

A field survey on the genus *Xenophrys* (Amphibia, Megophryidae) confirms underestimated diversity in the Gaoligong Mountains, with the description of a new species

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Abstract

The Gaoligong Mountains, located in the western part of China's Yunnan Province adjoining northern Myanmar, harbor a striking diversity of species and endemism. Previous studies have shown that amphibian diversity in this region remains underestimated. A field survey carried out in 2023 oversaw a collection of eight *Xenophrys* specimens from the Tongbiguan Provincial Nature Reserve, Yunnan Province, China. Subsequent molecular analyses revealed two distinct and previously undescribed lineages. Based on morphological evidence, we formally describe one of the lineages as a new species and tentatively assign the other lineage to *X. sp.* due to the absence of adult specimens for examination. Our results bring the total number of *Xenophrys* species to 29 and the number of *Xenophrys* species known to occur in China to 11. Furthermore, our study reveals that five species and putative species of *Xenophrys* (*X. dehongensis*, *X. glandulosa*, *X. periosa*, *X. yingjiangensis* sp. nov., and *X. sp.*) exhibit sympatric distribution. These findings highlight the need for future research to investigate the mechanisms of sympatric coexistence in *Xenophrys*. In addition, our study confirms that the amphibian diversity of the Gaoligong Mountains is undoubtedly underestimated. As a result, continued exploration of amphibians in the future is necessary to obtain a clearer understanding of the overall biodiversity in this region.

Key Words

Biodiversity, cryptic species, frog, sympatric distribution, Tongbiguan Provincial Nature Reserve, *Xenophrys yingjiangensis* sp. nov.

Introduction

The Asian horned toads of the subfamily Megophryinae (Bonaparte, 1850) are widely distributed in tropical Asia, from India and Bhutan to China and south to the Sundas and the Philippines (Frost 2024). It currently includes 136 recognized species, with more than half of the species

described in the last 10 years (Frost 2024). As a consequence of both morphological similarity among species and the complex patterns of genetic divergence, the generic classification of the subfamily Megophryinae has been constantly under debate (e.g., Delorme et al. 2006; Fei et al. 2009; Chen et al. 2017; Mahony et al. 2017; Lyu et al. 2023). In this study, we followed the classification

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system outlined in Frost (2024) and Lyu et al. (2023), in which Megophryidae was divided into 10 genera for the convenience of our comparisons.

The genus *Xenophrys* Günther, 1864, of the subfamily Megophryinae is distributed widely throughout southern China to the Indochina Peninsula and currently includes 28 recognized species (Frost 2024). These species inhabit primarily montane forests. To date, 10 species have been recorded in China (Frost 2024), with nearly half of the recognized species described in the last 10 years (e.g., Mahony et al. 2018; Lyu et al. 2023; Shu et al. 2023). Moreover, five species of *Xenophrys* have been recorded in Yunnan (AmphibiaChina 2024; Frost 2024).

The Gaoligong Mountains, located in the western part of China's Yunnan Province bordering northern Myanmar, form a long, narrow mountain range. It stretches 600 km from the Tibetan Plateau to Myanmar across a 5° latitude with a large elevation range of 210 m to 5000 m. Renowned as one of the world's most significant biodiversity hotspots outside of the tropics, its complex geography, hydrology, and climate have fostered many distinct habitat types that support diverse biotic components (Chaplin 2005). Several cryptic and novel amphibian species have been described from this mountain ecosystem in recent years (AmphibiaChina 2024), indicating that amphibian diversity in the region may still be diverse and largely underestimated.

A recent herpetological survey conducted at the Gaoligong Mountains, Yunnan Province, China, saw a collection of some *Xenophrys* specimens. Subsequent studies, including molecular data and morphological comparisons, revealed that these specimens represent five distinct evolutionary lineages, two of which could not be assigned to any known *Xenophrys* species. Therefore, we herein describe one of the two lineages as a new species.

Materials and methods

Sampling

Field surveys were conducted in August 2023. A total of eight *Xenophrys* specimens were collected in Tongbiguan Town, Yingjiang County, Yunnan, China (Fig. 1). After taking photographs, the toads were euthanized using benzocaine. Liver tissue was taken from the specimens and preserved in 95% ethanol at -80 °C. The specimens were then fixed in 10% formalin and subsequently stored in 75% ethanol after 24 hours. All the newly collected specimens were deposited in the herpetological collection of the Museum of the Kunming Institute of Zoology (KIZ), Chinese Academy of Sciences (CAS).

Molecular data and phylogenetic analyses

Total genomic DNA was extracted using the standard phenol-chloroform extraction protocol (Sambrook et al. 1989). The mitochondrial fragment 16S ribosomal RNA gene (16S rRNA) was amplified and sequenced using the primer pairs (5'–3') 16SAR (CGCCTGTTTAYCAAAAACAT) and 16SBR (CCGGTYTGAAGTCAGATCAYGT) (Kocher et al. 1989). The polymerase chain reaction (PCR) was performed in a 25 µl volume reaction with the following conditions: an initial denaturing step at 94 °C for 4 min; 35 cycles of denaturing at 94 °C for 40 s; annealing at 55 °C for 40 s; and extending at 72 °C for 1 min; and a final extending step of 72 °C for 10 min. PCR products were sequenced using the same forward and reverse primers as those used in PCR. Sequencing was conducted using an ABI 3730xl DNA automated sequencer (Applied Biosystems, UK). All sequences were assembled from forward and reverse reads

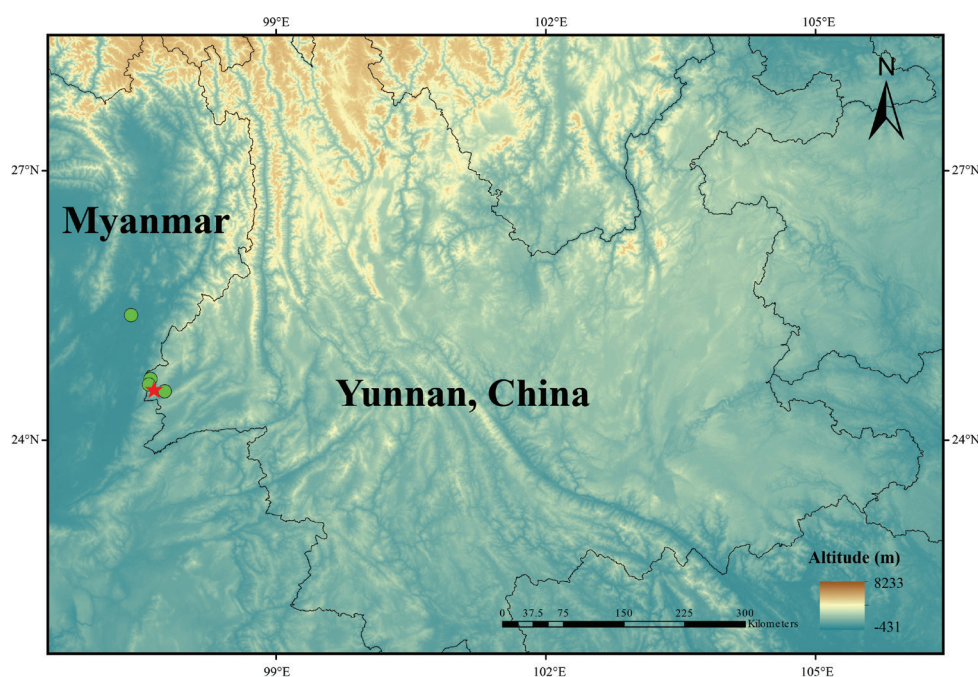


Figure 1. Known distribution of *Xenophrys yingjiangensis* sp. nov. The red pentagram indicates the type locality of *Xenophrys yingjiangensis* sp. nov.

and edited manually using DNASTAR LASERGENE 7.1. New sequences were deposited in GenBank (Table 1).

