

First record of *Theلودerma pyaukkya* Dever, 2017 (Anura: Rhacophoridae) in China, with range extension of *Theلودerma moloch* (Annandale, 1912) to Yunnan

DEAR EDITOR,

Theلودerma pyaukkya is recorded for the first time in China based on a specimen collected from western Yunnan. Morphologically, the specimen shows good agreement with the original description of *T. pyaukkya*, and phylogenetically is clustered with the type specimens and holotype of *T. pyaukkya* from Kachin State (northern Myanmar) with strong support. The taxonomic status of *T. pyaukkya* from Chin State (western Myanmar) needs further examination. In addition, *Theلودerma moloch* is also recorded in Yunnan for the first time. This brings the number of *Theلودerma* species recorded in Yunnan, China, to seven, namely, *T. albopunctatum*, *T. baibungense*, *T. bicolor*, *T. gordonii*, *T. moloch*, *T. pyaukkya*, and *T. rhododiscus*.

Theلودerma Tschudi, a genus of the family Rhacophoridae, is distributed widely throughout Southeast Asia, China, India, and Sri Lanka, and presently includes 26 recognized species (Frost, 2020). To date, eight species have been recorded in China, namely, *T. albopunctatum* (Liu and Hu), *T. baibungense* (Jiang, Fei, and Huang), *T. bicolor* (Bourret), *T. corticale* (Boulenger), *T. gordonii* Taylor, *T. lateriticum* Bain, Nguyen, and Doan, *T. moloch* (Annandale), and *T. rhododiscus* (Liu and Hu) (AmphibiaChina, 2020; Chen et al., 2019; Hou et al., 2017; Qi et al., 2018). Of these eight species, five occur in Yunnan, i.e., *T. albopunctatum*, *T. baibungense*, *T. bicolor*, *T. gordonii*, and *T. rhododiscus* (Qi et al., 2018).

Theلودerma pyaukkya Dever, a species highly similar in appearance and size to *T. albopunctatum* and *T. asperum* (Dever, 2017), was previously known only from Kachin State in northern Myanmar and Chin State in western Myanmar (Dever, 2017). Western Yunnan borders northern Myanmar (Figure 1A) and previous studies have shown these regions to be similar in amphibian fauna (e.g., Hui et al., 2019; Yu et al.,

2018, 2019). Thus, it is expected that *T. pyaukkya* could also be found in western Yunnan, where only one species of *Theلودerma* (*T. baibungense*) has been reported (Hou et al., 2017).

During recent field surveys in western Yunnan, China, we collected a *Theلودerma* specimen similar to members of the *T. asperum* complex (*T. asperum*, *T. albopunctatum*, *T. baibungense*, and *T. pyaukkya*) in appearance due to its splattered brown-and-white dorsal coloration. Molecular comparison indicated this individual to be *T. pyaukkya*. Herein, we describe this new record for China in detail.

Field surveys were conducted in Yingjiang County, Yunnan Province, China (Figure 1A). Specimens were collected and euthanized with ethyl acetate and then fixed in 80% ethanol for storage after taking photographs. Liver tissue samples were preserved in 99% ethanol for molecular analysis. The specimens were deposited in Guangxi Normal University (GXNU YU000115 and GXNU YU000116).

Total genomic DNA was extracted from the liver tissues. Tissue samples were digested using proteinase K, and subsequently purified following standard phenol/chloroform isolation and ethanol precipitation. Fragments encoding partial 16S rRNA (16S) and cytochrome c oxidase subunit I (COI) genes were amplified and sequenced (see Supplementary Methods). All new sequences were deposited in GenBank under accession Nos. MT509809, MT509810, and MT522176 (Supplementary Table S1). Phylogenetic relationships among *Theلودerma* species were inferred using Bayesian inference (Supplementary Methods). Uncorrected pairwise distances between species were calculated in MEGA 7 (Kumar et al., 2016).

Measurements were taken with a digital caliper to the

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nearest 0.1 mm. Morphological terminology followed Fei et al. (2009) (Supplementary Methods).

