

Article

Two Additional New Species of *Feihyla* Sensu Stricto (Anura, Rhacophoridae) from Yunnan Province, China [†]

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urn:lsid:zoobank.org:act:1CE52C75-EE6E-4A2C-B09D-C559DB33D0F2.

Abstract

Two new species of *Feihyla* sensu stricto are described based on specimens from western and southwestern Yunnan Province, China. Morphologically, the first new species from western Yunnan can be distinguished from other known species of *Feihyla* sensu stricto by the body size, the ratios of the distance between the snout tip and the nostril to the distance between the nostril and the eye, the internasal distance to the interorbital distance, the interorbital distance to the eye diameter, and whether the nuptial pad presents on the second finger in adult males. The second new species from southwestern Yunnan can be distinguished from other known species of *Feihyla* sensu stricto by the body size, the ratios of the distance between the snout tip and the nostril to the distance between the nostril and the eye, the internasal distance to the interorbital distance, the interorbital distance to the eye diameter, and the hindlimb length to the snout–vent length. The two new species can be distinguished from each other by the body size, the ratios of the distance between the snout tip and the nostril to the distance between the nostril and the eye, the internasal distance to the interorbital distance, and whether the nuptial pad presents on the second finger in adult males. In the phylogenetic analysis, the two new species formed two distinct clades in *Feihyla* sensu stricto and diverged from each other and from other species of *Feihyla* sensu stricto by 2.7% to 14.2% in the 16S ribosomal RNA gene.

Keywords: 16S rRNA; Changning; Menghai; morphology; phylogeny; taxonomy

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1. Introduction

Feihyla sensu lato currently contains 17 species, widely distributed from northeastern India, Myanmar, southern China, Laos, Thailand, Vietnam, Cambodia, to the Andaman Islands and Malaysia and Indonesia, whereas *Feihyla* sensu stricto contains four species only distributed in southern China and Vietnam, namely *F. fuhua* Fei, Ye & Jiang, 2010, *F. palpebralis* (Smith, 1924), *F. wenshanensis* Liu, Bu, Feng, Zhang, Yang, Ma, Chen, Chen, He, Zhong & Rao, 2026, and *F. zhaotongensis* Liu, Bu, Feng, Zhang, Yang, Ma, Chen, Chen, He, Zhong & Rao, 2026 [1–3].

Previously, *Feihyla palpebralis* was recorded to be distributed in China [4–6]. Later, Fei et al. [7] considered that the populations previously regarded as *F. palpebralis* from China do not belong to *F. palpebralis* and described *F. fuhua* based on specimens collected from Pingbian County, Yunnan Province, China. At this point, whether *F. palpebralis* is distributed in China became uncertain. The database of Chinese amphibians lists the species *F. palpebralis*, but marks its distribution in China as questionable [1]. Biju et al. [8] considered that *F. palpebralis* only occurs in southern Vietnam and the populations previously regarded as *F. palpebralis* from China are all likely to refer to *F. fuhua*. Liu et al. [3] restricted the range of *F. fuhua* to only southern Honghe Prefecture of Yunnan Province and described the populations from southeastern and northeastern Yunnan as *F. wenshanensis* and *F. zhaotongensis*, respectively. Unfortunately, due to the lack of specimens, Liu et al. [3] were unable to determine the taxonomic status of the populations of *Feihyla* sensu stricto from western Yunnan.

During our recent field surveys in Yunnan Province, China, some specimens which closely resemble *Feihyla fuhua* were collected from western and southwestern Yunnan. Morphological comparison cannot assign these specimens to any known species and analysis of molecular data revealed these specimens as two novel lineages in *Feihyla* sensu stricto. Herein, we describe these specimens as two new species.

2. Materials and Methods

The specimen collection was performed in accordance with the Wild Animals Protection Law of the People's Republic of China. The new specimens were collected by hand, anaesthetized, and euthanized in a closed vessel with a piece of cotton wool containing ethyl acetate. Liver tissue samples were preserved in analytical pure ethanol and specimens were preserved in approximately 70% ethanol solution and deposited in Kunming Natural History Museum of Zoology, Kunming Institute of Zoology, Chinese Academy of Sciences (KIZ).