Maximum likelihood (ML) and Bayesian inference (BI) were used to infer phylogenetic trees. Fifty-eight homologous sequences of *Xenophrys* and representative outgroups (*Brachytarsophrys feae*, *Leptobranchella ventripunctata*, and *Leptobranchium huashen*) were downloaded from the GenBank (Table 1). The dataset was aligned using MUSCLE v3.8 (Edgar 2004), checked by eye, and trimmed to minimize missing characters in MEGA v6.0.6 (Tamura et al. 2013). BI and ML analyses were performed on the CIPRES web server (Miller et al. 2010) using MrBayes v3.2.4 (Ronquist et al. 2012). For BI analyses, the best-fit model of evolution was determined using the Bayesian information criterion (BIC; Posada 2008) implemented in jModelTest

2.1.7 (Darriba et al. 2012). GTR+G was the best-fit model of evolution for 16S rRNA. The Monte Carlo Markov chain length was run for 10 million generations and sampled every 1000 generations, with a burn-in of 25%. Convergence was assessed by the average standard deviation of split frequencies (below 0.01) and ESS values (greater than or equal to 200) in TRACER 1.6 (Rambaut et al. 2014). ML analyses were performed using RAXML-HPG BlackBox 8.2.10 (Stamatakis 2014) with 1,000 bootstrap replicates and using the standard bootstrap search (random seed value 12,345) under the GTR+gamma nucleotide substitution model. Mean genetic distances between and within species were calculated using uncorrected pairwise distances (*p*-distance) by 16S rRNA implemented in MEGA v6.0.6, with complete deletion of missing data and gaps (Tamura et al. 2013).

Table 1. Localities, voucher information, and Genbank accession numbers for all specimens used in this study.

Species	Voucher	Locality	Accession No.	Reference
<i>Xenophrys ancræ</i>	SDBDU 2009.727	India, Arunachal, Changlang	KY022318	Mahony et al. 2018
<i>Xenophrys ancræ</i>	ZSI A 11606	India, Arunachal, Changlang	MN734391	Mahony et al. 2020
<i>Xenophrys auralensis</i>	NCSM 79599	Cambodia, Kampong Speu, Aural	KX811807	Chen et al. 2017
<i>Xenophrys awuh</i>	SDBDU 2007.192	India, Nagaland state, Kohima district, above New Ministers' Hill, Aradurah Forest	MN734399	Mahony et al. 2020
<i>Xenophrys awuh</i>	MZUHC 314	India, Mizoram	MT793046	Lalronunga et al. 2020
<i>Xenophrys dehongensis</i>	SYS a005823	China, Yunnan, Yingjiang	OQ180993	Lyu et al. 2023
<i>Xenophrys dehongensis</i>	KIZ 053847	China, Yunnan, Tongbiguan	PP989323	This study
<i>Xenophrys dzukou</i>	SDBDU 2007.106	India, Nagaland, Kohima	KY022324	Mahony et al. 2017
<i>Xenophrys flavipunctata</i>	SDBDU 2009.298	India, East Khasi Hills district, Meghalaya	MH647517	Mahony et al. 2018
<i>Xenophrys flavipunctata</i>	SDBDU 2007.134	India, Kohima district, Nagaland	MH647518	Mahony et al. 2018
<i>Xenophrys glandulosa</i>	KIZ 013609	China, Yunnan, Wenlong	KX811761	Chen et al. 2017
<i>Xenophrys glandulosa</i>	SYS a003757	China, Yunnan, Mt Gaoligong	MH406754	Liu et al. 2018
<i>Xenophrys glandulosa</i>	KIZ 053845	China, Yunnan, Tongbiguan	PP989322	This study
<i>Xenophrys himalayana</i>	SDBDU 2009.1227	India, West Kameng district, Arunachal Pradesh	MH647526	Mahony et al. 2018
<i>Xenophrys himalayana</i>	SDBDU 2009.1206	India, West Kameng district, Arunachal Pradesh	MH647527	Mahony et al. 2018
<i>Xenophrys lancangica</i>	SYS a007794	China, Yunnan, Simao	OQ180994	Lyu et al. 2023
<i>Xenophrys lancangica</i>	SYS a007825	China, Yunnan, Jinghong	OQ180997	Lyu et al. 2023
<i>Xenophrys lekaguli</i>	FMNH 265955	Thailand, Sa Kao, Mueang Sa Kao	KY022214	Mahony et al. 2017
<i>Xenophrys major</i>	RGK 0089	India, Manipur, Tamenglong	KY022308	Mahony et al. 2017
<i>Xenophrys major</i>	SDBDU 2007.229	India, Nagaland, Kohima	MH647514	Mahony et al. 2018
<i>Xenophrys mangshanensis</i>	KIZ 021786	China, Guangdong, Mt Nanling	KX811790	Chen et al. 2017
<i>Xenophrys mangshanensis</i>	SYS a002177	China, Guangdong, Huaiji	MH406666	Liu et al. 2018
<i>Xenophrys maosonensis</i>	KIZ 016045	China, Yunnan, Xichou	KX811780	Chen et al. 2017
<i>Xenophrys maosonensis</i>	SYS a008748	China, Guangxi, Mt Shiwandashan	OQ181000	Lyu et al. 2023
<i>Xenophrys maosonensis</i>	SYS a008766	China, Guangxi, Mt Shiwandashan	OQ181002	Lyu et al. 2023
<i>Xenophrys medogensis</i>	KIZ 06657	China, Xizang, Beibeng	KX811768	Chen et al. 2017
<i>Xenophrys medogensis</i>	SYS a002932	China, Xizang, Medog	MH406725	Liu et al. 2018
<i>Xenophrys megacephala</i>	ZSI A 11213	India, Meghalaya, Ri Bhoi	KY022315	Mahony et al. 2018
<i>Xenophrys montana</i>	SDBDU 2011.1047	India, West Bengal, Darjeeling	KY022312	Mahony et al. 2017
<i>Xenophrys montana</i>	SDBDU 2011.1049	India, West Bengal, Darjeeling	MH647506	Mahony et al. 2018
<i>Xenophrys numhumaeng</i>	SDBDU 2007.041	India, Manipur, Tamenglong	KY022316	Mahony et al. 2017
<i>Xenophrys numhumaeng</i>	BNHS 6076	India, Manipur, Tamenglong	MN734393	Mahony et al. 2020
<i>Xenophrys oreocrypta</i>	SDBDU 2008.1400	India, West Garo Hills district, Meghalaya	MH647520	Mahony et al. 2018
<i>Xenophrys oreocrypta</i>	SDBDU 2009.1108	India, West Garo Hills district, Meghalaya	MH647521	Mahony et al. 2018
<i>Xenophrys oropedion</i>	ZSI A 11601	India, Meghalaya state, East Khasi Hills district, Shillong, Malki Forest	MN734394	Mahony et al. 2020
<i>Xenophrys oropedion</i>	ZSI A 11603	India, Meghalaya state, East Khasi Hills district, Shillong, Malki Forest	MN734395	Mahony et al. 2020
<i>Xenophrys pangdaensis</i>	YBU 21248	China, Xizang, Yadong County, Pangda Village	OR026569	Shu et al. 2023
<i>Xenophrys pangdaensis</i>	YBU 21261	China, Xizang, Yadong County, Pangda Village	OR026572	Shu et al. 2023
<i>Xenophrys periosa</i>	SDBDU 2009.793	India, East Siang district, Arunachal Pradesh	MH647522	Mahony et al. 2018
<i>Xenophrys periosa</i>	SDBDU 2009.1285	India, West Kameng district, Arunachal Pradesh	MH647524	Mahony et al. 2018
<i>Xenophrys periosa</i>	CIB YN201909160	China, Yunnan, Gongshan, Dulongjiang	MT225581	Shi et al. 2020
<i>Xenophrys periosa</i>	KIZ 053849	China, Yunnan, Tongbiguan	PP989324	This study
<i>Xenophrys robusta</i>	K5207/ZSI11404	India, Sikkim, North Sikkim	KX894674	Deuti et al. 2017
<i>Xenophrys robusta</i>	SDBDU 2011.1057	India, West Bengal, Darjeeling	KY022314	Mahony et al. 2018
<i>Xenophrys serchhipii</i>	SDBDU 2009.612	India, Tripura, North Tripura	KY022323	Mahony et al. 2018
<i>Xenophrys serchhipii</i>	SDBDU 2008.1492	India, Manipur, Tamenglong	MN734405	Mahony et al. 2020
<i>Xenophrys "sp.17"</i>	KIZ 011940	Myanmar, Myitkyina	KX811792	Chen et al. 2017
<i>Xenophrys "sp.17"</i>	KIZ 048503	China, Yunnan, Tongbiguan Provincial Nature Reserve	KX811793	Chen et al. 2017