The obtained sequence alignments for the 16S and COI genes were 496 bp and 681 bp long, respectively. As shown in previous study (Dever, 2017), *T. pyaukkya* consists of two lineages, one containing the holotype and paratype from the type locality, Kachin State (clade I), and one containing the paratypes from Chin State (clade II). GXNU YU000116 clustered with clade I with strong support (Figures 1C and 1D), whereas clade II was sister to *T. baibungense* with strong support (Figure 1D). The genetic distances between GXNU YU000116 and *T. pyaukkya* in clade I were 1.2%–2.1% for

16S and 0.6%–1.4% for COI, whereas clade I differed from clade II by 5.8%–6.2% for 16S and 10.1%–10.4% for COI (Table 1) and from *T. baibungense* by 5.7%–6.4% for 16S. The distance between *T. baibungense* and clade II was 4.0%–4.6% for 16S, slightly higher than the conventional threshold of species-level divergence in other groups of Anura (3.0% of divergence in the 16S rRNA gene; Vences et al., 2005a, 2005b; Vieites et al., 2009). Therefore, we considered GXNU YU000116 to belong to *T. pyaukkya* (type locality: Kachin State).

GXNU YU000115 was clustered with *T. moloch* from Tibet with strong support and short branches (Figure 1D).

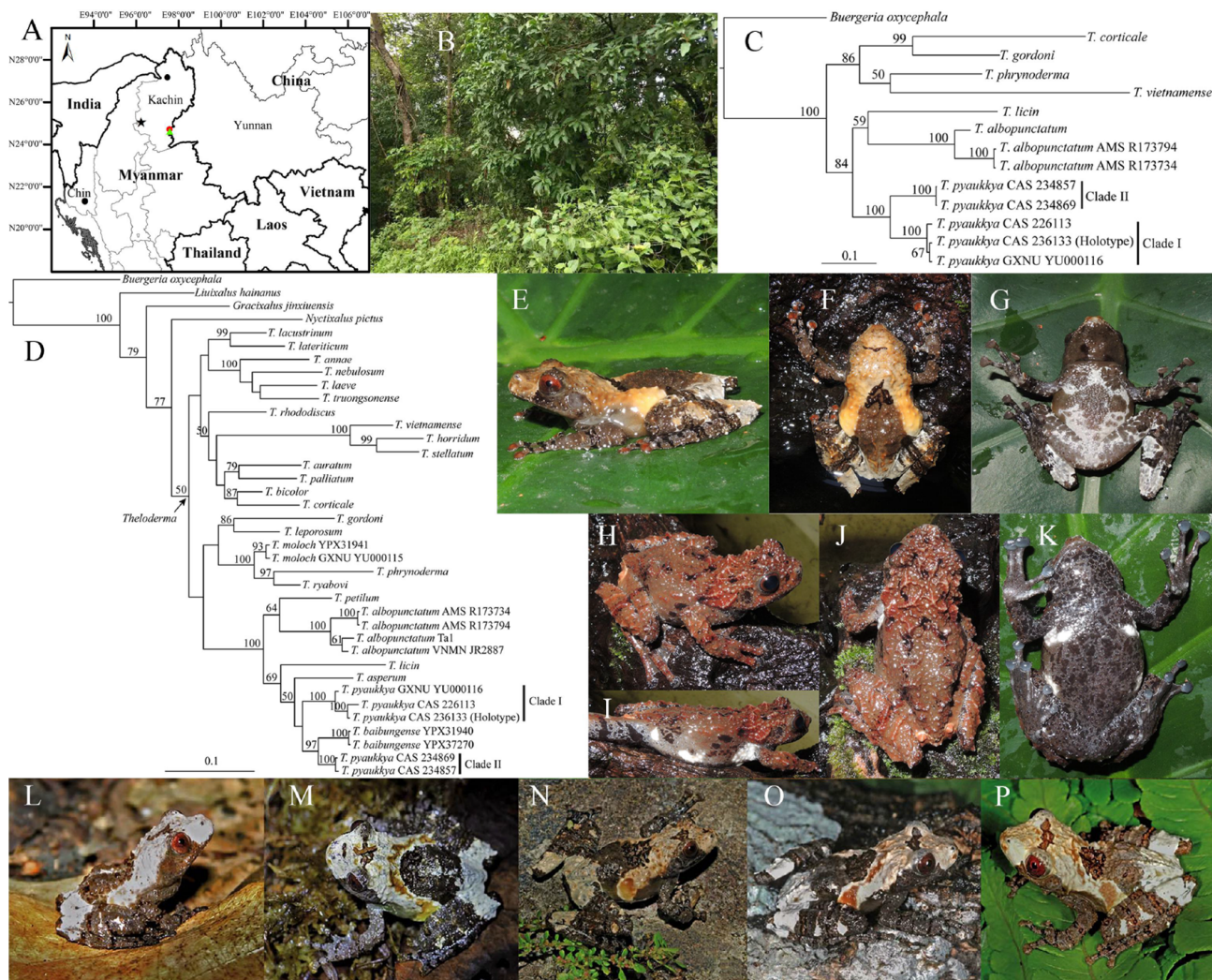


Figure 1 Collection sites, habitat, phylogeny, and photos of *Theiaderma pyaukkya* (GXNU YU000116), *T. moloch* (GXNU YU000115), and *T. albopunctatum*

A: Collection sites of *T. pyaukkya* (red circle) and *T. moloch* (green triangle) in Yunnan, China (black star and black circles indicate type locality and other known distributions of *T. pyaukkya* in Myanmar, respectively); B: Habitat at collection site of *T. pyaukkya* in Yunnan; C, D: Bayesian phylogenies of *Theiaderma* inferred from COI gene and 16S rRNA gene, respectively; E–K (Photos by G.H.Y.): Lateral, dorsal, and ventral views of *T. pyaukkya* (E–G) and *T. moloch* (H–K) from Yunnan, China; L–P (reproduced from Hou et al., 2017): *T. albopunctatum* from Yinggeling, Hainan (L), Dayaoshan, Guangxi (M), Lao Cai, Vietnam (N), Vinh Phuc, Vietnam (O), and Kon Tum, Vietnam (P).

