection permits were issued by the Chengdu Institute of Biology, Chinese Academy of Sciences (permit No.: 2021-130).

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

C.P.G. and J.H.H. designed the study. M.J.Z., X.Y.W., and C.P.G. collected specimens in the field. C.P.G., M.J.Z., K.W.L., and J.H.H. performed data analyses. C.P.G. measured the specimens and wrote the draft with assistance

from M.J.Z., K.W.L., X.Y.W., and J.H.H. All authors read and approved the final manuscript.

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