Species	Voucher	Locality	Accession No.	Reference
<i>Xenophrys</i> "sp.17"	KIZ 048504	China, Yunnan, Tongbiguan Provincial Nature Reserve	KX811794	Chen et al. 2017
<i>Xenophrys</i> "sp.17"	KIZ 048505	China, Yunnan, Tongbiguan Provincial Nature Reserve	KX811795	Chen et al. 2017
<i>Xenophrys yingjiangensis</i> sp. nov.	KIZ 053814	China, Yunnan, Tongbiguan Provincial Nature Reserve	PP989318	This study
<i>Xenophrys yingjiangensis</i> sp. nov.	KIZ 053815	China, Yunnan, Tongbiguan Provincial Nature Reserve	PP989320	This study
<i>Xenophrys yingjiangensis</i> sp. nov.	KIZ 053828	China, Yunnan, Tongbiguan Provincial Nature Reserve	PP989319	This study
<i>Xenophrys yingjiangensis</i> sp. nov.	KIZ 053848	China, Yunnan, Tongbiguan Provincial Nature Reserve	PP989317	This study
<i>Xenophrys</i> sp.	KIZ 053846	China, Yunnan, Tongbiguan Provincial Nature Reserve	PP989321	This study
<i>Xenophrys takensis</i>	FMNH 261711	Thailand, Kampaeng, Khlong Lan	KY022215	Mahony et al. 2017
<i>Xenophrys truongsoneensis</i>	IEBRA 4943	Vietnam, Dak Lak	ON146200	Luong et al. 2022
<i>Xenophrys truongsoneensis</i>	IEBRA 4948	Vietnam, Lam Dong	ON146201	Luong et al. 2022
<i>Xenophrys truongsoneensis</i>	IEBRA 4952	Vietnam, Ninh Thuan	ON146202	Luong et al. 2022
<i>Xenophrys zhang</i>	KIZ 014278	China, Xizang, Nyalam	KX811765	Chen et al. 2017
<i>Xenophrys zhang</i>	SYS a008204	China, Xizang, Nyalam	OQ180998	Lyu et al. 2023
<i>Xenophrys zunhebotoensis</i>	RGK41	India, Nagaland, Zunheboto	KY022322	Mahony et al. 2017
<i>Xenophrys zunhebotoensis</i>	SDBDU 2009.374	India, Nagaland, Kohima	MN734418	Mahony et al. 2020
Outgroups				
<i>Brachytarsophrys feae</i>	KIZ YN070570	China, Yunnan, Longchuan	KX811809	Chen et al. 2017
<i>Leptobranchella ventripunctata</i>	KIZ 046940	China, Yunnan, Wenlong	KX811929	Chen et al. 2017
<i>Leptobranchium huashen</i>	KIZ 049025	China, Yunnan, Mengyang	KX811931	Chen et al. 2017

Morphology and morphometrics

All the measurements were recorded with digital calipers to the nearest 0.1 mm. Morphological terminology followed Fei et al. (2009). Twenty-five measurements included the following: **SVL**: snout-vent length (measured from tip of snout to vent); **HDL**: head length (measured from tip of snout to rear of jaw); **HDW**: maximum head width (measured width of head at its widest point); **SNT**: snout length (measured from tip of snout to anterior corner of ocular aperture); **ED**: eye diameter (diameter of exposed portion of eyeball); **UEW**: width of upper eyelid (maximum width of upper eyelid); **TD**: tympanum diameter (measured as maximal diameter of tympanum); **DNE**: distance from nostril to eye (distance from the front of the eye to the center of the nostril); **SN**: distance from the center of the nostril to the tip of the snout; **IND**: internarial distance (distance between nares); **TEY**: distance from anterior edge of tympanum to posterior corner of eye; **IOD**: interorbital distance (measured at narrowest point between eyes on top of the head); **FAL**: forearm length (measured from the elbow to the wrist); **LAD**: (diameter of lower arm); **FHL**: forearm and hand length (distance from elbow to the tip of the third finger); **TL**: tibia length (distance from knee to heel); **HL**: hand length (distance from the posterior end of the inner metacarpal tubercle to tip of third finger); **HLL**: hindlimb length; **FL**: foot length (distance from the proximal end of inner metatarsal tubercle to the tip of fourth toe); **THL**: thigh length (from the cloaca to the knee); **TAL**: tarsus length (measured as the distance from knee to heel); **FLI-IV**: first to fourth finger length.

Results

The aligned sequence matrix of the 16S gene contained 490 bp, among which 215 sites were variable and 159 were parsimony-informative (including outgroups). Both BI and ML trees had almost identical topologies with relatively robust

support for most nodes, differing mainly at terminal nodes identified as weakly supported or collapsed. The genus *Xenophrys* was recovered as monophyletic with strong support from both analyses (BPP=1; BS=96; Fig. 2). The newly collected sympatric samples from Tongbiguan Nature Reserve, Yunnan, China, were divided into five highly divergent clades with strong nodal supports: three were nested within a clade containing recognized species, while the other two formed their own previously unknown lineages (Fig. 2).

The newly collected specimen (KIZ 053849) and *X. periosa* (including the type specimens) nested into a single clade with strong support (BPP=1; BS=81; clade A), and the within-group mean genetic *p*-distance was 1.7% (0%–3.4%, Suppl. material 1). The newly collected specimen (KIZ 053845) strongly clustered with *X. glandulosa* (BPP=1; BS=100; clade B), with maximal uncorrected pairwise 16S distances of merely 0.0%. The newly collected specimen (KIZ 053847) formed a monophyletic clade with *X. dehongensis* from the paratype (BPP=1; BS=99; clade E), with shallow within-group genetic differentiation (0.7%, Suppl. material 1).

