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A new species of the genus *Amolops* (Amphibia: Ranidae) and the first national record of *Amolops vitreus* from China

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Abstract

The torrent frogs of the genus *Amolops* represent a great anuran diversification in southern China and Southeast Asia. Previous studies have shown that, the diversity of this genus still remains underestimated. During herpetological surveys from 2021 to 2022, several *Amolops* specimens were collected from the international border regions of southwestern Yunnan Province, China. Herein, we utilized molecular phylogenetic and morphological data to identify these specimens. Our findings indicate the presence of a separate and previously unknown lineage in the *A. viridimaculatus* group, which we formally describe as a new species. Furthermore, the specimen from Xishuangbanna National Nature Reserve clustered with *A. vitreus* from the paratype, supporting the morphological diagnosis. Therefore, we describe a new species and a new species record for China. Our study contributes to the species richness of the genus *Amolops* as well as the diversity of amphibians in China. Notably, our discovery brings the total number of *Amolops* species to 85 and the total number of torrent frog species known to occur in China to 53. In addition, our study further confirmed that Yunnan and Indochina Peninsula have similar faunal composition, implying that more studies are needed to achieve a complete understanding of the species diversity and distribution pattern.

Key words

Amolops yangi **sp. nov.**, *Amolops vitreus*, new species, new species record, Southwest border of China, Yunnan Province

Introduction

Yunnan is located at the intersection of three global biodiversity hotspots: the eastern Himalayas region, the mountains of Southwest China, and the Indo-Burma region (Myers et al. 2000). It is recognized globally as one of the richest and most diverse regions due to its species richness and wide range of habitats and ecosystems (Yang et al. 2004; Pu et al. 2007). Although Yunnan only comprises 4.1% of China's total land area, it is home to more than 19,365 higher plant and 2,273 vertebrate species (54.8% of China's total) (Liu et al. 2021). Moreover, this area harbors more than 3,400 endemic species and about 60% of the endangered species in China (Yang et al. 2004; Liu et al. 2021). Notably, current research still indicates that the vertebrate diversity of Yunnan has been underestimated (e.g., Chen et al. 2015; Su et al. 2020; Wang et al. 2022). The gap may be even larger in invertebrates (e.g., Yu et al. 2022). In particular, it shares an extensive 4,060-kilometer border with Vietnam, Laos and Myanmar. Since, these areas are located in the same zoogeographic region (Holt et al. 2013), and their habitats on either side of the border are continuous with no known biogeographic barriers, their species composition is similar. Furthermore, many new records have been reported in Yunnan in recent years (e.g., Jiang et al. 2022; Yu et al. 2022). Similarly, many new records have been reported from Vietnam, Laos and Myanmar, for species initially described from China (e.g., Nguyen et al. 2020; Rahman et al. 2020; Liang et al. 2023).

Amphibians face a greater risk of rapid loss of diversity and are considered the most threatened group of vertebrates (Alroy 2015). Yunnan Province is the region with the richest amphibian diversity in China, and the number of threatened amphibian species has increased significantly in recent years (Yuan et al. 2022). Therefore, a clear number and distribution of species is both warranted and of great conservation importance. Recent studies on *Megophrys* sensu lato, *Leptobrachella*, and *Amolops* have shown that the diversity of amphibians in this region is underestimated (Chen et al. 2017, 2018; Wu et al. 2020). Intensified fieldwork surveys in recent years have led to a continuous description of new amphibian species (Chen et al. 2020; Liu et al. 2021, 2023; Shi et al. 2021; Wu et al. 2019; Yang et al. 2019a,b). Furthermore, a series of new genera records and new species records of amphibians from China have been described along the border region in the recent past, such as the genus *Wijayarana* (Wu et al. 2023a), *Nidirana chapaensis* (Yuan et al. 2018), *Amolops putaoensis* (Zhang et al. 2022), and *Microhyla hmongorum* (Wu et al. 2023b). These findings highlight a largely underestimated amphibian diversity in the border region of Yunnan Province.

The genus *Amolops* Cope, 1865 of the family Ranidae is the most species-rich genus within the family Ranidae, distributed widely throughout Nepal, northern India, western and southern China to Malay Peninsula, and currently includes 84 recognized species (Frost 2023). *Amolops* is characterized by possessing a gastromyzophorus adhesive disk (ventral sucker), dorsal and ventral poison glands in their tadpoles and enlarged digital discs in adults (Yang 1991; Fei et al. 2009; Wu et al. 2020). These montane species mainly inhabit fast-flowing, rocky stream environments, such as torrents and waterfalls (Fei et al. 2009; Wu et al. 2020). In recent years, extensive taxonomic and phylogenetic studies have been conducted on the genus *Amolops*, delimiting 10 species groups (e.g., Wu et al. 2020; Zeng et al. 2020; Jiang et al. 2021), which greatly improves our understanding on the taxonomy and species diversity of this genus. In China, 51 species have been recorded, and assigned into eight species groups: *A. chayuensis* group, *A. daiyunensis* group, *A. hainanensis* group, *A. mantzorum* group, *A. marmoratus* group, *A. monticola* group, *A. ricketti* group and *A. viridimaculatus* group (AmphibiaChina 2023). Nearly half of the currently recognized Chinese *Amolops* species have been described in the last 10 years (AmphibiaChina 2023). Moreover, 22 species of *Amolops* have been recorded in Yunnan, 13 of which were first discovered in Yunnan in the past 10 years (AmphibiaChina 2023). This is ascribed to intensified survey efforts and the adoption of more integrative taxonomic approaches.

During our recent herpetological surveys at the Yunnan border area, four specimens of the genus *Amolops* were collected. Molecular data and morphological comparisons revealed that these specimens included a new species and a new species record for China which we herein describe.

Materials and Methods

Sampling

A total of four individuals including three adult males and one adult female were collected from the Yunnan border, China, (Fig. 1). Following euthanasia, all specimens were fixed in 10% formalin for 24 hours for preservation after extraction of liver tissues from the adults in 95% ethanol, and subsequently transferred to 70% ethanol for permanent preservation. Voucher specimens were deposited in the herpetological collection of the Museum of the Kunming Institute of Zoology (KIZ), Chinese Academy of Sciences (CAS).

DNA extraction, PCR amplification, and sequencing

Total genomic DNA was extracted using standard phenol-chloroform protocols (Sambrook et al. 1989), and three partial fragments of the mitochondrial 16S rRNA gene (16S), cytochrome oxidase subunit I (*COI*), and NADH dehydrogenase subunit 2 (*ND2*) genes were amplified and sequenced using the following primers: 16SAR (5'-CGCCTGTTTAYCAAAAACAT-3') and 16SBR (5'-CCGGTYTGAACTCAGATCAYGT-3) (Kocher et al. 1989),

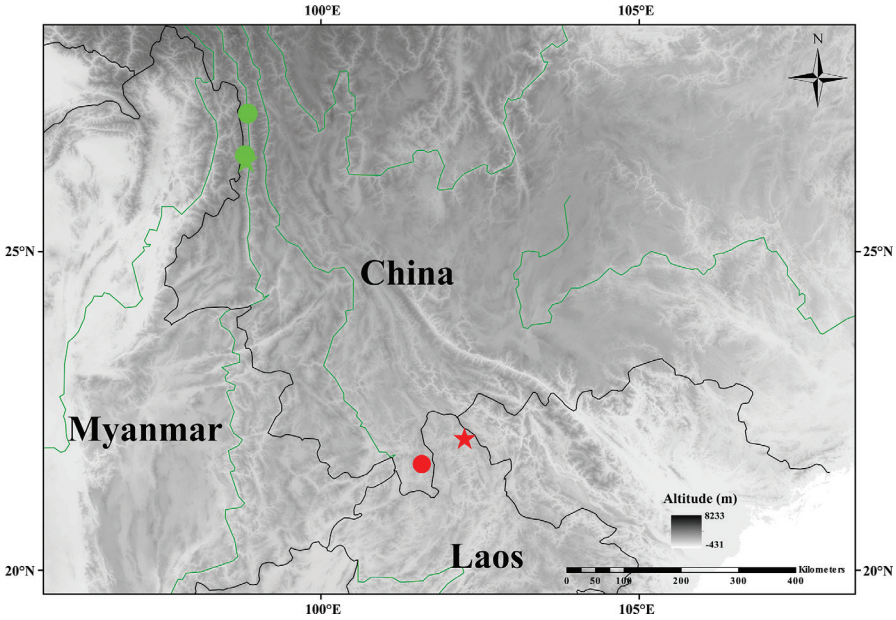

Figure 1. Sampling localities of *Amolops* used in this study. The green pentagram indicates the type locality of *Amolops yangi* **sp. nov.**, the green circles indicate the other distribution localities of *Amolops yangi* **sp. nov.**, the red pentagram indicates the type locality of *A. vitreus*, and the red circle indicates the new locality record of *A. vitreus*.