Genomic DNA was extracted from liver tissues. A partial fragment of the mitochondrial 16S ribosomal RNA gene (16S) was amplified and sequenced using the primers L2188 [9] and 16H1 [10]. The amplification conditions of polymerase chain reaction (PCR) were the same as those in Tang et al. [11]: an initial denaturing step at 95 °C for 4 min and 35 cycles of denaturing at 94 °C for 60 s, annealing at 51 °C for 60 s, extending at 72 °C for 60 s, and a final extending step of 72 °C for 10 min. The molecular experiment was completed by Sangon Biotech (Shanghai, China) Co., Ltd. *Ghatixalus variabilis* (Jerdon, 1853) and *Tamixalus calcadensis* (Ahl, 1927) were used as outgroups according to Dubois et al. [12]. The new sequences have been deposited on GenBank. Available genetic data of 13 species of *Feihyla* sensu lato and the two outgroups were obtained from GenBank (Table 1).

Table 1. The specimen voucher numbers, localities, and GenBank accession numbers of the sequences used in the phylogenetic analysis of this study.

Species	Voucher	Locality	Accession	Reference
<i>Feihyla changningensis</i> sp. nov.	KIZ2026091	Changning, Yunnan, China	QB149279	This study
<i>Feihyla changningensis</i> sp. nov.	KIZ2026092	Changning, Yunnan, China	QB149280	This study
<i>Feihyla changningensis</i> sp. nov.	KIZ2026093	Changning, Yunnan, China	QB149281	This study
<i>Feihyla changningensis</i> sp. nov.	KIZ2026094	Changning, Yunnan, China	QB149282	This study
<i>Feihyla changningensis</i> sp. nov.	KIZ2026095	Changning, Yunnan, China	QB149283	This study
<i>Feihyla changningensis</i> sp. nov.	KIZ2026096	Changning, Yunnan, China	QB149284	This study
<i>Feihyla changningensis</i> sp. nov.	KIZ2026097	Changning, Yunnan, China	QB149285	This study
<i>Feihyla changningensis</i> sp. nov.	KIZ2026098	Changning, Yunnan, China	QB149286	This study
<i>Feihyla menghaiensis</i> sp. nov.	KIZ2026099	Menghai, Yunnan, China	QB149287	This study
<i>Feihyla menghaiensis</i> sp. nov.	KIZ2026100	Menghai, Yunnan, China	QB149288	This study
<i>Feihyla menghaiensis</i> sp. nov.	KIZ2026101	Menghai, Yunnan, China	QB149289	This study
<i>Feihyla menghaiensis</i> sp. nov.	KIZ2026102	Menghai, Yunnan, China	QB149290	This study
<i>Feihyla menghaiensis</i> sp. nov.	KIZ2026103	Menghai, Yunnan, China	QB149291	This study
<i>Feihyla menghaiensis</i> sp. nov.	KIZ2026104	Menghai, Yunnan, China	QB149292	This study
<i>Feihyla menghaiensis</i> sp. nov.	KIZ2026105	Menghai, Yunnan, China	QB149293	This study
<i>Feihyla menghaiensis</i> sp. nov.	KIZ2026106	Menghai, Yunnan, China	QB149294	This study
<i>Feihyla menghaiensis</i> sp. nov.	KIZ2026107	Menghai, Yunnan, China	QB149295	This study
<i>Feihyla baladika</i>	MZBAmpH23920	Tapanuli, North Sumatra, Indonesia	MW054232	Biju et al. [8]
<i>Feihyla baladika</i>	MZBAmpH23951	Tapanuli, North Sumatra, Indonesia	MW054233	Biju et al. [8]
<i>Feihyla fuhua</i>	KIZ040270	Pingbian, Yunnan, China	QB149296	This study
<i>Feihyla fuhua</i>	KIZ040271	Pingbian, Yunnan, China	QB149297	This study
<i>Feihyla fuhua</i>	KIZ040272	Pingbian, Yunnan, China	QB149298	This study
<i>Feihyla fuhua</i>	KIZ040273	Pingbian, Yunnan, China	QB149299	This study
<i>Feihyla fuhua</i>	KIZ040274	Pingbian, Yunnan, China	QB149300	This study