For the two new distinct clades, the newly collected samples and samples proposed as *Megophrys* sp. 17 in Chen et al. (2017) clustered into a monophyletic clade with a strong nodal support (BPP=1; BS=99; clade D). This clade was recovered as a sister taxon to *X. dehongensis*. In addition, the remaining sample (KIZ 053846) formed an independent monophyletic clade, which clustered with *X. awuh*, *X. zunhebotoensis*, *X. serchhipii*, *X. numhumaeng*, *X. oropedion*, *X. ancræ*, *X. megacephala*, and *X. dzukou* with moderate support (BPP=0.96; BS=81; clade C). The two putative new species showed obvious genetic divergence from the other congeners. The genetic distance between the new populations and the other congeneric species ranged from 5.3% (with *X. megacephala*) to 12.0% (with *X. lancangica*) for clade D, 7.1% (with *X. ancræ*), and 12.2% (with *X. robusta*) for clade C (Suppl. material 1). It is comparable to the divergences among the nearest neighbor genetic distances of the described *Xenophrys* species, which ranged from 2.1% (*X. mangshanensis* and *X. maosonensis*) to 15.7% (*X. dzukou* and *R. awuh*). In addition, these levels

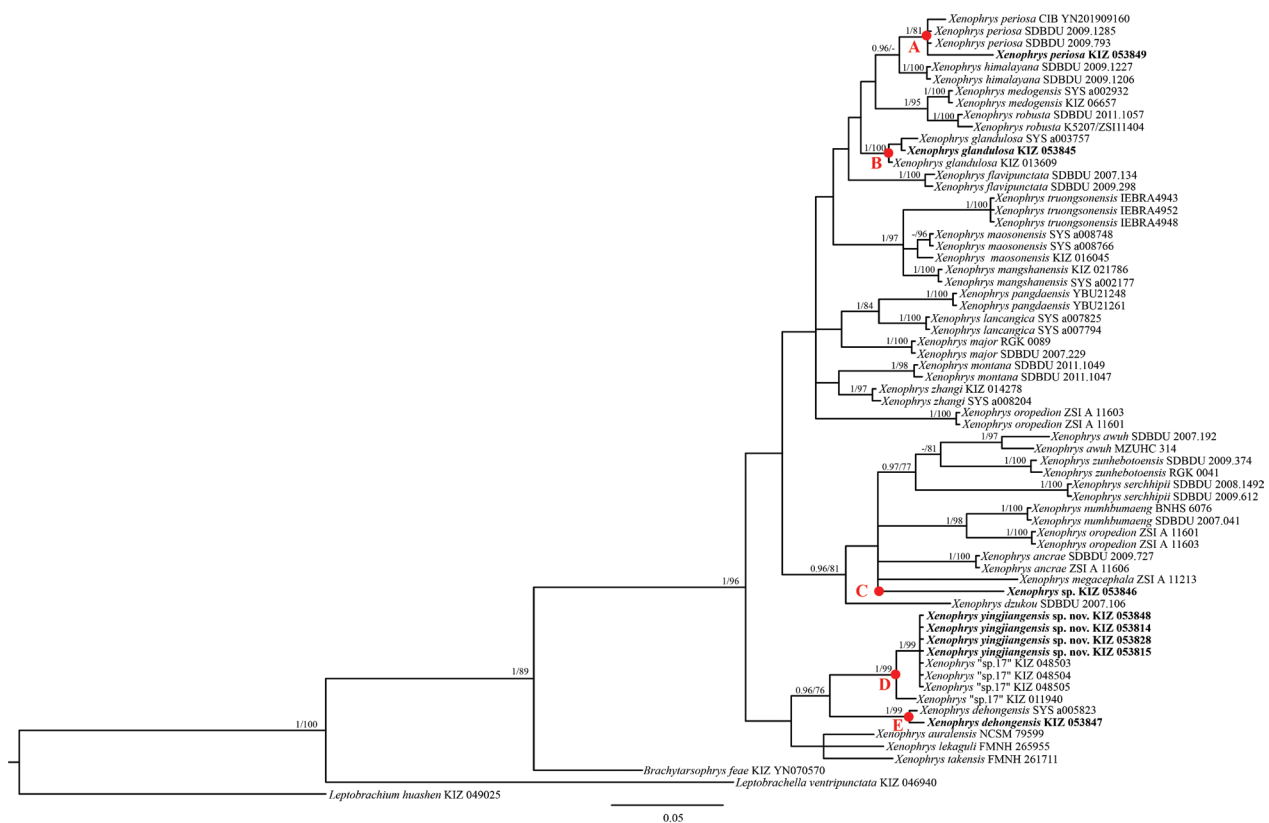


Figure 2. Phylogram of *Xenophrys* based on the mitochondrial 16S rRNA gene. Node values with Bayesian posterior probabilities (BPP) < 0.95 and Bootstrap support (BS) < 70 are not shown. A “.” denotes Bayesian posterior probabilities (BPP) < 0.95 and bootstrap support (BS) < 70. New samples for the present study are indicated in bold font.

of pairwise divergence of the 16S rRNA gene exceeded the acceptable threshold (3%) of species-level genetic divergence in anurans (Vences et al. 2005).

Morphologically, these specimens from Clade D differed from recognized congeners; therefore, we formally describe them as new. However, Clade C contains only one subadult specimen, and further surveys are needed to retrieve adult specimens.

Taxonomic account

Xenophrys yingjiangensis Wu, Yu, Chen & Che, sp. nov.

<https://zoobank.org/C2E61BB7-426B-448F-8A9C-66B993E7201A>

Figs 3, 4, Table 2

Chresonymy. *Megophrys* sp17., Chen et al. 2017.

Type material. Holotype: KIZ 053848, an adult male collected from Tongbiguan Provincial Nature Reserve, Yingjiang County, Yunnan, China (24.563°N, 97.639°E; elevation 1478 m a.s.l.), collected by Zhong-Bin Yu, Dong An, Tian-En Chen, and Xian-Kun Huang on 12 August 2023.

Paratypes: KIZ 048503–KIZ 048505, three adult males, from Tongbiguan Provincial Nature Reserve, Yingjiang County, Yunnan, China (24.546°N, 97.759°E; elevation 809 m a.s.l.), collected by Jin-Min Chen and Mian Hou on 11 August 2013; KIZ 053828, one adult male, collected at the same locality and with the same collection information as the holotype.

Etymology. The specific epithet “yingjiang” is a Latinized adjective derived from the name of Yingjiang County, Yunnan Province, China, where the new species occurs. We propose the English common name “Yingjiang horned toad” and the Chinese common name “Ying Jiāng Jiǎo Chán (盈江角蟾)”.

Diagnosis. *Xenophrys yingjiangensis* sp. nov. differs from its congeners by a combination of the following morphological characters: (1) medium adult size, adult male SVL 44.6–49.8 mm (N=5); (2) head slightly longer than wide; (3) tympanum distinct, narrow anteriorly, slightly widening posteriorly; (4) pupil vertically elliptical; (5) vomerine ridges and vomerine teeth present; (6) tongue large, oval-shaped, feebly notched posteriorly; (7) relative finger lengths: II < IV < I < III; (8) the heels slightly overlapping when the tibias are positioned at right angles to the body axis; (9) tibio-tarsal articulation of straightened limb reaching the nostril; (10) lateral dermal fringes on toes distinct, narrow; (11) toes with rudimentary webbing; (12) inner metatarsal tubercle large, elongate; (13) a distinct narrow ‘\’-shaped parietoscapular ridge present; (14) flesh pink ventral surface of thighs.