Table 1 Divergence (*P*-distance; %) between and within *Theلودerma pyaukkya* and *T. baibungense* estimated from 16S (lower triangle) and COI (upper triangle) sequences

Species	Clade	Voucher	1	2	3	4	5	6	7
<i>T. pyaukkya</i>	I	1. YU000166		0.6	1.4	10.1	10.1	N/A	N/A
	I	2. CAS 236 133	1.2		1.3	10.4	10.4	N/A	N/A
	I	3. CAS 226 113	2.1	1.0		10.3	10.3	N/A	N/A
	II	4. CAS 234 869	6.2	6.0	6.0		0	NA	N/A
	II	5. CAS 234 857	6.2	5.8	5.8	0.2		N/A	N/A
<i>T. baibungense</i>		6. YPX37270	5.7	6.1	5.9	4.0	4.2		N/A
		7. YPX31940	6.0	6.4	6.2	4.3	4.6	0	

N/A: Not available.

Taxonomic account

Theلودerma pyaukkya Dever, 2017 (Figure 1E–G)

Burmese Bug-eyed Frog/缅甸棱皮树蛙 (Miǎn Diàn Léng Pí Shù Wā)

Specimen examined: Adult male (GXNU YU000116) collected on 03 September 2018 by Shuo Liu from Nabang Township, Yingjiang County, Yunnan Province, China (N24°42'49.9", E97°35'6.5", 313 m a.s.l.; Figure 1A).

Morphological description: Morphological characters of the specimen from Yunnan agreed well with the descriptions of Dever (2017). Body size small, SVL 31.0 mm; head flat and triangular, width (HW 11.6 mm) nearly equal to length (HL 11.4 mm); snout truncate in dorsal view, sloping slightly from eye to nostril, nostril at tip of snout; slightly concave between nostrils at tip of snout; canthus rostralis rounded, curving from eye to nostril; loreal region oblique and concave; internarial distance (IND 2.4 mm) narrower than interorbital distance (IOD 3.8 mm); tympanum rounded, distinct (TD 2.6 mm), more than half eye diameter (ED 3.5 mm), separated from eye by 1.0 mm; supratympanic fold present; vomerine teeth absent; choanae oval; tongue attached anteriorly, deeply notched posteriorly; small vocal slits present on floor of mouth at each corner; nuptial pads absent.