<i>Feihyla fuhua</i>	SCUM0606132L	Dawei Mountain, Yunnan, China	EU215546	Li et al. [13]
<i>Feihyla fuhua</i>	NMNS3104	/	AF458130	Wilkinson et al. [14]
<i>Feihyla hanseneae</i>	ZMKUAm00782	Ban Ta Khun, Surat Thani, Thailand	KC357628	Aowphol et al. [15]
<i>Feihyla hanseneae</i>	ZMKUAm00781	Ban Ta Khun, Surat Thani, Thailand	KC357625	Aowphol et al. [15]
<i>Feihyla inexpectata</i>	BORNENSIS22421	Sandakan, Sabah, Malaysia	AB813160	Matsui et al. [16]
<i>Feihyla inexpectata</i>	ZMHA12647	Sandakan, Sabah, Malaysia	MG909569	Haas et al. [17]
<i>Feihyla kajau</i>	NMBE1056500	Sarawak, Borneo, Malaysia	JN377362	Unpublished
<i>Feihyla kajau</i>	FMNH269090	Sarawak, Borneo, Malaysia	KC465789	Li et al. [18]
<i>Feihyla palpebralis</i>	KIZ712	Lam Dong, Vietnam	GQ285681	Li et al. [19]
<i>Feihyla senapatiensis</i>	SDBDU41657	Manipur, India	MW054216	Biju et al. [8]
<i>Feihyla senapatiensis</i>	SDBDU41659	Nagaland, India	MW054218	Biju et al. [8]
<i>Feihyla shyamrupus</i>	SDBDU41662	Manipur, India	MW054221	Biju et al. [8]
<i>Feihyla shyamrupus</i>	SDBDU41665	Nagaland, India	MW054224	Biju et al. [8]
<i>Feihyla vittata</i>	CASHERP241274	Myitkyina, Kachin, Myanmar	KC692881	Aowphol et al. [15]
<i>Feihyla vittata</i>	CASHERP241113	Myitkyina, Kachin, Myanmar	KC692882	Aowphol et al. [15]
<i>Feihyla vittiger</i>	MZBAmpH27238	Telaga Warna, Sukabumi, Indonesia	MW054231	Biju et al. [8]
<i>Feihyla wenshanensis</i>	KIZ2015119	Xichou, Yunnan, China	PX934992	Liu et al. [3]
<i>Feihyla wenshanensis</i>	KIZ2015120	Xichou, Yunnan, China	PX934993	Liu et al. [3]
<i>Feihyla wenshanensis</i>	KIZ2015121	Xichou, Yunnan, China	PX934994	Liu et al. [3]
<i>Feihyla wenshanensis</i>	KIZ2015122	Xichou, Yunnan, China	PX934995	Liu et al. [3]
<i>Feihyla wenshanensis</i>	KIZ2015123	Xichou, Yunnan, China	PX934996	Liu et al. [3]
<i>Feihyla wuguanfui</i>	SWU001964	Cangyuan, Yunnan, China	OR500735	Liu et al. [20]
<i>Feihyla wuguanfui</i>	SWU001965	Cangyuan, Yunnan, China	OR500736	Liu et al. [20]
<i>Feihyla zhaotongensis</i>	KIZ2015149	Yiliang, Yunnan, China	PX934997	Liu et al. [3]
<i>Feihyla zhaotongensis</i>	KIZ2015150	Yiliang, Yunnan, China	PX934998	Liu et al. [3]
<i>Ghatixalus variabilis</i>	SDBDU2008.4409	Nilgiris, Tamil Nadu, India	KU169981	Biju et al. [21]
<i>Tamixalus calcadensis</i>	SDBDU2010.257	Palaghat, Kerala, India	KC571274	Biju et al. [22]

DNA sequences were aligned using ClustalW [23] integrated in MEGA 11.0.11 [24] with default parameters. The best substitution model GTR + F + I + G4 was selected in ModelFinder [25] using Akaike information criterion [26]. A Maximum likelihood phylogenetic tree was constructed using IQ-TREE 1.6.12 [27] with bootstrap support evaluated by 1000 ultrafast bootstrap replicates. Bayesian phylogenetic construction was done in MrBayes version 3.2.6 [28], two independent runs of four Markov Chains for 1,000,000 generations were sampled every 100 generations and a consensus topology was calculated for 7500 trees after discarding the first 2500 trees using the relative burn-in option. The genetic distances (uncorrected pairwise distances) between and within species were calculated in MEGA 11.0.11 with the pairwise deletion option for handling alignment gaps and missing data.