Description of the holotype (measurements in Table 2). KIZ 053848, mature male, sized medium body (SVL 45.0 mm); head moderate (HDL/SVL 39.6%, HDW/SVL 38.9%), slightly longer than wide (HDW/HDL 98.3%); snout obtusely rounded in dorsal view, obtusely projecting beyond the lower jaw in profile, without rostral appendage; triangular in dorsal view; top of head flat; loreal



Figure 3. Views of the Holotype KIZ 053848 in life. **A.** Lateral view; **B.** Lateral view of head; **C.** Dorsal view of hindlimbs; **D.** Ventral view; **E.** Ventral view of hand, and **F.** Ventral view of foot. Photos by Zhong-Bin Yu.

region vertical and concave; canthus rostralis angular; eyes large (ED/HDL 31.5%); eye less than twice as long as maximum tympanum diameter (ED/TD 207.4%) and shorter than snout length (SNT 6.8 mm, ED/SNT 82.4%); tympanum distinct, circular in shape, relatively small (TD/HDL 15.2%), with upper border concealed by supratympanic ridge; eye-tympanum distance (TYE 3.3 mm) longer than tympanum diameter (TD 2.7 mm); nostril rounded, laterally positioned, nostril closer to the tip of snout than to the anterior corner of the eye (SN/DNE 81.6%); internarial distance greater than interorbital distance (IND/IOD 109.4%) and width of upper eyelid (IND/UEW 126.1%); pineal ocellus absent; vomerine teeth in two oblique series, positioned between choanae, separated from each other by distance equal to distance from choanae; maxillary teeth present; choanae oval; tongue large, oval-shaped, feebly notched posteriorly; single internal vocal sac, with a sac slit opening on floor of mouth at each corner; pupil vertically elliptical (Fig. 3B).

Forelimbs moderately long and thin; forearm not enlarged relative to the upper arm, its length shorter than the hand length (FAL/HL 86.4%); fingers long and narrow, lateral fringes on fingers absent, relative finger lengths: II < IV < I < III; tips of all fingers rounded, slightly expanded relative to digit widths, with subcircular pads, terminal grooves absent; no webbing between fingers; subarticular tubercle absent; supernumerary tubercle absent; metacarpal tubercle absent (Fig. 3E).

Hindlimbs relatively long and thin, thigh length (THL 22.3 mm) shorter than the tibia length (TL 23.8 mm) but slightly longer than the foot length (FL 21.4 mm); the heels slightly overlapping when the tibias are positioned at right angles to the body axis; tibio-tarsal articulation of straightened limb reaching the nostril; toes long and thin, relative toe lengths: I < II < V < III < IV; tips of all toes rounded, slightly dilated, terminal grooves absent; notably expanded relative to digit widths forming circular

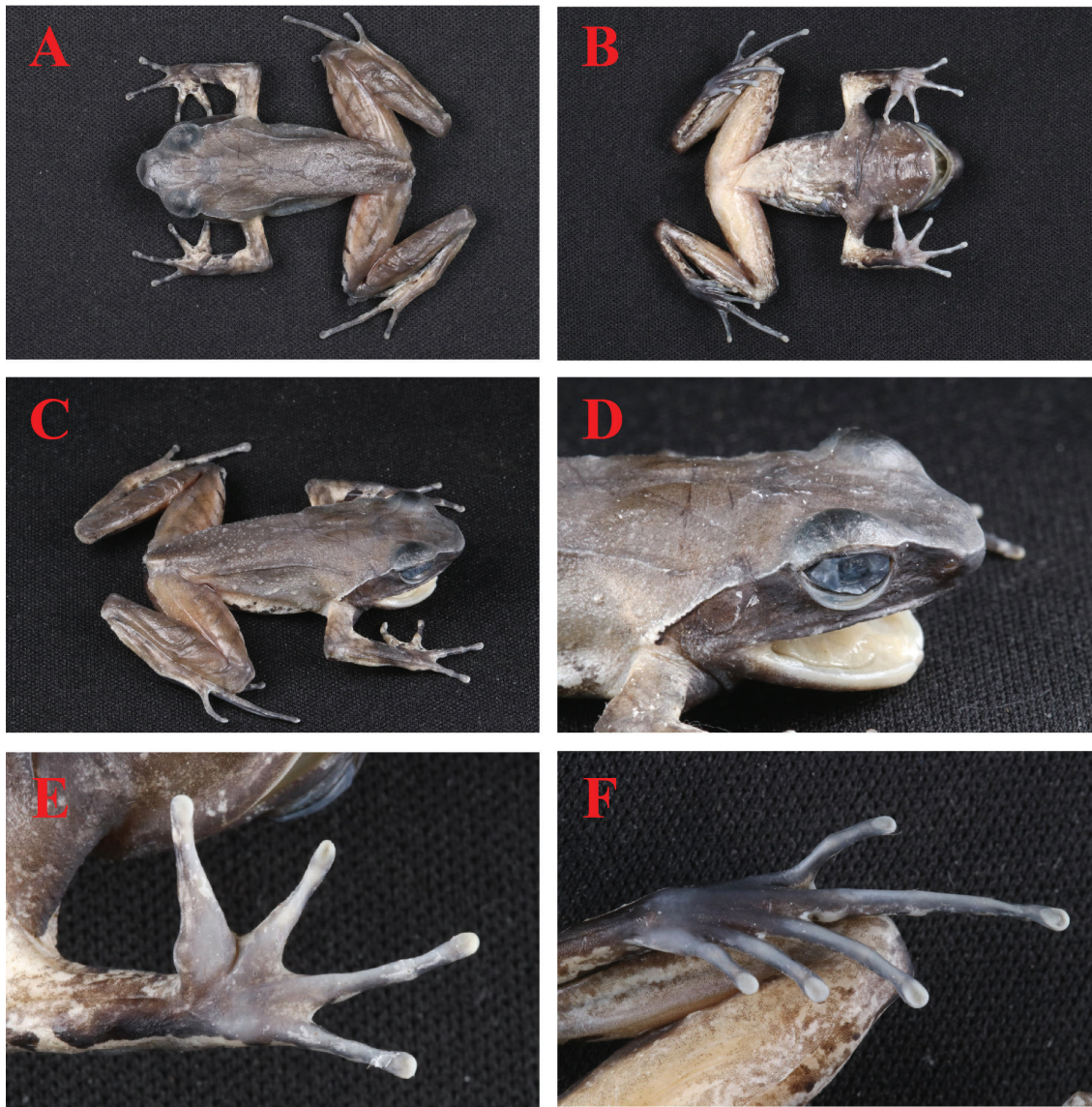


Figure 4. Views of the Holotype KIZ 053848 in preservative. **A.** Dorsal view; **B.** Ventral view; **C.** Lateral view; **D.** Lateral view of head; **E.** Ventral view of hand, and **F.** Ventral view of foot. Photos by Zhong-Bin Yu.

pads; lateral dermal fringes on toes distinct, narrow; toes with rudimentary webbing; tarsal fold absent; subarticular tubercle, supernumerary tubercle, and outer metatarsal tubercle absent; inner metatarsal tubercle large, elongate, ca. one and a half times longer than wide (Fig. 3F).