ND2AR (5'-CAATGTTGGTTAAAATCCTTCC-3') and NDBR (5'-AGGCTTTGAAGGCCTTTGGTC-3') (Stuart et al. 2006), and Chmf4 (5'-TYTCWACWAAYCAYAAAGAYATCGG-3'), and Chmr4 (5'-ACYTCRGGRTGRCCRAARAATCA-3') (Che et al. 2012). The polymerase chain reaction (PCR) was performed in a 25 µl reaction volume with the following cycling conditions: initial denaturing step at 95°C for 4 min, 35 cycles of denaturing at 94°C for 40 s, annealing at 55°C for 16S rRNA for 45 s, 50°C for *ND2* for 1 min, and 55°C for *COI* for 45 s, extending at 72°C for 1 min and a final extension at 72°C for 10 min. PCR products were directly sequenced by an ABI 3730xl DNA automated sequencer (Applied Biosystems, UK) with both forward and reverse primers. All sequences were assembled from forward and reverse reads and edited manually using DNASTAR LASERGENE 7.1. All new sequences were deposited in GenBank (Table S1).

Phylogenetic analysis

To study the historical relationships among *Amolops* species, phylogenetic trees were reconstructed based on the 16S, *COI*, and *ND2* fragments. Homologous sequences of *Amolops* and representative outgroups (*A. spinapectoralis* and *A. yatseni*) were downloaded from GenBank (Table S1). Phylogenetic relationships were inferred using maximum likelihood (ML) and Bayesian inference (BI) methods. The best-fit nucleotide substitution model was selected using the JMODELTEST v2.1.7 (Darriba et al. 2012) following the Bayesian information criterion (BIC; Posada 2008) for BI. The best-fit models were summarized in Table S2. BI analysis was implemented by the CIPRES web server (Miller et al. 2010). For BI analyses, the Monte Carlo Markov chain length was run, sampling one tree every 1,000 generations for 10,000,000 generations with a burn-in of 25%. Convergence was assessed in TRACER 1.5 (Rambaut et al. 2009) by the average standard deviation of split frequencies (below 0.01) and the effective sample size (over 200). Maximum likelihood (ML) analyses were conducted using RAxML v8.0.0 with 1,000 bootstrap replicates and using the standard bootstrap search (random seed value 12,345) under GTR+I+G nucleotide substitution model (Stamatakis et al. 2014). We also calculated pairwise sequence divergence using uncorrected pairwise distances (p distances) implemented in MEGA 6.0.6 (Tamura et al. 2013).

Morphology

The four preserved adult specimens were measured with digital calipers to the nearest 0.1 mm. Measurements followed Fei et al. (2009). Measurements included the following: **SVL** (Snout-vent length): measured from tip of snout to vent; **HL** (head length): measured from tip of snout to jaw angle; **HW** (head width): measured as head width at its widest point; **SL** (snout length): measured from tip of snout to anterior corner of eye; **INS** (internasal space): measured as distance between nares; **IOS** (interorbital space): measured at narrowest point between eyelids on top of head; **NED** (nasal to eye distance): measured as distance from the anterior corner of eye to nostril center; **UEW** (upper eyelid width): maximum width of upper eyelid; **ED** (eye diameter): measured as the distance between corners of eye; **TD** (tympanum diameter): measured as maximal diameter of tympanum; **LAHL** (length of lower arm and hand): distance from elbow to the tip of the third finger; **HND** (hand length): measured as the distance from the proximal edge of inner metacarpal tubercle to the tip of third finger; **LAD**: (diameter of lower arm); FEM (femoral length): measured from the cloaca to the knee; **TIB** (tibia length): measured as the distance from knee to heel; **FTL** (foot length): measured as the distance from proximal end of inner metatarsal tubercle to the tip of fourth toe. Sexual maturity was determined

by the presence of nuptial pads or vocal sacs (males), or eggs or enlarged oviducts (females).

To compare the morphometrics of *A. viridimaculatus* group, we extracted data from the published literature for all species available (Table S3). Principal Component Analysis (PCA) was used to explore the morphological differences between the undescribed species and other species of *A. viridimaculatus* group. The morphometric analyses were conducted separately for the male and female groups. The analyses were carried out in R 4.0.2.