Measurements were taken by S.L. with digital calipers to the nearest 0.1 mm. Symmetrical values were measured on the left side. The morphological characteristics and abbreviations followed Fei et al. [5]. The webbing formula followed Myers and Duellman [29]. We measured snout–vent length (SVL), from snout tip to vent; head length (HL), from snout tip to rear of mandible; head width (HW), at widest point of head; snout length (SL), from snout tip to anterior margin of eye; snout–nostril distance (SN), from snout tip to center of nostril; nostril–eye distance (NE), from center of nostril to anterior margin of eye; internasal distance (INS), between centers of nostril; interorbital distance (IOS), minimum distance between upper eyelids; upper eyelid width (UEW), maximum width of upper eyelid; eye diameter (ED), diameter of exposed eyeball; lower arm and hand length (LAHL), from elbow to tip of third finger; hand length (HAL), from wrist to tip of third finger; hindlimb length (HLL), from vent to tip of fourth toe; tibia length (TL), from knee to tibiotarsal articulation; tarsus and foot length (TFL), from tibiotarsal articulation to tip of fourth toe; and foot length (FL), from proximal end of inner metatarsal tubercle to tip of fourth toe.

We measured 17 newly collected specimens from western and southwestern Yunnan, the type specimens of *Feihyla wenshanensis* and *F. zhaotongensis*, and four male topotypic specimens of *F. fuhua*. Detailed measurement data can be found in the following tables and Table S1. We conducted principal component analysis (PCA) to test whether there are morphological differences between the two potential new species and its closely related species and each other. Raw measurements were used in the analysis, including SVL, HL, HW, SL, SN, NE, INS, IOS, UEW, ED, LAHL, HAL, HLL, TL, TFL, and FL, and no transformation or sample size correction and covariance or correlation matrix were used. Due to the limited sample size of females, only data of males were analyzed. The PCA was performed using the `prcomp` command in R 4.2.2, and the scatter plot was plotted using the `ggplot2` package in R 4.2.2 [30].

3. Results

3.1. Phylogenetic Relationship

The obtained sequence alignment is 945 bp in length, of which 603 sites were conserved and 336 sites were variable, including 303 parsimony-informative sites. Bayesian inference and maximum likelihood analysis yielded similar results. The newly collected specimens were recovered as two distinct lineages in *Feihyla* sensu stricto; however, the phylogenetic relationships among the two novel lineages and *F. fuhua* and *F. zhaotongensis* were unresolved due to the quite low nodal support rates (Figure 1). The uncorrected pairwise distances between the two novel clades and other species of *Feihyla* sensu stricto ranged from 2.7% to 14.2%, and the uncorrected pairwise distance between the two novel clades was 3.2% (Table 2).