Skin of dorsal surfaces of head, body and limbs relatively smooth, with very small granules; posterior back densely-distributed with numerous small to medium sized granules and tubercles; flanks with small scattered tubercles (Fig. 3A); supratympanic fold distinct, narrow anteriorly, slightly widening posteriorly, extending from the posterior corner of the eye to a level above the insertion of the arm; tympanum skin smooth, tympanic rim slightly elevated relative to skin of temporal region (Fig. 3B); two opposing “V”-shaped parietoscapular ridge present on dorsum joined by a ca. 10 mm long dorsomedial fold in a hourglass-shape; dorsolateral fold absent; a distinct narrow “\”-shaped parietoscapular ridge present, its two sides extending posteriorly from above tympanum, terminating beyond level of axilla; dorsal surface of thighs,

shanks and upper forearms with distinct transverse ridges (Fig. 3A, C); ventral surfaces of limbs, throat, chest, and abdomen smooth; pectoral glands small, rounded, slightly raised, closer to the axilla than to the mid-ventral line; femoral gland distinct, extend longitudinally, positioned equidistant from the knee and cloacal opening on rear of each thigh.

Coloration in life. For the coloration of the holotype in life, see Fig. 3. Dorsal surface reddish brown, with a complete inverted triangle bordered with a light edge present between eyes; lateral surface of trunk of body and anterior surface of the thighs near the groin pinkish; throat purplish grey with white flecking; chest and anterior half of abdomen purplish grey with yellowish flecking and grey-brown blotches; posterior half of abdomen white with irregular lighter greyish blotches; ventral surface of thighs pinkish; ventral surface of feet and shanks brown-black; brown nuptial pads present on the base of first and second finger; supratympanic fold, light colored, bordered by a black lower margin; iris copper-brown, with tiny dark

Table 2. Measurements (in mm) of the type series of *Xenophrys yingjiangensis* sp. nov. Bold font and an asterisk (*) indicate the holotype.

	KIZ 053848*	KIZ 053828	KIZ 048503	KIZ 048504	KIZ 048505
Sex	♂	♂	♂	♂	♂
SVL	45.0	49.8	46.8	44.6	47.9
HDL	17.8	17.6	17.9	18.4	18.2
HDW	17.5	17.5	16.4	17.3	17.4
SNT	6.8	7.0	6.5	7.0	7.1
ED	5.6	5.7	5.2	6.0	5.8
IOD	5.3	4.7	5.3	5.3	4.9
UEW	4.6	5.2	4.5	4.9	5.2
IND	5.8	6.1	5.7	5.6	5.8
DNE	3.8	3.2	3.4	3.4	3.4
SN	3.1	2.9	3.0	3.1	3.5
TD	2.7	3.2	4.3	3.3	3.3
TYE	3.3	3.0	3.1	2.6	3.1
FHL	23.3	23.5	22.8	22.5	21.8
FAL	10.8	10.9	10.0	10.0	9.9
HL	12.5	12.6	12.8	12.5	12.0
LAD	4.3	4.6	4.3	4.1	3.9
FLI	5.9	6.1	3.8	3.8	4.1
FLII	4.5	5.0	3.7	3.1	3.8
FLIII	8.6	8.2	6.1	5.9	6.0
FLIV	5.2	5.4	4.6	4.6	4.0
HLL	75.9	80.4	76.0	78.7	72.6
THL	22.3	25.1	23.9	23.0	20.7
TL	23.8	24.8	24.0	25.7	24.0
TAL	32.7	34.3	12.3	12.9	12.9
FL	21.4	21.6	20.7	21.2	20.4

reticulations spreading from pupil; pectoral and femoral glands creamy white; inner metatarsal tubercle off-white.

Coloration in preservative. For coloration of the holotype in preservative, see Fig. 4. After eight months of storage in ethanol, dorsal and lateral surfaces of head and body fading to greyish-brown; slightly darker brown triangular marking between the eyes; two opposing “V”-shaped parietoscapular ridges present on two sides of dorsum becoming less distinct; the “/”-shaped parietoscapular ridge present dorsum still clear; lateral surfaces of head below supratympanic ridges and canthus rostralis dark brown; supratympanic ridges whitish-cream; dorsal surfaces of forelimbs and hindlimbs primarily light greyish-brown; granules and tubercles on posterior half of back and flanks more distinct; throat and chest faded greyish-brown with several scattered white dots; ventral thighs and shank faded to pale yellow, with several dark brown blotches on the anterior thigh and shank; pectoral and femoral glands white; inner metatarsal tubercle still off-white.

Sexual dimorphism. All adult males with nuptial pads covering most of the dorsal surface of the bases of fingers I and II; male with single internal vocal sac (Fig. 5), with a sac slit opening on floor of mouth at each corner.

Distribution and ecology. *Xenophrys yingjiangensis* sp. nov. is only known from the Tongbiguan Provincial Nature Reserve, Tongbiguan Town, Yingjiang County, Yunnan, China, and Myitkyina, Myanmar (Fig. 1). All individuals were discovered in a mountainous area surrounded by shrubland at elevations of approximately 800–1200 m (Fig. 6). This species is in sympatric distribution with *X. periosa*, *X. dehongensis*, *X. glandulosa*,

and *Xenophrys* sp. of congeners. In addition, other frog species also found at the site include *Leptobrachella yingjingensis*, *Jingophrys feii*, and *Kurixalus yangi*.

Comparison. We compared *Xenophrys yingjiangensis* sp. nov. with other congeneric species (Ohler et al. 2002; Stuart et al. 2006; Mahony 2011; Mahony et al. 2011; Mahony et al. 2013, 2018; 2020; Che et al. 2020; Luong et al. 2022; Lyu et al. 2023; Shu et al. 2023).

Xenophrys yingjiangensis sp. nov. is obviously different from its four most phylogenetically close congeners (*X. dehongensis*, *X. auralensis*, *X. lekaguli*, and *X. takensis*). It differs from *X. dehongensis* by adult male SVL 44.6–49.8 mm, $n = 5$ (vs. adult male SVL 34.8–36.7 mm, $n = 5$), metacarpal tubercle absent (vs. two metacarpal tubercles indistinct), tibio-tarsal articulation of straightened limb reaching the nostril (vs. tibio-tarsal articulation reaching posterior corner of eye), inner metatarsal tubercle large, elongate, ca. one and a half times longer than wide (vs. inner metatarsal tubercle indistinct), relative finger lengths: $II < IV < I < III$ (vs. $II < I < IV < III$); from *X. auralensis* by medium adult size, adult male SVL 44.6–49.8 mm, $n = 5$ (vs. large sized species, adult male SVL 71.0–76.9 mm, $n = 9$), head longer than wide (vs. head wider than long), internarial distance greater than interorbital distance and width of upper eyelid (vs. interorbital distance larger than internarial distance and width of upper eyelid), relative finger lengths: $II < IV < I < III$ (vs. $II < I < IV < III$), transverse crossbar in hindlimbs absent (vs. forelimb, dorsal parts of thigh, tibia and foot greyish brown with darker brown bands); from *X. lekaguli* by adult male SVL 44.6–49.8 mm, $n = 5$ (vs. adult male SVL 55.6–66.6 mm, $n = 8$), head longer than wide (vs. head slightly wider than long), relative finger lengths: $II < IV < I < III$ (vs. $IV < II < I < III$); tongue feebly notched posteriorly (vs. tongue unnotched), vertical bar below eye absent (vs. wide, dark vertical bar below eye), transverse crossbar in limbs absent (vs. limbs with narrow dark brown crossbars); from *X. takensis* by head longer than wide (vs. head wider than long), tongue large, oval-shaped, feebly notched posteriorly (vs. tongue oval, not notched posteriorly), relative finger lengths: $II < IV < I < III$ (vs. $IV \leq II < I < III$ or $IV = I < II < III$), lateral dermal fringes on toes distinct, narrow (vs. absent).