Forearm and hand length (FHL 16.6 mm) 53.5% of snout-vent length; relative length of fingers I<II<IV<III; tips of all fingers expanded into discs with circummarginal and transverse ventral grooves, disk of third finger largest; webbing absent between fingers; subarticular tubercles prominent and round, formula 1, 1, 2, 2; supernumerary tubercles on palms absent; two metacarpal tubercles, inner rounded and large, outer oval and small.

Legs long, heels overlapping when legs positioned at right angles to body, tibiotarsal articulation reaching snout, tibia (TL 16.6 mm) 53.5% of snout-vent length; foot (FL 14.2 mm) 45.8% of body length; tips of toes expanded into discs with circummarginal and transverse grooves, slightly smaller than those of fingers; toes fully webbed, webbing formula I1–2II1–2III1–2IV2–1V; subarticular tubercles distinct, rounded, formula 1, 1, 2, 3, 2; supernumerary tubercles present; inner metatarsal tubercle oval, outer metatarsal tubercles absent.

Dorsal skin rough, covered with numerous single fine,

calcified, white-tipped asperities; head side and body flank also with fewer tubercles; chin and chest smooth, with few asperities; venter granular with many small asperities aggregated in fine clumps.

Measurements of GXNU YU000116 (in mm): SVL 31.0; HL 11.4; HW 11.6; SL 4.9; IND 2.4; IOD 3.8; UEW 2.5; ED 3.5; TD 2.6; DNE 3.3; FHL 16.6; TL: 16.6; FL 14.2; TFL 21.7.

Color in life: Color pattern similar to holotype. Top of head light white-cream, continuing onto sides and lateral region of dorsum; loreal region also white-cream; beneath eyes and tympanum brown; dark brown stripe between eyes; dark brown patch on middorsal between shoulders; posterior region near urostyle covered by irregular white-cream blotches, extending to thigh, tibia, and tarsal; limbs with dark brown banding; discs rust brown; ventral surface brown with cream marbling on chest, venter, thigh, tibia, and tarsal; metacarpal tubercles light cream; iris rust colored, similar to finger and toe discs.

Ecological notes: The specimen was found on a leaf of a protosomatic tree near a stream. The leaf was about 2 m above the ground, with several shrubs (e.g., *Eupatorium adenophorum*) around the base of the tree (Figure 1B).

Theلودerma moloch (Annandale, 1912) (Figures 1H–K)

Tibetan Bug-eyed Frog/西藏棱皮树蛙 (Xī Zàng Léng Pí Shù Wā)

Specimen examined: Adult male (GXNU YU000115) collected on 01 September 2018 by Shuo Liu from Tongbiguan Township, Yingjiang County, Yunnan Province, China (N24°37'0.9", E97°35'13.5", 1 265 m a.s.l.; Figure 1A).

Morphological description: Morphological characters of the Yunnan specimen agreed well with the descriptions of Annandale (1912), Fei et al. (2009), and Hou et al. (2017). Body size medium, SVL 40.2 mm; head strongly depressed, triangular, width (HW 14.2 mm) greater than length (HL 11.1 mm); snout truncate in dorsal view, oblique vertically in lateral view, snout length (SL 4.2 mm) nearly equal to eye diameter (ED 4.3 mm); nostril prominent, close to tip of snout; canthus rostralis indistinct; loreal region oblique and concave; interorbital distance (IOD 3.7 mm) equal to width of upper eyelid (UEW 3.7 mm) and broader than internarial distance (IND 3.2 mm); tympanum rounded, fairly distinct (TD 3.2 mm); supratympanic fold absent; vomerine teeth in two short

oblique ridges between choanae; tongue large, pyriform, notched posteriorly; vocal opening present on floor of mouth at each corner; nuptial pads absent.

Forearm and hand length (FHL 20.7 mm) 51.5% of snout-vent length; fingers free, relative length of fingers I<II<IV<III; tips of all fingers expanded into discs with circummarginal and transverse ventral grooves; discs of third and fourth fingers equal to each other (2.8 mm) and narrower than tympanum diameter; subarticular tubercles round, formula 1, 1, 2, 2, proximal one obviously smaller than distal one on two outer fingers; supernumerary tubercles on palms present; two metacarpal tubercles present.