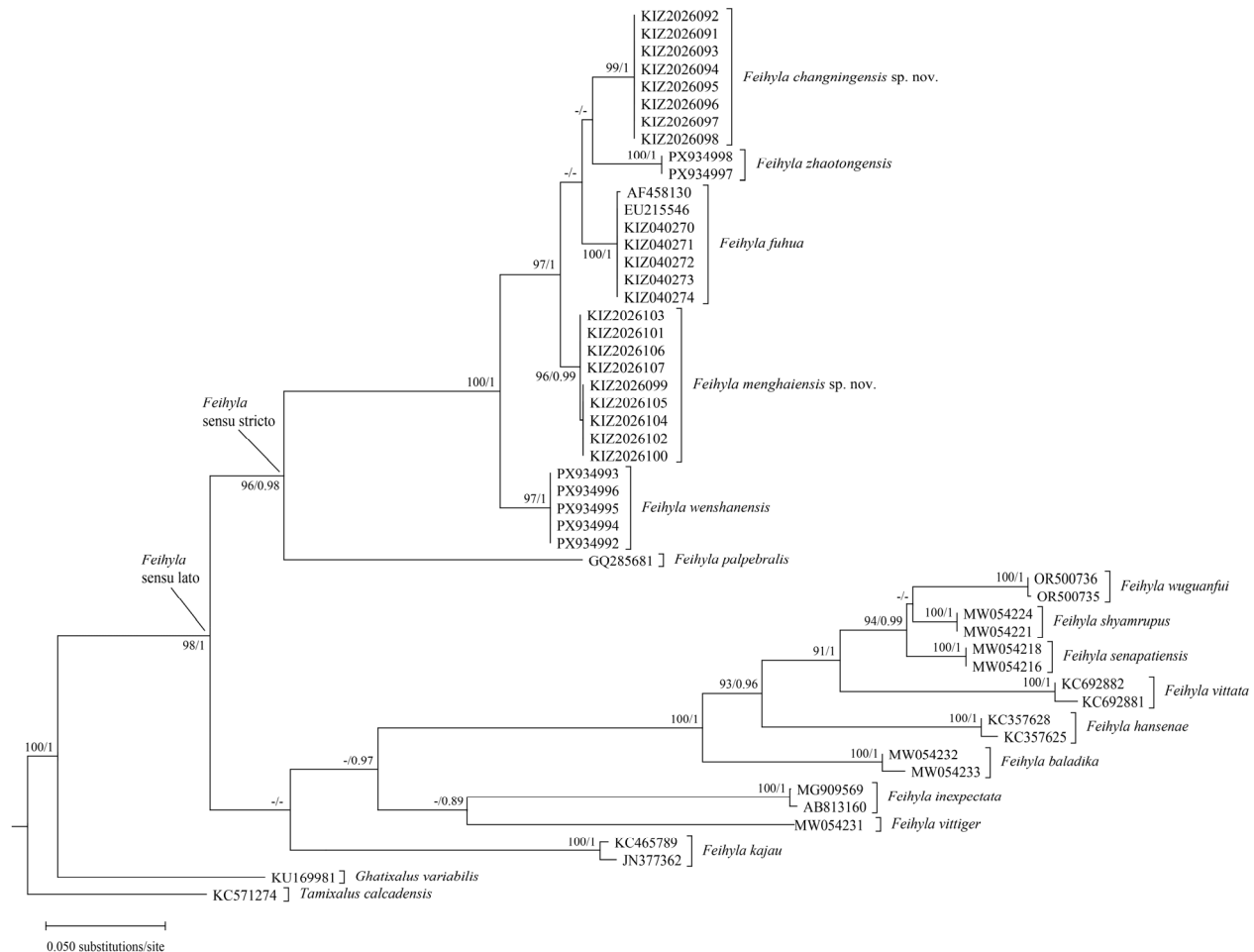


Figure 1. Maximum likelihood phylogram of the genus *Feihyla* inferred from 16S sequences. Node values are ultrafast bootstrap support for maximum likelihood analysis/Bayesian posterior probabilities, “-” indicates a value below 80/0.80.

Table 2. The interspecific and intraspecific mean uncorrected pairwise distances (%) based on 16S sequences.

Species	1	2	3	4	5	6
1 <i>Feihyla changningensis</i> sp. nov.	0					
2 <i>Feihyla menghaiensis</i> sp. nov.	3.2	0.1				
3 <i>Feihyla fuhua</i>	3.1	2.7	0			
4 <i>Feihyla palpebralis</i>	13.5	14.2	13.9	0		
5 <i>Feihyla wenshanensis</i>	5.3	4.6	5.5	12.4	0	
6 <i>Feihyla zhaotongensis</i>	4.1	4.1	3.8	14.0	5.6	0

3.2. Morphological Analysis

The first two major principal components from PCA were retained, which accounted for 83.90% of the total variances with the loadings for PC1 and PC2 accounted for 75.74% and 8.16%, respectively. The scatterplot based on the first two major principal components showed separations among the two potential new species and the three known species of *Feihyla sensu stricto* distributed in Yunnan (Figure 2).