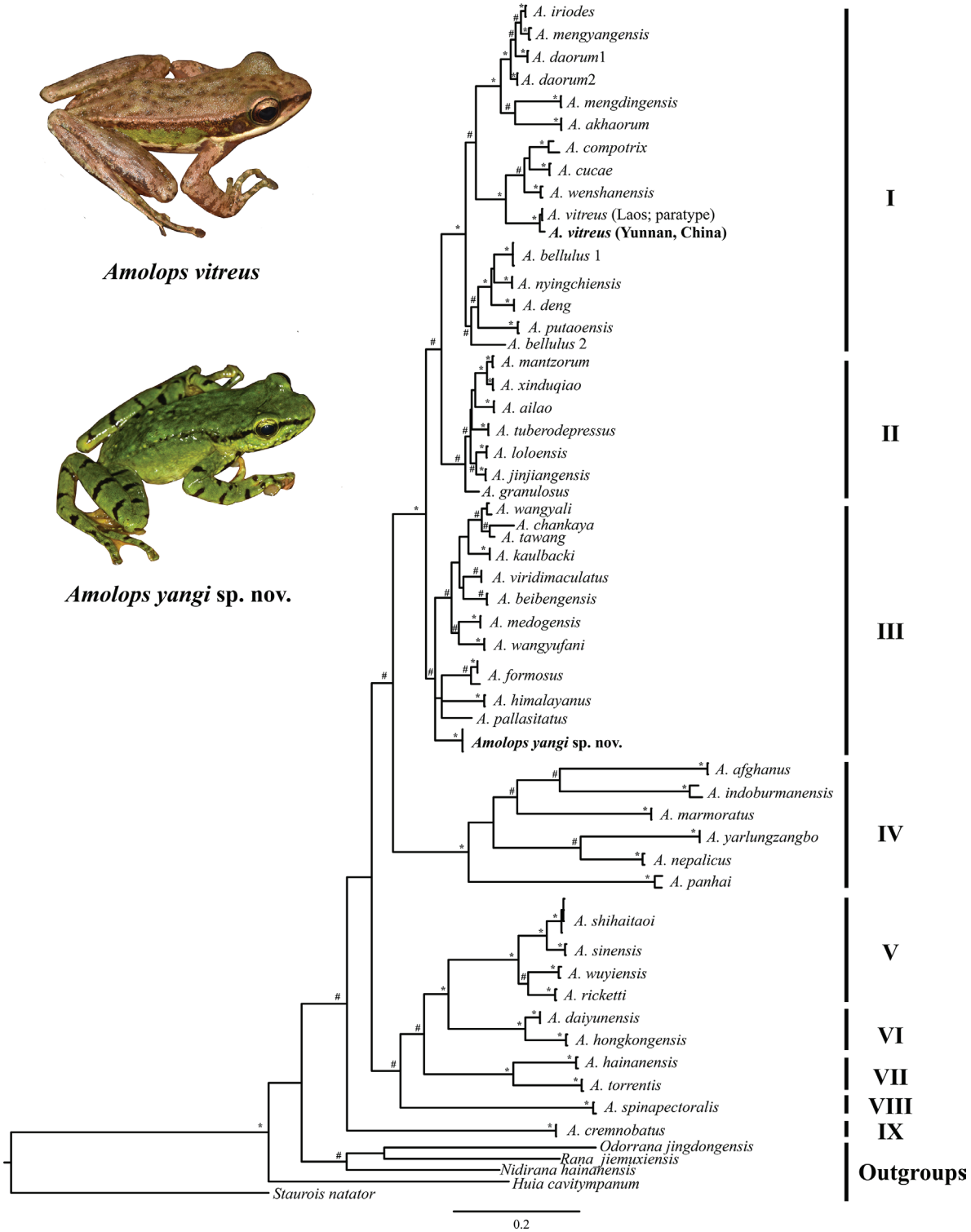

Figure 2. Phylogram of *Amolops* based on mitochondrial 16S, *COI*, and *ND2*. The "*" denote Bayesian posterior probabilities (BPP) = 1.00 and bootstrap support (BS) = 100%; "#" denote Bayesian posterior probabilities (BPP) ≥ 0.95 and bootstrap support (BS) ≥ 70%. Node values with Bayesian posterior probabilities (BPP) < 95 or Bootstrap support (BS) < 70 are not shown.

Results

Phylogenetic data

Our concatenated mtDNA alignment (***ND2***: 890 base pairs (bp), *COI*: 570 bp, 16S: 541 bp) contained 108 individuals with a total of 2002 bp, with 900 conserved sites and 1098 variable sites. Of the variable sites in the alignment, 1008 were parsimony-informative (559 bp, 247 bp, and 202 bp including the outgroup sequences).

Both BI and ML analyses resulted in essentially identical topologies, with relatively robust support for most nodes. The genus *Amolops* was recovered as monophyletic with strong support from both analyses (BPP = 1; BS = 96; Fig. 2). The genus forms nine monophyletic clades (clade I–IX, Fig. 2), corresponding to nine different species groups. Among these, three collected specimens from Fugong and Lushui formed a separate lineage with strong support (BPP = 1; BS = 100; Fig. 2) and nested within the *A. viridimaculatus* group (clade III). The genetic distance between the new population and other species of the *A. viridimaculatus* group ranged from 2.3% (with *A. kaulbacki*) to 3.5% (with *A. beibengensis*) for 16S, 8.3% (with *A. medogensis*) to 11.1% (with *A. beibengensis*) for *COI*, and 9.4% (with *A. formosus*) to 12.9% (with *A. kaulbacki*) for *ND2* (Table S4). It is comparable to the divergences among the nearest neighbour genetic distances of this group, which ranged from 1.0% (*A. wangyali* and *A. tawang*) to 6.1% (*A. himalayanus* and *A. wangyufani*) for 16S, 6.1% (*A. viridimaculatus* and *A. kaulbacki*) to 10.2% (*A. beibengensis* and *A. formosus*) for *COI*, and 6.6% (*A. viridimaculatus* and *A. beibengensis*) to 14.3% (*A. kaulbacki* and *A. formosus*) for *ND2* (Table S4). Morphologically, the new specimens differed from presently recognized congeners. In the PCA analysis, the first two principal components explained 85.7% of the total variation in the males, where PC1 and PC2 eigenvectors accounted for 75.1% and 10.7% of the total variance, respectively (Table S5). Similarly, the first two principal components occupied a considerable proportion in the females, 80.9% of the total, whereas PC1 and PC2 eigenvectors accounted for 65.8% and 15.1% of the total variance, respectively (Table S5). Regardless of the sex of the species, the samples showed no overlap between the new specimens and other species of *A. viridimaculatus* group on the two-dimensional graphs for PC1 and PC2 (Fig. 3). In addition, one collected male specimen (KIZ 050452) from Xishuangbanna National Nature Reserve was nested in the *A. monticola* group (Fig. 2, clade I) and formed a monophyletic clade with *A. vitreus* from the paratype confirming shared genetic traits therefore validating the morphological diagnosis. As a result, below we describe a new species and a new species record for China.

Taxonomic account

Amolops yangi Wu, Yu, Lu, Yuan & Che sp. nov.

https://zoobank.org/5B4E8AC7-37AD-4192-83E3-A05E49393B7B

Holotype. Adult female (KIZ 038643), from Ega, Lushui, Nujiang Lisu Autonomous Prefecture, Yunnan Province, China (26.43744°N, 98.75044°E; elevation 3496 m a.s.l.), collected by Zhong-Bin Yu, Dong An, Tian-En Chen on 07, August, 2021.

Paratypes. One adult male KIZ 038645 from Ega, Lushui, Nujiang Lisu Autonomous Prefecture, Yunnan Province, China (26.44924°N, 98.76762°E; elevation 2915 m a.s.l.), collected by Zhong-Bin Yu, Dong An, Tian-En Chen on 07, August, 2021; one adult male KIZ 050788 from Yaping road, Lumadeng Township, Fugong county, Nujiang Lisu Autonomous Prefecture, Yunnan Province, China (27.15890°N, 98.79712°E; elevation 2451 m a.s.l.), collected by Zhong-Bin Yu, Dong An on 05, August, 2022.

Etymology. The specific epithet "*yangi*" is a patronymic noun in the genitive singular; derived from the name of Prof. Da-Tong Yang of the Kunming Institute of Zoology, CAS, China. We acknowledge his great contributions

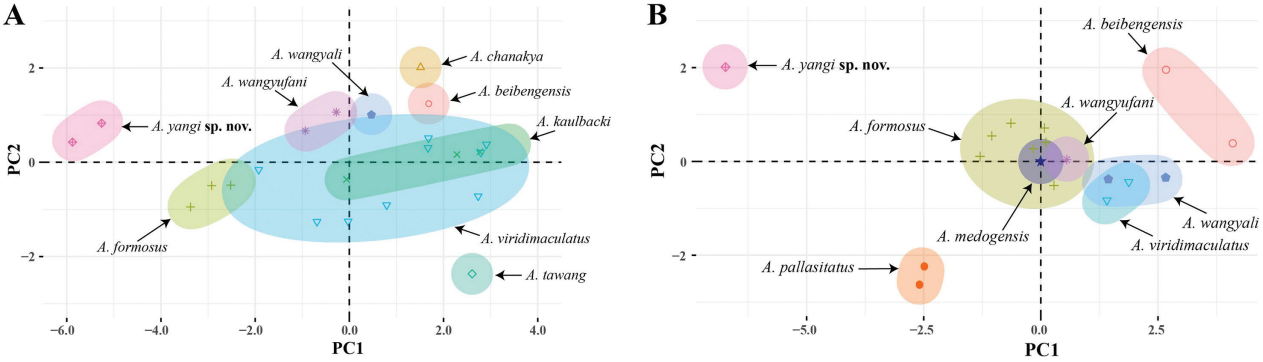

Figure 3. Plots of the first principal component (PC1) versus the second (PC2) for *A. yangi* **sp. nov.** and other species of *A. viridimaculatus* group from the principal component analysis. **A** Male, **B** female.

Table 1. Measurements (mm) of *Amolops yangi* **sp. nov**. and *A. vitreus*. The asterisk (*) indicates the holotype.