Xenophrys yingjiangensis sp. nov. is different from other congeneric species. The new species differs from *X. ancrae* by inner metatarsal tubercle large, elongate, ca. one and a half times longer than wide (vs. inner metatarsal tubercle very weak), pupil horizontally orientated (vs. pupil vertically elliptical), relative finger lengths: $II < IV < I < III$ (vs. $I < II < IV < III$), lateral dermal fringes on toes distinct, narrow (vs. absent); from *X. awuh* by adult male SVL 44.6–49.8 mm, $n = 5$ (vs. adult male SVL 35.7–41.1 mm, $n = 4$), nostril closer to the tip of snout than to the anterior corner of the eye (vs. nostril closer to eye than to snout), vomerine teeth present (vs. absent), inner metatarsal tubercle large, elongate, ca. one and a half times longer than wide (vs. inner metatarsal tubercle indistinct), lateral dermal fringes on toes distinct, narrow (vs. absent); from *X. damrei* by adult male SVL 44.6–49.8 mm, $n = 5$ (vs. adult male SVL 57.1 mm, $n = 1$), head longer than wide (vs. head wider than long), nostril



Figure 5. Advertisement calls for Paratype KIZ 048505. Photo by Jin-Min Chen.



Figure 6. Habitat of *Xenophrys yingjiangensis* sp. nov. at the type locality in Tongbiguan Provincial Nature Reserve, Yingjiang County, Yunnan, China. Photo by Zhong-Bin Yu.

closer to the tip of snout than to the anterior corner of the eye (vs. nostril closer to eye than snout), lateral dermal fringes on toes distinct, narrow (vs. absent), male with single internal vocal sac (vs. external vocal sac indistinct); from *X. dzukou* by adult male SVL 44.6–49.8 mm, $n = 5$ (vs. adult male SVL 34.2–35.3 mm, $n = 4$), nostril closer to the tip of snout than to the anterior corner of the eye (vs. nostril closer to eye than snout), inner metatarsal tubercle large, elongate, ca. one and a half times longer than wide (vs. inner metatarsal tubercle indistinct), toes with rudimentary webbing (vs. webbing absent); from *X. flavipunctata* by adult male SVL 44.6–49.8 mm, $n = 5$ (vs. adult male SVL 56.9–68.4 mm, $n = 4$), head longer than wide (vs. head wider than long), tongue large, oval-shaped, feebly notched posteriorly (vs. tongue moderately large, deeply notched posteriorly), inner metatarsal tubercle large, elongate, ca. one and a half times longer than wide (vs. inner metatarsal tubercle indistinct), transverse crossbar in hindlimbs absent (vs. dorsal surfaces of hindlimbs with distinct mid brown transverse crossbars); from *X. himalayana* by adult male SVL 44.6–49.8 mm, $n = 5$ (vs. adult male SVL 68.0–73.5 mm, $n = 6$), lateral dermal fringes on toes distinct, narrow (vs. absent), outer metacarpal tubercle absent (vs. outer metacarpal tubercle weakly developed), transverse crossbar in hindlimbs absent (vs. dorsal surfaces of thighs and shanks with distinct dark brown transverse

crossbars); from *X. megacephala* by the heels slightly overlapping when the tibias positioned at right angles to the body axis (vs. not meeting), inner metatarsal tubercle large, elongate, ca. one and a half times longer than wide (vs. inner metatarsal tubercle indistinct), relative finger lengths: $II < IV < I < III$ (vs. $IV < II < I < III$); dorsal surface of thighs, shanks and upper forearms with distinct transverse ridges (vs. absent), transverse crossbar in limbs absent (vs. dorsal surface of the fore and hind limbs with faint dark cross bars); from *X. numhumaeng* by adult male SVL 44.6–49.8 mm, $n = 5$ (vs. adult male SVL 33.8–34.6 mm, $n = 2$), pupil vertically elliptical (vs. pupil horizontally orientated), inner metatarsal tubercle large, elongate, ca. one and a half times longer than wide (vs. inner metatarsal tubercle weak), lateral dermal fringes on toes distinct, narrow (vs. absent), pupil vertically elliptical (vs. pupil horizontally orientated), inner metatarsal tubercle large, elongate, ca. one and a half times longer than wide (vs. inner metatarsal tubercle very weak), relative finger lengths: $II < IV < I < III$ (vs. $I < II < IV < III$); from *X. oropedion* by adult male SVL 44.6–49.8 mm, $n = 5$ (vs. adult male SVL 32.8–39.6 mm, $n = 7$), lateral dermal fringes on toes distinct, narrow (vs. absent), inner metatarsal tubercle large, elongate, ca. one and a half times longer than wide (vs. inner metatarsal tubercle indistinct); from *X. pangdaensis* by adult male SVL 44.6–49.8 mm, $n = 5$ (vs. adult male SVL 17.9–22.2 mm, $n = 6$), tympanum distinct (vs. indistinct), inner metatarsal tubercle large, elongate, ca. one and a half times longer than wide (vs. inner metatarsal tubercle indistinct), transverse crossbar in limbs absent (vs. two dark transverse bands on each forearm, three dark transverse bands on anterior surface of thigh and shank), iris copper-brown (vs. iris orange-red); from *X. periosa* by medium adult size, adult male SVL 44.6–49.8 mm, $n = 5$ (vs. medium adult size, adult male SVL 71.3–93.8 mm, $n = 12$), outer metacarpal tubercle absent (vs. outer metacarpal tubercle weakly developed), inner metatarsal tubercle large, elongate, ca. one and a half times longer than wide (vs. inner metatarsal tubercle weakly defined), iris copper-brown (vs. iris very dark orange), transverse crossbar in hindlimbs absent (vs. hindlimbs with distinct transverse crossbars); from *X. truongsongensis* by adult male SVL 44.6–49.8 mm, $n = 5$ (vs. adult male SVL 58.8–71.4 mm, $n = 14$), internarial distance greater than interorbital distance and width of upper eyelid (vs. internarial distance narrower than interorbital distance but wider than upper eyelid), external vocal sac indistinct (vs. absent), upper lip dark brown (vs. upper lip with a continuous white stripe, running from the nostril to shoulder), hindlimbs (vs. dorsal surface of fore and hind limbs reddish brown with dark crossbars); from *X. lancangica* by adult male SVL 44.6–49.8 mm, $n = 5$ (vs. adult male SVL 64.0–65.4 mm, $n = 3$), metacarpal tubercle absent (vs. two metacarpal tubercles indistinct), tibio-tarsal articulation of straightened limb reaching the nostril (vs. tibiotarsal articulation reaching region between nostril and tip of snout), inner metatarsal tubercle large, elongate, ca. one and a half times longer than wide (vs. inner metatarsal tubercle indistinct), transverse crossbar in limbs absent (vs. dorsal limbs with transverse bands), relative finger lengths: $II < IV$