Legs long, heels overlapping when legs positioned at right angles to body; tibiotarsal articulation reaching anterior corner of eye; tibia (TL 19.9 mm) 49.5% of snout-vent length; foot (FL 17.7 mm) 44% of body length; tips of toes expanded into discs with circummarginal and transverse grooves, smaller than finger discs; toes fully webbed, webbing formula I1–1.5II1–2III1–2IV2–1V; subarticular tubercles rounded, formula 1, 1, 2, 3, 2; inner metatarsal tubercle oval, outer metatarsal tubercles absent.

Dorsal surface very rough with prominent irregular ridges and warts, more so on neck and across shoulders; head side and body flank rough with smaller warts; large tubercles on back of thighs near vent, with one fairly enlarged conical tubercle on each side of vent; ventral surface granular.

Measurements of GXNU YU000115 (in mm): SVL 40.2; HL 11.1; HW 14.2; SL 4.2; IND 3.2; IOD 3.7; UEW 3.7; ED 4.3; TD 3.2; FHL 20.7; TL: 19.9; FL 17.7; TFL 27.2.

Color in life: Dorsum coffee-brown, mottled with irregular black blotches and reddish brown patches; iris reddish brown; tympanum black; three white patches on each side of body, one in axilla, one in groin, and one between them; several black blotches on anterior and posterior parts of flank; anterior and posterior surface of thigh marbled with black, white, and gray; ventral surface black with faint reticulation running all over body and onto ventral surface of limbs; two white patches connected to that in axilla on each side of chest.

Ecological notes: The specimen was found on a leaf in an evergreen broad-leaf forest near a road. The leaf was about 1.7 m above the ground and there was no water body within hundreds of meters of the site.

Remarks: The taxonomic status of the *T. pyaukkya* paratypes from Chin State (western Myanmar) needs further examination, as the clade containing them (clade II) was recovered as sister to *T. baibungense* with strong support based on 16S sequences (Figure 1D). Although nuclear sequences recovered *T. pyaukkya* as a monophyly in Dever (2017), neither *T. baibungense* nor *T. asperum* were included. Poyarkov et al. (2018) considered that synonymy of *T. pyaukkya* with *T. baibungense* could be assumed. However, the distance between clade II and *T. baibungense* reached 4.0%–4.6%, which is slightly higher than the conventional threshold of species-level divergence in other groups of Anura (3.0% of divergence in the 16S rRNA gene according to Vences et al., 2005a, 2005b, and Vieites et al., 2009), and

they obviously differed from each other in body size according to the original descriptions (SVL 30.0–31.5 mm in clade II vs. 15.0–16.2 mm in *T. baibungense*) (see Dever, 2017; Fei et al., 2009). Thus, *T. pyaukkya* from Chin State (western Myanmar; clade II) might represent a cryptic, undescribed species pending further investigation of nuclear data from *T. baibungense*, additional morphological characters, and new material sampling.

In addition, *T. moloch*, a species known previously from India and southern Tibet in China (Hou et al., 2017), is also reported to occur in Yunnan in this study based on GXNU YU000115 from Yingjiang County (Figure 1D). Thus, there are seven *Theleiderma* species currently known from Yunnan, i.e., *T. albopunctatum*, *T. baibungense*, *T. bicolor*, *T. gordonii*, *T. moloch*, *T. pyaukkya*, and *T. rhododiscus*. *Theleiderma pyaukkya* resembles *T. albopunctatum* in both appearance and size, but *T. pyaukkya* can be distinguished from *T. albopunctatum* by small, yet noticeable asperities covering entire dorsum (Dever, 2017) and discs red (vs. brown; Figure 1). In addition, *T. pyaukkya* can be easily distinguished from *T. baibungense* by its larger body size (SVL 28–31 mm vs. 15–16.2 mm).

SCIENTIFIC FIELD SURVEY PERMISSION INFORMATION

Permission for field surveys in Nabang Township was granted by the Tongbiguan Provincial Nature Reserve.

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

G.H.Y. and M.H. conceived and designed the study. L.N.D. performed the experiments, measured the specimens, and wrote the manuscript. S.L. conducted the field surveys. G.H.Y. and M.H. identified the specimens. All authors read and approved the final version of the manuscript.