	***A. yangi* sp. nov.**	***A. yangi* sp. nov.**	***A. yangi* sp. nov.***	***A. vitreus***
Sex	♂	♂	♀	♂
Catalog No.	**KIZ** 050788	**KIZ** 038645	**KIZ** 038643	**KIZ** 050452
SVL	51.8	46.3	51.5	38.9
HL	17.0	16.8	17.6	15.4
HL/SVL	32.8%	36.3%	34.2%	39.6%
HW	16.7	15.4	16.8	13.9
HW/SVL	32.2%	33.3%	32.6%	35.7%
SL	7.2	7.7	8.1	6.5
SL/SVL	13.9%	16.6%	15.7%	16.7%
SL/HL	42.4%	45.8%	46.0%	42.2%
INS	6.7	6.5	6.9	4.6
INS/SVL	12.9%	14.0%	13.4%	11.8%
IOS	4.1	4.8	4.3	4.1
INS/IOS	163.4%	135.4%	160.5%	112.2%
NED	3.3	3.2	3.3	3.4
UEW	4.1	3.6	4.1	3.8
INS/UEW	163.4%	180.6	168.3%	121.1%
UEW/IOS	100.0%	75.0%	95.3%	92.7%
ED	5.0	4.9	5.1	4.4
ED/HL	29.4%	29.2%	29.0%	28.6%
ED/SL	69.4%	63.6%	63.0%	67.7%
TD	2.6	2.4	1.8	2.7
TD/ED	52.0%	49.0%	35.3%	61.4%
LAHL	26.9	25.0	29.1	19.6
LAHL/SVL	51.9%	54.0%	56.5%	50.4%
HND	17.1	15.4	18.4	12.2
HND/SVL	33.0%	33.3%	35.7%	31.4%
LAD	5.0	4.8	4.4	3.8
LAD/SVL	9.7%	10.4%	8.5%	9.8%
FEM	26.3	22.7	25.3	22.2
TIB	27.1	24.3	27.2	23.5
FEM/TIB	97.0%	93.4%	93.0%	94.5%
FTL	27.7	26.8	28.0	20.3
FEM/FTL	94.9%	84.7%	90.4%	109.4%

to the herpetological research in southwestern China. We suggest the Chinese formal name as "杨氏湍蛙".

Diagnosis. *Amolops yangi* **sp. nov.** is assigned to the genus *Amolops* based on molecular phylogenetic analyses and can be distinguished from its congeners by a combination of the following characters: (1) medium body size (SVL 46.3–51.8 mm in males and at least 51.5 mm in female); (2) vomerine teeth developed, on two short oblique between choanae, equal in distance from each other as to choanae; (3) supernumerary tubercles present at the base of each finger; (4) tympanum indistinct; (5) three metacarpal tubercles, inner metacarpal tubercle long, outer metacarpal tubercle relatively small, oval, median one rounded; (6) supratympanic fold indistinct; (7) discontinuous glandular dorsolateral fold from rear of eye to near vent; (8) circummarginal grooves present on tips of outer three fingers, absent on first finger; (9) iris distinctly bicolored, golden-yellow in upper one-fourth and reddish brown in lower three-fourths, black reticulations throughout; (10) rictal gland absent; (11) dorsal surface of the head, back, limbs, fingers, and toes green, interspersed with irregular black spots; (12) dorsal parts of limbs, fingers and toes with black crossbars; (13) vocal sac absent in males; (14) male with orange nuptial pad at the base of first finger.

Description of holotype (all measurements in mm; see Table 1). KIZ 038643, sexually mature female, body size moderate, adult female (SVL 51.5 mm); head length larger than wide (HL/SVL 34.2%, HW/SVL 32.6%); top of head flat; snout short (SL/HL 46.0%), snout rounded in dorsal view (Fig. 4A), obtusely rounded in profile, projecting beyond margin of lower jaw; loreal region and concave and oblique; canthus rostralis distinct, slightly constricted behind nostrils; dorsal region of snout flattened; eyes relatively large (ED/HL 29.0%), slightly protuberant in dorsal view and notably protruding in profile (Fig. 4A), eye diameter shorter than snout length (ED/SL 63.0%); nostrils oval, laterally orientated, slightly protuberant, closer to anterior corner of eye than to tip of snout; pupil oval, horizontal; tympanum indistinct, circular in shape, rela-

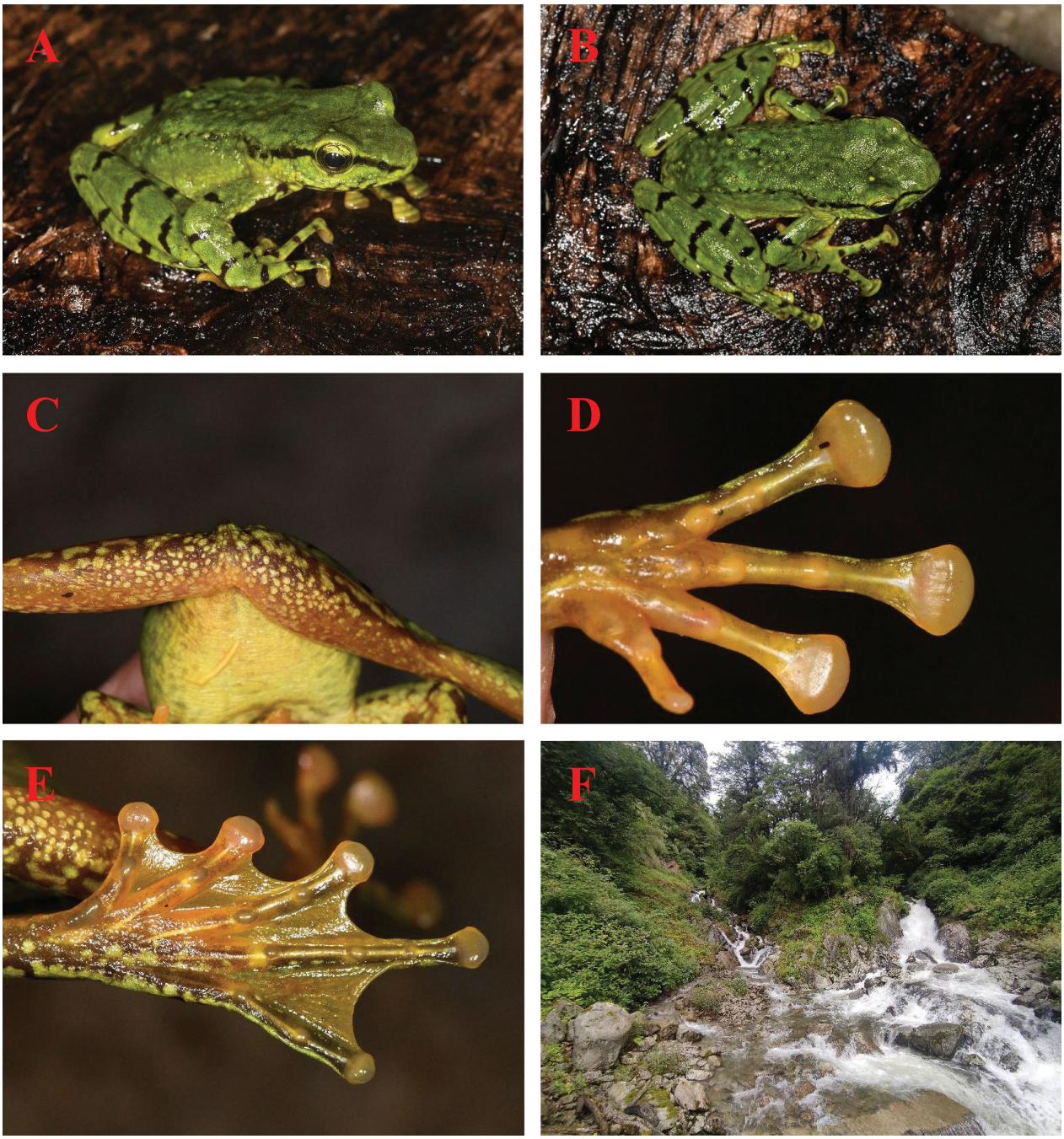

Figure 4. *Amolops yangi* **sp. nov.** (Holotype KIZ 038643). **A** Lateral view, **B** dorsal view, **C** ventral view of thighs, **D** ventral view of hand, **E** foot, **F** habitat. Photos by Zhong-Bin Yu.

tively small (TD/HL 10.2%), tympanum diameter about one third of eye diameter (TD/ED 35.3%); internarial distance (INS/SVL 13.4%) larger than width of upper eyelid (INS/UEW 168.3%) and interorbital distance (INS/IOS 160.5%); tongue cordiform, deeply notched posteriorly; vomerine teeth developed, on two short oblique between choanae, equal in distance from each other as to choanae; choanae oval; maxillary teeth developed; a small tooth-like projection on anteromedial edge of mandible.