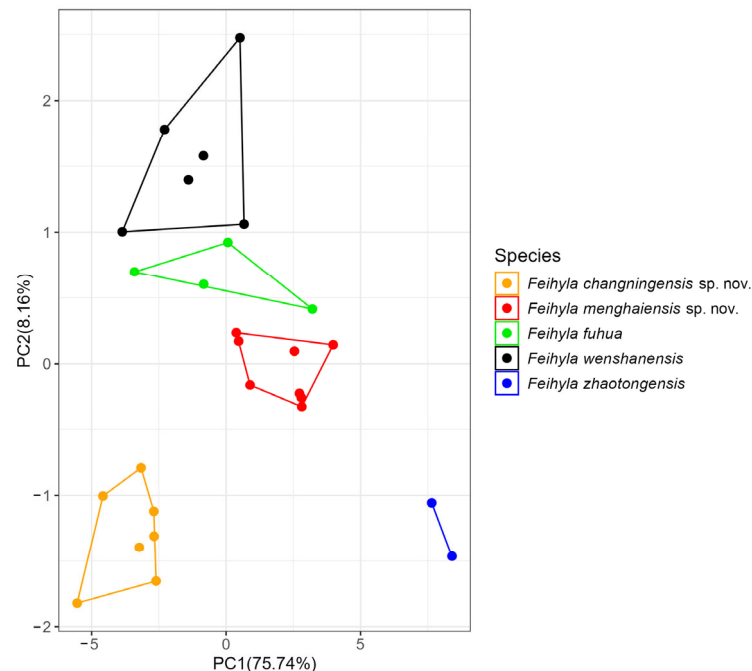


Figure 2. Scatterplot of the first two major principal components of morphometric data for males of *Feihyla changningensis* sp. nov., *Feihyla menghaiensis* sp. nov., *F. fuhua*, *F. wenshanensis*, and *F. zhaotongensis*.

3.3. Taxonomic Account

Feihyla changningensis sp. nov.

urn:lsid:zoobank.org:act:67F07545-2036-4FC1-BC56-7C847AAEC5F3

Holotype. KIZ2026096, adult male, collected on 4 August 2026 by Shuo Liu from Xincheng Village, Tianyuan Town, Changning County, Baoshan City, Yunnan Province, China (24°53'51" N, 99°36'02" E, 2200 m, WGS84).

Paratypes. KIZ2026091–KIZ2026095 and KIZ2026097, six adult males, KIZ2026098, adult female, all the same collection information as the holotype.

Diagnosis. Body size small, SVL 24.6–26.9 mm in adult males and 32.5 mm in single adult female; nostril closer to snout tip than to eye; internasal distance approximately equal to interorbital distance; tibiotarsal articulation reaching center of eye when hindlimbs pressed forward; inner metatarsal tubercle length approximately one fourth of first toe length; a dark transverse stripe between eyes, a curved dark stripe from behind eye to crotch on each side of dorsum, a discontinuous dark stripe on midline of posterior dorsum; nuptial pad present on base of first finger in adult males.

Description of holotype. Adult male, SVL 26.5 mm; head length greater than head width (HL/HW 1.13); snout relatively sharp, longer than eye diameter (SL/ED 1.29); canthus rostralis distinct; interorbital region flat; pineal ocellus absent; internasal distance equal to interorbital distance; interorbital distance greater than upper eyelid width (IOS/UEW 1.13) and smaller than eye diameter (IOS/ED 0.87); nostril slightly closer to snout tip than to eye (SN/NE 0.86); loreal region slightly concave; tympanum indistinct; supratympanic fold distinct, narrow; vomerine teeth absent; tongue elongated triangle, narrow anteriorly and wide posteriorly, notched posteriorly; choanae small, round; single subgular vocal sac, vocal sac slit opening at bottom of mouth on each side.

Forelimbs slender, moderately long; relative lengths of fingers I < II < IV < III; tips of fingers dilated into discs; subarticular tubercles formula 1, 1, 2, 2, ones on first and second fingers and distal ones on third and fourth fingers large and distinct, proximal ones on

third and fourth fingers small and indistinct; supernumerary tubercles indistinct; webbing between fingers absent; inner metacarpal (thenar) tubercle distinct, no distinct outer metacarpal tubercle; nuptial pad yellowish white, composed of tiny granules, present on base of first finger.