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Li-Na Du^{1,2,#}, Shuo Liu^{3,#}, Mian Hou^{4,*}, Guo-Hua Yu^{1,2,*}

¹ Key Laboratory of Ecology of Rare and Endangered Species and Environmental Protection (Guangxi Normal University), Ministry of Education, Guilin, Guangxi 541004, China

² Guangxi Key Laboratory of Rare and Endangered Animal Ecology, College of Life Science, Guangxi Normal University, Guilin, Guangxi 541004, China

³ Kunming Natural History Museum of Zoology, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan

650223, China

⁴ Institute of Continuing Education, Sichuan Normal University,
Chengdu, Sichuan 610068, China

*Authors contributed equally to this work

*Corresponding authors, E-mail: turtlechina@126.com;
yugh2018@126.com

REFERENCES

- AmphibiaChina. 2020. The database of Chinese amphibians. Kunming Institute of Zoology (CAS), Kunming, Yunnan, China. (2020-04-10). <http://www.amphibiachina.org/>. (in Chinese)
- Annandale N. 1912. Zoological results of the Abor Expedition, 1911-1912. I. Batrachia. *Records of the Indian Museum*, **8**: 7–36.
- Chen WC, Liao XW, Zhou SC, Mo YM. 2019. First record of *Theلودerma lateriticum* Bain, Nguyen et Doan, 2009 (Anura: Rhacophoridae) from China with redescribed morphology. *Biodiversity Journal*, **10**(1): 25–36.
- Dever JA. 2017. A new cryptic species of the *Theلودerma asperum* complex (Anura: Rhacophoridae) from Myanmar. *Journal of Herpetology*, **51**(3): 425–436.
- Fei L, Hu S, Ye C, Huang Y. 2009. Fauna Sinica. Volume 2. Amphibia Anura. Beijing: Science Press. (in Chinese)
- Frost DR. 2020. Amphibian species of the world 6.0, an online reference. American Museum of Natural History, New York, USA. (2020-02-01). <http://research.amnh.org/herpetology/amphibia/index.html/>.
- Hou M, Yu GH, Chen HM, Liao CL, Zhang L, Chen J, et al. 2017. The taxonomic status and distribution range of six *Theلودerma* species (Anura: Rhacophoridae) with a new record in China. *Russian Journal of Herpetology*, **24**(2): 99–127.
- Hui H, Yu GH, Yang JX, Rao DQ. 2019. First record of *Minervarya chiangmaiensis* (Anura: Dicroglossidae) from China and Myanmar. *Russian Journal of Herpetology*, **26**(5): 261–266.
- Kumar S, Stecher G, Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, **33**(7): 1870–1874.
- Poyarkov NA Jr, Kropachev II, Gogoleva SS, Orlov NL. 2018. A new species of the genus *Theلودerma* Tschudi, 1838 (Amphibia: Anura: Rhacophoridae) from Tay Nguyen Plateau, central Vietnam. *Zoological Research*, **39**(3): 158–184.
- Qi S, Yu GH, Lei B, Fan Y, Zhang DL, Dong ZW, et al. 2018. First record of *Theلودerma gordonii* Taylor, 1962 from Yunnan Province, China. *Russian Journal of Herpetology*, **25**(1): 43–55.
- Vences M, Thomas M, Bonett RM, Vieites DR. 2005a. Deciphering amphibian diversity through DNA barcoding: chances and challenges. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **360**(1462): 1859–1868.
- Vences M, Thomas M, Van Der Meijden A, Chiari Y, Vieites DR. 2005b. Comparative performance of the 16S rRNA gene in DNA barcoding of amphibians. *Frontiers in Zoology*, **2**(1): 5.
- Vieites DR, Wollenberg KC, Andreone F, Köhler J, Glaw F, Vences M. 2009. Vast underestimation of Madagascar's biodiversity evidenced by an integrative amphibian inventory. *Proceedings of the National Academy of Sciences of the United States of America*, **106**(20): 8267–8272.
- Yu GH, Hui H, Hou M, Wu ZJ, Rao DQ, Yang JX. 2019. A new species of *Zhangixalus* (Anura: Rhacophoridae), previously confused with *Zhangixalus smaragdinus* (Blyth, 1852). *Zootaxa*, **4711**(2): 275–292.
- Yu GH, Hui H, Rao DQ, Yang JX. 2018. A new species of *Kurixalus* from western Yunnan, China (Anura, Rhacophoridae). *ZooKeys*, **770**: 211–226.