Forelimbs moderately long and robust, forelimb and hand length (29.1 mm) longer than half body size (LAHL/SVL 56.5%); relative length of fingers: I<II<IV<III; circummarginal grooves present on tips of outer three fingers, absent on first finger; subarticular tubercles prominent and oval, formula 1, 1, 2, 2; supernumerary tubercles present at the base of each finger; webbing between fingers absent; narrow lateral fringes of fingers III and IV; three metacarpal tubercles, inner metacarpal tubercle long, outer metacarpal tubercle relatively small, oval, median one rounded (Fig. 4D).

Hindlimbs long and robust, femoral length shorter than the tibia length (FEM/TIB 93.0%) and the foot length (FEM/FTL 90.4%); tibiotarsal articulation of adpressed limb reaching between nostrils and eyes when hindlimb stretched alongside of body; the heels overlapping when the tibias are perpendicular to the body axis; relative toes lengths: I<II<III<V<IV; narrow lateral fringes of preaxial side of toe I and postaxial side of toe V; tips of all toes

expanded into discs with circummarginal grooves; toes fully webbed except for fourth toe, in which web reaches beyond distal subarticular tubercle; subarticular tubercles oval and distinct, formula 1, 1, 2, 3, 2; supernumerary tubercles absent; inner metatarsal tubercle long, outer metatarsal tubercle absent (Fig. 4E).

Dorsal surface of head, body, limbs, fingers, toes and flank of body relatively smooth; loreal region densely scattered with raised tubercles; temporal region and posterior angle of the jaw with dense tubercles; skin ventrally smooth, including throat, chest, abdomen, and ventral surface of limbs; discontinuous glandular dorsolateral fold from rear of eye to near vent; supratympanic fold indistinct; rictal gland absent (Fig. 4A–E).

Color of holotype in life. For coloration of the holotype in life see Figure 4A-E. Dorsal surface of the head, back, limbs, fingers, and toes green, interspersed with irregular black spots; throat, ventral surface of the head, chest and anterior abdomen mostly yellow, with scattered grayish spots; upper part of flanks green, lower part of flanks green-yellow; a black stripe below edge of the canthus rostralis extending from the snout tip across the eyes, to the anterior edge of supratympanic fold; upper lips with three dark bars; ventral surface of thighs orangish, densely scattered with small yellow spots; dorsal parts of limbs, fingers and toes with black crossbars; ventral surface of fingers and toes orange; toes webbing yellowish-gray; the inside of lower arm with black stripe; ventral surface of all fingers discs, subarticular tubercles, supernumerary tubercles, and metacarpal tubercles orange, ventral surface of outer three toes discs, subarticular tubercles, and metatarsal tubercle grey; ventral surface of inner two toes discs orange; iris distinctly bicolored, golden-yellow in upper one-fourth and reddish brown in lower three-fourths, black reticulations throughout.

Color of holotype in preservative. For coloration of the holotype in preservative see Fig. 5A–D. After two years of storage in ethanol, the dorsal surface fading to metallic blue with irregular black spots; black crossbars present on dorsal surfaces of limbs, fingers and toes still clear; throat, chest, belly, and ventral surface of limbs fading to cream-yellow, with irregular gray pigmentations; ventral surface of the hands and toes cream-yellow, digit tips and subarticular tubercles fading to cream-yellow or grayish-white.

Male secondary sexual characteristics. Adult males possess orange nuptial pads covering the base of first finger; absence of vocal sacs in males.

Distribution and ecology. *Amolops yangi* **sp. nov.** is currently known from two localities in the Gaoligong Mountains. These are Fugong and Lushui County, both in Yunnan Province, China. These two localities are separated by a straight-line distance of approximately 80 km. The new species inhabits the banks of rocky, fast-flowing streams or perches on shrubs (ca. 0.5 m above the ground) along the swift flowing streams (Fig. 4F). The

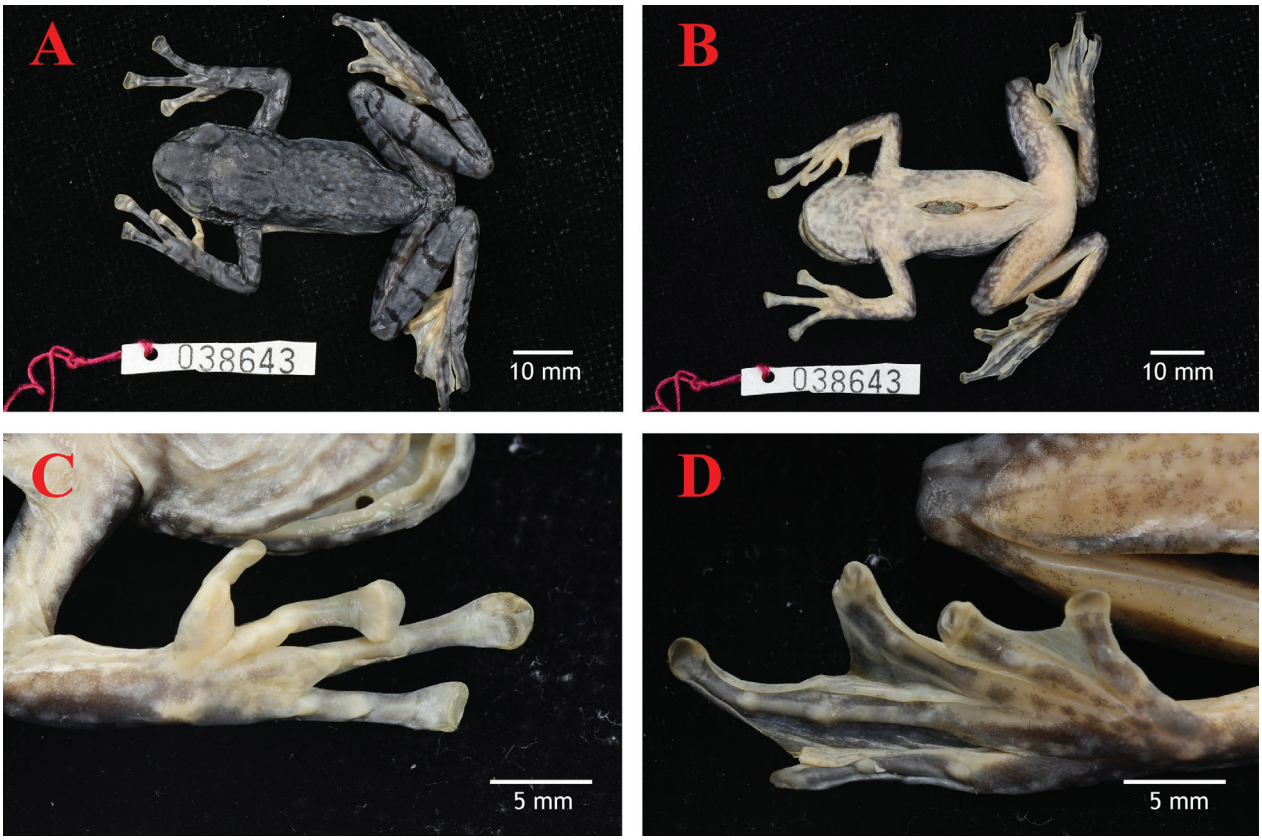

Figure 5. A Dorsal view, **B** ventral view, **C** ventral view of hand, **D** ventral view of foot, (**D**) *Amolops yangi* **sp. nov.** (Holotype KIZ 038643) in preservative. Photos by Zhong-Bin Yu.

new species primarily inhabits high altitude mountainous areas with elevations ranging from 2500 to 3500 m. The breeding season is currently uncertain. Other sympatric amphibian species found in the same habitat included *Nanorana chayuensis*, *A. viridimaculatus*, *Scutiger gongshanensis*, and *Xenophrys glandulosa*.