< I < III (vs. II < IV < I < III); from *X. glandulosa* by adult male SVL 44.6–49.8 mm, $n = 5$ (vs. adult male SVL 77.0–81.0 mm, $n = 3$), head longer than wide (vs. head wider than long), tongue large, feebly notched posteriorly (vs. tongue distinctly notched posteriorly), lateral dermal fringes on toes distinct, narrow (vs. moderately wide lateral fringes present on all toes), transverse crossbar in limbs absent in preservative (vs. dorsal surfaces of hindlimbs with distinct brown transverse crossbars in preservative), sides of head smooth (vs. sides of head finely granular); from *X. monticola* by vomerine teeth present (vs. vomerine teeth absent), inner metatarsal tubercle large, elongate, ca. one and a half times longer than wide (vs. inner metatarsal tubercle indistinct), toes with rudimentary webbing (vs. absent), lateral dermal fringes on toes distinct, narrow (vs. absent), pupil vertically elliptical (vs. pupil horizontally orientated), tongue large, feebly notched posteriorly (vs. tongue large, appears rounded posteriorly without notch); from *X. robusta* by adult male SVL 44.6–49.8 mm, $n = 5$ (vs. adult male SVL 73.5–83.1 mm, $n = 6$), head longer than wide (vs. head wider than long), vomerine teeth present (vs. absent), lateral dermal fringes on toes distinct, narrow (vs. absent); from *X. medogensis* by inner metacarpal tubercle absent (vs. distinct), toes with rudimentary webbing (vs. absent), lateral dermal fringes on toes distinct, narrow (vs. absent), relative finger lengths: II < IV < I < III (vs. I < II < IV < III); dark brown stripe in lower margin of the supratympanic folds absent (vs. lower margin of the supratympanic folds with dark brown stripe); from *X. major* by medium adult size, adult male SVL 44.6–49.8 mm, $n = 5$ (vs. large sized species, adult male SVL 75.0–87.5 mm, $n = 12$), throat purplish grey with white flecking; chest and anterior half of abdomen purplish grey with yellowish flecking and grey-brown blotches (vs. light-edged wide dark brown stripe extending from posterior edge of mandible onto base of forearms), dorsolateral surface of forearms without blotch (vs. three dark brown blotches on dorsolateral surface of forearms); from *X. maosonensis* by adult male SVL 44.6–49.8 mm, $n = 5$ (vs. adult male SVL 66.2 mm, $n = 1$), metacarpal tubercle absent (vs. two metacarpal tubercles indistinct), relative finger lengths: II < IV < I < III (vs. I < II < IV < III), tibio-tarsal articulation of straightened limb reaching the nostril (vs. tibio-tarsal articulation reaching center of eye), inner metatarsal tubercle large, elongate, ca. one and a half times longer than wide (vs. inner metatarsal tubercle indistinct); from *X. mangshanensis* by adult male SVL 44.6–49.8 mm, $n = 5$ (vs. adult male SVL 60.4–71.6 mm, $n = 10$), metacarpal tubercle absent (vs. two metacarpal tubercles indistinct), relative finger lengths: II < IV < I < III (vs. II < I < IV < III), tibio-tarsal articulation of straightened limb reaching the nostril (vs. tibio-tarsal articulation reaching center of eye), lateral dermal fringes on toes distinct, narrow (vs. absent), toes with rudimentary webbing (vs. absent), inner metatarsal tubercle large, elongate, ca. one and a half times longer than wide (vs. inner metatarsal tubercle indistinct); from *X. zhangii* by adult male SVL 44.6–49.8 mm, $n = 5$ (vs. adult male SVL 32.5–40.0 mm, $n = 7$), metacarpal tubercle absent (vs. two metacarpal tubercles indistinct), relative finger lengths: II < IV < I < III (vs. I < II < IV

< III), tibio-tarsal articulation of straightened limb reaching the nostril (vs. tibio-tarsal articulation reaching anterior corner of eye), toes with rudimentary webbing (vs. absent); from *X. zunhebotoensis* by adult male SVL 44.6–49.8 mm, $n = 5$ (vs. adult male SVL 28.4–33.9 mm, $n = 23$), vomerine teeth present (vs. absent), lateral dermal fringes on toes distinct, narrow (vs. absent), toes with rudimentary webbing (vs. absent), the heels slightly overlapping when the tibias positioned at right angles to the body axis (vs. meeting); absent (vs. dense orange speckling on chest and anterior abdomen); from *X. serchhipii* by lateral dermal fringes on toes distinct, narrow (vs. absent), relative finger lengths: II < IV < I < III (vs. IV < I = II < III), two opposing “V”-shaped parietoscapular ridge present on dorsum joined by a ca. 10 mm long dorsomedial fold in a hourglass-shape (vs. unconnected inverted “V”-shaped sacral ridge).

Discussion

Our study demonstrates that species diversity within the genus *Xenophrys* remains largely underestimated. Recent phylogenetic analysis has revealed multiple genetic lineages of *Xenophrys* that may represent new species (Chen et al. 2017). Among these, *X. yingjiangensis* sp. nov. was previously suggested to be a putative new species based on molecular phylogenetic analysis. In our present study, we combined morphological and molecular lines of evidence to confirm its species status and formally describe it. Our results bring the total number of *Xenophrys* to 29, and the number of *Xenophrys* known from China to 12. In addition to the new taxon described herein, the following species of *Xenophrys* are known from China: *X. dehongensis*, *X. glandulosa*, *X. himalayana*, *X. lancangica*, *X. mangshanensis*, *X. maosonensis*, *X. medogensis*, *X. pangdaensis*, *X. parva*, *X. periosa*, and *X. zhangii* (Frost 2024). Furthermore, our study also reveals a new distinct lineage from Tongbiguan Provincial Nature Reserve that we consider a putative species. However, at present, we only have one subadult specimen. Further fieldwork is needed to collect more adult specimens and compare their morphological characteristics to determine their taxonomic status.

During our field work in the Tongbiguan Provincial Nature Reserve, adults and subadults of five species and putative species of *Xenophrys* (*X. dehongensis*, *X. glandulosa*, *X. periosa*, *X. yingjiangensis* sp. nov., and *X. sp.*) were found at the same site at the same time. A sympatric distribution pattern has been observed in other amphibians, such as *L. flaviglandulosa*, *L. nyx*, *L. feii*, and *L. bourreti*, in the Xiaoqiaogou Nature Reserve (Chen et al. 2020). However, research on the mechanism of sympatric distribution in amphibians is not well understood. Further evolutionary studies that integrate life history (e.g., advertisement call and breeding season) with genome data will be critical in the future to explore the mechanisms of sympatric coexistence among multiple lineages in *Xenophrys*.

The Gaoligong Mountains may harbor more hidden amphibian diversity than previously postulated. Recent

intensive surveys have significantly enhanced our understanding of the amphibian diversity of the Gaoligong Mountains, with discoveries of multiple new species, new national record species, and a series of new regional record species (e.g., Liu et al. 2021; Zhang et al. 2022; Hou et al. 2023; Wu et al. 2024, 2021). Our new findings of *X. yingjiangensis* sp. nov. and putative species further confirm that amphibian diversity in this mountain ecosystem is undoubtedly underestimated. Future amphibian exploration will hopefully continue to discover more new taxa in the region. In addition, the classification and diversity of some species distributed in the Gaoligong Mountains have long been disputed, such as *Amolops bellulus* (Liu et al. 2000; Wu et al. 2020) and *Nanorana arnoldi* (AmphibiaChina 2024). Considering that the Gaoligong mountains are located at the China-Myanmar border, future international collaborations between herpetologists from both countries are necessary to clarify the distribution and classification of these species.

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Supplementary material 1

Average uncorrected p-distances (percentage) among *Xenophrys* species calculated from 16S rRNA gene sequences (below the diagonal) and standard error estimates (above the diagonal)

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Data type: xls

Explanation note: The ingroup mean uncorrected p-distances are shown on the diagonal.

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