Hindlimbs slender, moderately long; tibiotarsal articulation reaching center of eye when hindlimbs pressed forward; relative length of toes $I < II < III < V < IV$; tips of toes dilated into discs, discs on toes smaller than on fingers; subarticular tubercles formula 1, 1, 2, 3, 2, all small but distinct; supernumerary tubercle absent; webbing between toes undeveloped, formula $II-2\frac{1}{3}III-3\frac{1}{3}IV-3\frac{1}{3}V$; inner metatarsal tubercle oval, relatively short, length approximately one fourth of length of first toe, outer metatarsal tubercle absent (Figure 3).



Figure 3. The holotype (KIZ2026096) of *Feihyla changningensis* sp. nov. in preservative. (A) Dorsal view; (B) ventral view; (C) close-up view of the left volar; (D) close-up view of the left plantar.

Dorsal skin relatively smooth with many tiny tubercles; ventral skin of head relatively smooth with indistinct tiny tubercles, skin of abdomen with flat granules, ventral skin of forelimbs smooth, ventral skin of hindlimbs smooth except for some flat granules on bases of ventral thigh; some protruding tubercles around vent; dermal calcars at heels absent.

Coloration of holotype in life. Dorsal surface light brownish yellow with some tiny black spots, a transverse grayish brown stripe between eyes, a longitudinal curved grayish brown stripe from behind eye to crotch on each side, a row of indistinct grayish brown spots forming a discontinuous stripe on midline of posterior dorsum, some grayish brown transverse bands on limbs; loreal region grayish brown, upper lip yellowish white, a brown stripe from behind eye through tympanum to above shoulder, a light brown stripe from above shoulder to groin; ventral surface of head yellowish white with some indistinct dark spots, surface of abdomen light yellowish white with some dark spots, ventral surface of limbs flesh color (Figure 4).

Variation. The male paratypes are very similar to the holotype in morphometric characteristics, the female paratype is also similar to the holotype in morphometric characteristics except that it has a larger body size (Table 3). The coloration of the paratypes is also very similar to that of the holotype except that the background color is darker and the stripes on dorsum and limbs are more distinct in some individuals (Figure 5).



Figure 4. The holotype (KIZ2026096) of *Feihyla changningensis* sp. nov. in life. (A) Dorsal view; (B) ventral view; (C) left view; (D) right view.

Table 3. Measurements (in mm) of the type specimens of *Feihyla changningensis* sp. nov. For abbreviations, see Section 2.

	KIZ2026096 Holotype Male	KIZ2026091 Paratype Male	KIZ2026092 Paratype Male	KIZ2026093 Paratype Male	KIZ2026094 Paratype Male	KIZ2026095 Paratype Male	KIZ2026097 Paratype Male	KIZ2026098 Paratype Female
SVL	26.5	26.9	26.8	26.3	26.4	24.9	24.6	32.5
HL	8.8	8.8	8.8	8.8	8.8	8.4	8.5	10.8
HW	7.8	7.7	7.8	7.7	7.8	7.5	7.6	9.7
SL	4.0	4.0	4.3	4.0	3.9	3.8	3.6	4.4
SN	1.8	1.8	1.9	1.8	1.8	1.5	1.7	2.0
NE	2.1	2.1	2.3	2.2	2.1	2.0	1.9	2.3
INS	2.7	2.6	2.7	2.7	2.6	2.5	2.6	3.2
IOS	2.7	2.7	2.7	2.7	2.6	2.6	2.7	3.2
UEW	2.4	2.4	2.4	2.3	2.2	2.3	2.4	2.8
ED	3.1	3.2	3.2	3.1	3.1	3.0	3.0	3.6
LAHL	12.9	12.9	13.0	13.3	13.2	12.4	12.8	15.6
HAL	8.2	8.2	8.2	8.3	8.0	7.9	8.0	9.1
HLL	42.2	40.8	39.6	41.9	41.8	39.4	40.8	49.3
TL	13.1	12.4	12.3	12.8	12.6	12.3	12.9	15.2
TFL	19.1	18.5	18.7	19.1	19.4	17.5	18.4	22.2
FL	11.9	11.8	11.7	12.0	12.4	11.4	11.6	13.9



Figure 5. The paratypes of *Feihyla changningensis* sp. nov. in life. (A) The male KIZ2026092; (B) the male KIZ2026094; (C) the male KIZ2026097; (D) the female KIZ2026098.