Comparisons. Phylogenetic analyses indicated that the new species belongs to the *A. viridimaculatus* group with strong support. Geographically, the new species is found in Fugong and Lushui, Yunnan Province, China that belong to Gaoligong Mountains, and close to northern Myanmar. Therefore, we compared *Amolops yangi* **sp. nov.** with morphologically, geographically, and molecularly similar species, which include *A. chayuensis*, *A. bellulus*, *A. putaoensis*, *A. binchachaensis*, *A. deng*, *A. jinjiangensis*, *A. viridimaculatus*, *A. kaulbacki*, *A. marmoratus*, *A. afghanus*, and *A. tuberodepressus*, *A. beibengensis*, *A. wangyufani*, *A. formosus*, *A. medogensis*, *A. pallasitatus*, *A. nidorbellus*, *A. himalayanus*, *A. wangyali*, *A. longimanus*, *A. ailao*, *A. chanakya*, and *A. tawang* (Andersson 1938; Jiang et al. 1983; Yang and Rao 2008; Fei et al. 2009; Biju et al. 2010; Dever et al. 2012; Sun et al. 2013; Nidup et al. 2016; Qi et al. 2019; Che et al. 2020; Gan et al. 2020; Liu and Yang 2000; Liu et al. 2000; Mahony et al. 2022; Rao et al. 2022; Saikia et al. 2022; Tang et al. 2023).

Amolops yangi **sp. nov.** is significantly different from *A. viridimaculatus* by discontinuous glandular dorsolateral fold (vs. absent), supratympanic fold indistinct (vs. distinct), SVL 46.3–51.8 mm in males and 51.5 mm in female (vs. 72.7–82.3 in males and 83.0–94.3 in females), dorsal surface of the head, back, limbs, fingers, and toes green, interspersed with irregular black spots (vs. dorsum and flank with nearly round green or yellowish green spots, scattered with small green spots); from *A. kaulbacki* by SVL 46.3–51.8 mm in males and 51.5 mm in female (vs. SVL 72.6–82.6 mm in males and 82.7–87.2 mm in females), discontinuous glandular dorsolateral fold (vs. absent), supratympanic fold indistinct (vs. distinct), iris distinctly bicolored, golden-yellow in upper one-fourth and reddish brown in lower three-fourths, black reticulations throughout (vs. eyes brownish black with scattered yellow spotting and yellow ring around iris); from *A. beibengensis* by SVL 46.3–51.8 mm in males and 51.5 mm in female (vs. SVL 75.8 mm males and 90.2–93.2 mm in females), supratympanic fold indistinct (vs. distinct, wide and thick), discontinuous glandular dorsolateral fold from rear of eye to near vent (vs. absent); from *A. wangyufani* by SVL 46.3–51.8 mm in males and 51.5 mm in female (vs. SVL 68.3–69.0 mm males and 83.4 mm in female), vomerine teeth developed, on two short oblique between choanae, equal in distance from each other as to choanae (vs. vomerine teeth developed, the two rows are almost in touch), discontinuous glandular dorsolateral fold from rear of eye to near vent (vs. absent); from *A. medogensis* by SVL 46.3–51.8 mm in males and 51.5 mm in female (vs. SVL 95.0 mm male and 72.4–96.9 mm in females), tibiotarsal articulation of adpressed limb reaching between nostrils and eyes (vs. beyond tip of snout), supratympanic fold indistinct (vs. distinct, wide and thick); from *A. himalayanus* by dorsal surface of the head, back, limbs, fingers, and toes green, interspersed with irregular black spots (vs. dark brown, interspersed with irregular yellow spots), vocal sac absent in males (vs. externally visible vocal sacs present); from *A. pallasitatus* by SVL 51.5 mm in female (vs. SVL 70.6–72.3 mm in females), discontinuous glandular dorsolateral fold from rear of eye to near vent (vs. absent), dorsal surface of the head, back, limbs, fingers, and toes green, interspersed with irregular black spots (vs. dorsum yellow-green, with irregular dark brown blotches without margins); from *A. wangyali* by SVL 46.3–51.8 mm in males and 51.5 mm in female (vs. SVL 71.4–76.7 mm males and 80.5–89.6 mm in females), dorsal surface of the head, back, limbs, fingers, and toes green, interspersed with irregular black spots (vs. large brown irregularly shaped blotches on dorsum of head and body), rictal gland absent (vs. a distinct patch of rictal glands at rear of jaw on either side); from *A. nidorbellus* by SVL 46.3–51.8 mm in males and 51.5 mm in female (vs. SVL 76.4–82.3 mm males and 85.4–98.0 mm in females), dorsum green, interspersed with irregular black spots (vs. dorsally brown with small irregularly arranged cobalt green spots), discontinuous glandular dorsolateral fold (vs. absent); from *A. longimanus* by SVL 46.3–51.8 mm in males and 51.5 mm in female (vs. SVL 30 mm), nostrils closer to anterior corner of eye than to tip of snout (vs. nostrils a litter nearer to tip of snout than the eye), above tympanum to the forelimb a thick glandular parotoid-like swelling absent (vs. present), eye diameter (ED/SL 63.0%) shorter than snout length (vs. snout about as long as the eye diameter, ED/SL 97.7%); from *A. formosus* by dorsal parts of limbs, fingers and toes with black crossbars (vs. legs and toes with black white-dotted crossbars), inner metatarsal tubercle long, outer metatarsal tubercle absent (vs. metatarsal tubercle indistinct), dorsum green, interspersed with irregular black spots (vs. upper parts green, marbled with black, the black spots enclosing a number of small whitish dots); from *A. chanakya* by SVL 46.3–51.8 mm in males (vs. SVL 76.4 mm), tympanum indistinct (vs. tympanum distinct, about 40% of eye length), absence of vocal sacs in males (vs. vocal sac externally visible), dorsal color green, interspersed with irregular black spots (vs. dorsal color dull brick-red, spotted with irregular cocoa-brown spots, these cocoa-brown spots enclosing a number of smaller dull brick-red spots); from *A. tawang* by SVL 46.3–51.8 mm in males (vs. SVL 82.5 mm), tibiotarsal articulation of adpressed limb reaching between nostrils and eyes (vs. reaching up to snout), dorsal color green, interspersed with irregular black spots (vs. dorsal color olivegreen, spotted with large, irregular shaped dark-brown spots, brown spots enclosing a number of small olivegreen dots).

Amolops yangi **sp. nov.** is significantly different from *A. chayuensis* by absence of vocal sacs in males (vs. pair of external subgular vocal sacs); discontinuous glandular dorsolateral fold from rear of eye to near vent (vs. dorsolateral fold prominent); upper part of flanks green, lower part of flanks green-yellow (vs. upper part of flanks brown, lower part of flanks light green or white with dark

brown blotches); from *A. bellulus* by tympanum indistinct (vs. tympanum distinct), iris distinctly bicolored, golden-yellow in upper one-fourth and reddish brown in lower three-fourths, black reticulations throughout (vs. upper half of iris golden yellow with some irregular brown spots, lower half dark brown); from *A. putaoensis* by SVL of adult male 46.3–51.8 mm (vs. 37.6–40.2 mm), rictal gland absent (vs. two rictal glands present), iris distinctly bicolored, golden-yellow in upper one-fourth and reddish brown in lower three-fourths, black reticulations throughout (vs. upper one-fourth of iris bronze with black reticulations, lower three-fourths dark), absence of vocal sacs in males (vs. pair of internal subgular vocal sacs present); from *A. binchachaensis* by SVL of adult female 51.5 mm (vs. 65.0 mm), tympanum indistinct, relatively small, about one third of eye diameter (vs. tympanum big), supratympanic fold indistinct (vs. supratympanic fold absent), dorsal surface of the head, back, limbs, fingers, and toes green (vs. light yellow); from *A. deng* by SVL of adult female 51.5 mm (vs. 68.5–72.0 mm), tympanum indistinct, relatively small, about one third of eye diameter (vs. tympanum distinct, slightly less than half of eye diameter), tibiotarsal articulation of adpressed limb reaching between nostrils and eyes (vs. tibiotarsal articulation of adpressed limb reaching beyond tip of snout); from *A. jinjiangensis* by supratympanic fold indistinct (vs. distinct), dorsal surface of head, body, limbs, fingers, toes and flank of body relatively smooth (vs. rough with tubercles), the absence of a pair of large tubercles on sides of cloaca (vs. present); from *A. tuberodepressus* by supratympanic fold indistinct (vs. present, wide), flanks smooth (vs. with flatter tubercles), relative length of fingers I<II<IV<III (vs. II<IV<I<III), vomerine teeth developed (vs. weak); from *A. ailao* by SVL 46.3–51.8 mm in males (vs. SVL 33.0–35.1 mm in males), vomerine teeth developed (vs. absent), tibiotarsal articulation of adpressed limb reaching between nostrils and eyes (vs. beyond anterior corner of eye), iris distinctly bicolored, golden-yellow in upper one-fourth and reddish brown in lower three-fourths (vs. iris light brown with dark wash); from *A. marmoratus* by tibiotarsal articulation of adpressed limb reaching between nostrils and eyes (vs. beyond tip of snout), discontinuous glandular dorsolateral fold from rear of eye to near vent (vs. distinct dorsolateral fold absent), rictal gland absent (vs. multiple small globular rictal glands on right side, single rictal gland on left side, just posterior to jaw); from *A. afghanus* by SVL 51.5 mm in female (vs. SVL 67.7–94.1 mm in females), rictal gland absent (vs. indistinct rictal glands present on one side of head at posterior end of jaw), absence of vocal sacs in males (vs. males with dual gular pouches).

Amolops vitreus (Bain, Stuart & Orlov, 2006)

Common name. vitreous cascade frog, glass torrent frog

Type locality. collected on a stream bank near Nam Khang River in hilly evergreen forest, Phou Dendin National Biodiversity Conservation Area, Phongsaly District, Phongsaly Province, Laos.

Figure 6. *Amolops vitreus* (KIZ 050452). **A** Lateral view, **B** dorsal view, **C** ventral view of hand, **D** foot. Photos by Shao-Bing Hou.

Conservation status. IUCN: VU.

Type specimens of *A. vitreus*. Holotype: FMNH 258182, based on original designation.

Specimen examined. Adult male (KIZ 050452) collected on 01, August, 2022 by Yun-He Wu, Shao-Bin Hou, and Zhong-Xiong Fu from Xishuangbanna National Nature Reserve, Mengla, Yunnan Province, China (21.73742°N, 101.53935°E, elevation 899 m a.s.l.).

Chinese name. Based on the type locality, we suggest the Chinese formal name as "丰沙里湍蛙".

Morphological description (measurements in mm; provided in Table 1). Morphological characters of the specimen from China agreed well with the original description of Bain et al. (2006). Adult male with SVL 38.9 mm; head length (HL 15.4 mm, 39.6% of SVL) longer than width (HW 13.9 mm, 35.7% of SVL); snout obtusely pointed in dorsal view, projecting beyond lower jaw, round in profile, its length longer than horizontal diameter of eye (ED/SL 67.7%); canthus rostralis rounded, loreal region slightly concave, oblique; interorbital space slightly larger than width of upper eyelid (UEW/IOS 92.7%) and internarial distance (INS/IOS 112.2%); tympanum distinct (TD 2.7 mm), rounded, more than half eye diameter (ED 4.4 mm); vomerine teeth developed; tongue cordiform, deeply notched posteriorly; pupil horizontal (Fig. 6A); external subgular vocal sacs present, vocal sac opening on floor of mouth at each corner.

Forelimbs slender; length of lower arm and hand (LAHL 19.6 mm, 50.4% of SVL), about half SVL; relative finger lengths: I<II<IV<III; tips of all fingers expanded into discs with circummarginal grooves; webbing between fingers absent; subarticular tubercles distinct, formula 1, 1, 2, 2; two metacarpal tubercles; velvety nuptial pad on first finger (Fig. 6C).

Hindlimbs long, tibia (TIB 23.5 mm) more than half SVL, longer thigh length (22.2 mm) and foot length (FTL 20.3 mm); tibiotarsal articulation beyond the snout when the leg is stretched forward; relative length of toes: I<II<III<V<IV; heels overlapping when thighs are positioned at right angles to the body; tips of all toes expanded into discs with circummarginal grooves; fully webbing between toes; subarticular tubercles prominent and rounded, formula 1, 1, 2, 3, 2; inner metatarsal tubercle distinct and oval, outer metatarsal tubercle absent (Fig. 6D).

Dorsal skin and ventral surfaces of head, body, limbs, and flanks relatively smooth, with exception of small tubercles posterior surface of thigh; supratympanic fold absent; dorsolateral fold distinct, from posterior corner of upper eyelid to near vent; two rictal glands present; humeral gland absent (Fig. 6).

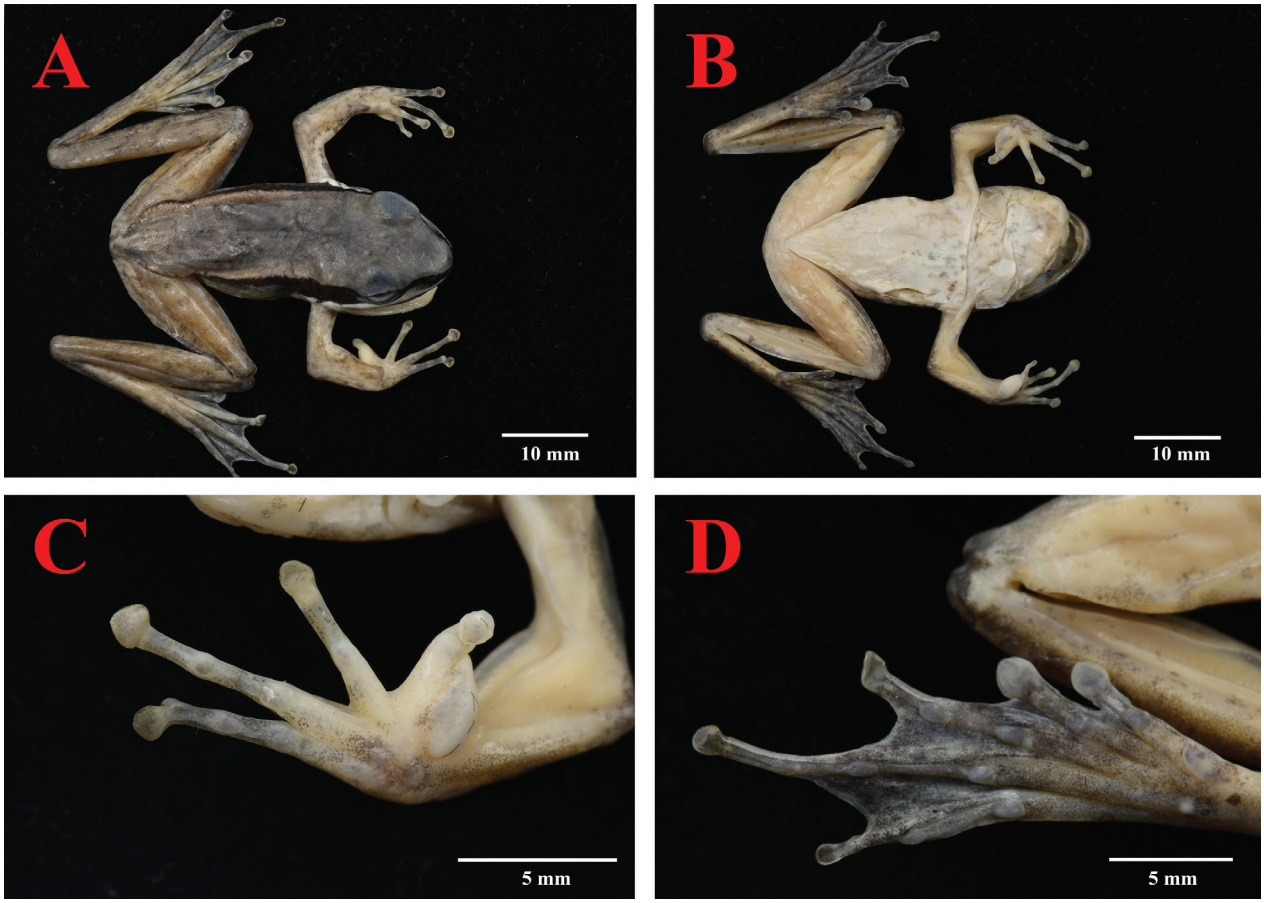

Figure 7. *Amolops vitreus* (KIZ 050452) in preservative. **A** Dorsal view, **B** ventral view, **C** ventral view of hand, **D** ventral view of foot. Photos by Zhong-Bin Yu.

Color in in life. Dorsal surface brown, with some dark brown spots; upper lip stripe white, extending from tip of snout to posterior of arm insertion; narrow, reddish brown stripe on edge of canthus from tip of snout among margin of upper eyelid, continuing along upper edge of dorsolateral fold; dorsal surface of limbs light brown with dark brown crossbars, interspersed with small dark brown spots; tympanic region dark brown; throat, chest and anterior part of belly light cream; flank dark brown upper one-third, green lower two-third; expanded finger tips reddish, except for first finger tips yellow; subarticular tubercles on toes, expanded toe tips, and inner metatarsal tubercle dark brown; iris distinctly bicolored, silvery-white in upper one-fourth and reddish brown in lower three-fourths, black reticulations throughout (Fig. 6).

Color in preservative. After one year of storage in ethanol, dorsal surface fading to grayish brown; black crossbars present on dorsal surfaces of limbs, fingers and toes becoming indistinct; dorsolateral fold yellowish; throat, chest, and abdomen cream-white; ventral surface of limbs light yellow; ventral surface of the hands cream-white; digit tips, subarticular tubercles of fingers, metacarpal tubercles, and nuptial pad fading to cream-yellow or grayish-white; toe webbing greyish brown with dark gray flecking; ventral surface of the toes greyish brown, digit tips, subarticular tubercles of toes and inner metatarsal tubercle fading to greyish brown (Fig. 7).

Ecological notes. *Amolops vitreus* was found in rocky, fast-flowing streams at night (20:30–23:30 h) on 01 August 2022, surrounded by evergreen broad-leaved forest. Other frog species observed along the stream included *Leptobrachella eos*, *Polypedates megacephalus*, and *Xenophrys* sp.

Distribution. This study further extends the geographical range of *A. vitreus* to approximately 82.6 km from the nearest known locality of the species. *Amolops vitreus* is currently known in Phou Dendin National Biodiversity Conservation Area, Phongsaly Province, Laos; Muong Nhe Nature Reserve, Dien Bien Province, northwestern Vietnam; and Xishuangbanna National Nature Reserve, Mengla, Yunnan province, China.

Discussion

Currently, there are 22 recognized species of *Amolops* known to exist in Yunnan Province (AmphibiaChina 2023). The new species and new species record described in this study brings the total number of *Amolops* species from Yunnan to 24. Our study provides more evidence that the amphibian diversity has been largely underestimated along the border regions of southern China (e.g., Yuan et al. 2019; Chen et al. 2020; Wu et al. 2020, 2021, 2023a,b; Zhang et al. 2022). It further unveils the taxonomy of some species of the genus *Amolops* such as *A. mengyangensis* which have long been controversial (Wu et al. 2020). Future intensified surveys and international collaboration along these international borders combined with molecular and acoustic data, will likely uncover more new amphibia species and new country records. In addition, integrating a more detailed morphological investigation as well as assimilation of multi-loci nuclear genomic markers to resolve taxonomic disputes will speed up species discovery. This study increases the total number of *Amolops* species to 85, and the number of *Amolops* species known from China to 53.

The Gaoligong Mountains consist of a long, narrow, mountain range running from north to south in the western part of China's Yunnan Province adjoining northern Myanmar. It spans a 5° latitude with a large elevation range of 210 m to 5000 m (Chaplin 2005). It has been shown to have an isolating effect on many *Amolops* species. For instance, *A. kaulbacki* and *A. putaoensis* are only known to be distributed on the western slope of the Gaoligong Mountains based on our field work and related researches (Frost 2023). Currently, *A. kaulbacki* is distributed in Kachin, Northern Myanmar and Pianma, Yunnan, China, and *A. putaoensis* in Kachin State, Myanmar and upper Dulong River, Yunnan, China. In addition, Wu et al. (2020) indicated that *A. bellulus* was paraphyletic, forming two distinct monophyletic clusters, one of which is distributed on the western slope of Gaoligong Mountains, and the other clade is distributed on the eastern slope of Gaoligong Mountains. Since the type locality of *A. bellulus* is on the western slope (Liu et al. 2000), the clade from the eastern slope is speculated to be a potential new species. A similar situation was also found in *A. yangi* **sp. nov.** and *A. longimanus*. *Amolops yangi* is distributed on the western slope of Gaoligong Mountains, while *A. longimanus* is distributed on the eastern slope of Gaoligong Mountains. The Gaoligong Mountains present a large climate difference in the eastern slope and the western slope due to the influence of the Indian Ocean monsoon climate, and the rainfall on the western slope which is significantly greater than that on the eastern slope (Li and Li 2020). Therefore, this distribution pattern may be due to the unique topographic and climatic characteristics of the Gaoligong Mountains. Future studies on comparative phylogeography of these species can be carried out to explore the formation mechanism.

Acknowledgments

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Supplementary Material 1

Tables S1–S5

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Data type: .zip

Explanation notes: Table S1. Localities, voucher information, and Genbank accession numbers for all specimens used in this study. — **Table S2.** Best-fitting models and partitions selected by JMODELTEST v2.1.7 for phylogeny analysis. — **Table S3.** Morphological data from *Amolops yangi* sp. nov. and other species of *A. viridimaculatus* group used in the PCA analyses. — **Table S4.** Uncorrected *p*-distance (percentage) of *Amolops* species included in phylogenetic analyses and standard error estimates. — **Table S5.** Summary statistics and principal component analysis scores for the mensural characters of *Amolops yangi* sp. nov. and other species of *A. viridimaculatus* group.

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