Comparisons. *Feihyla changningensis* sp. nov. differs from *F. palpebralis* by head length being greater than head width (vs. head being as long as broad) in females, interorbital distance being greater than width of upper eyelid (vs. interorbital distance being as broad as upper eyelid) in females, webbing between fingers being absent (vs. rudimentary webbing being present between outer two fingers) in females, and toes being one third webbed (vs. toes being half webbed) in females.

Feihyla changningensis sp. nov. differs from *F. fuhua* by nostril being closer to snout tip than to eye (vs. nostril being closer to eye than to snout tip) in males, having a smaller ratio of interorbital distance to eye diameter (IOS/ED 0.84–0.90 vs. 0.94–1.03) in males, tibiotarsal articulation reaching center of eye when hindlimbs pressed forward (vs. tibiotarsal articulation reaching anterior corner of eye) in males, and nuptial pad present on base of first finger (vs. nuptial pads present on bases of first and second fingers) in males.

Feihyla changningensis sp. nov. differs from *F. wenshanensis* by nostril being closer to snout tip than to eye (vs. nostril being closer to eye than to snout tip) in males, having a smaller ratio of internasal distance to interorbital distance (INS/IOS 0.96–1.00 vs. 1.04–1.24) in males, a greater ratio of interorbital distance to eye diameter (IOS/ED 0.84–0.90 vs. 0.64–0.79) in males, tibiotarsal articulation reaching center of eye when hindlimbs pressed forward (vs. tibiotarsal articulation reaching anterior corner of eye) in males, and nuptial pad present on base of first finger (vs. nuptial pads present on bases of first and second fingers) in males.

Feihyla changningensis sp. nov. differs from *F. zhaotongensis* by having a smaller body size (SVL 24.6–26.9 mm vs. 32.0–33.5 mm) in males, a greater ratio of head length to head width (HL/HW 1.12–1.14 vs. 1.05–1.07) in males, and nuptial pad present on base of first finger (vs. nuptial pads present on bases of first and second fingers) in males.

Etymology. The name refers to Changning County, the locality where the new species was found. We propose “Changning Jelly-nest Treefrog” for the common English name and “昌宁费树蛙” for the common Chinese name of the new species.

Distribution. This species is currently only known to be distributed in Changning County, Baoshan City, Yunnan Province, China, and may also occur in other areas of western Yunnan.

Natural history notes. The specimens of *Feihyla changningensis* sp. nov. were collected beside a pond at night. There were many grasses, trees, and bamboos around the pond. Males were calling on the leaves of the trees or bamboo stems. Some jelly-nests of eggs were found on the leaves of plants (Figure 6). *Nidirana occidentalis* Lyu, Yang & Wang, 2020 was found inhabiting the same pond.



Figure 6. The habitat and the jelly-nests of eggs of *Feihyla changningensis* sp. nov.

***Feihyla menghaiensis* sp. nov.**

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Holotype. KIZ2026103, adult male, collected on 6 August 2026 by Shuo Liu from Manwa Village, Xiding Township, Menghai County, Xishuangbanna Prefecture, Yunnan Province, China (21°51'07" N, 100°04'10" E, 1910 m, WGS84).

Paratype. KIZ2026099–KIZ2026102 and KIZ2026104–KIZ2026106, seven adult males, KIZ2026107, adult female, the same collection information as the holotype.

Diagnosis. Body size small, SVL 27.4–29.9 mm in adult males and 36.7 mm in single adult female; distance between snout tip and nostril equal to distance between nostril and eye; internasal distance greater than interorbital distance; tibiotarsal articulation reaching

center of eye when hindlimbs pressed forward; inner metatarsal tubercle length approximately one third of first toe length; a dark transverse stripe between eyes, a curved dark stripe from behind eye to crotch on each side of dorsum, a continuous or discontinuous dark stripe on midline of posterior dorsum; nuptial pads present on bases of first and second fingers in adult males.

Description of holotype. Adult male, SVL 29.9 mm; head length slightly greater than head width (HL/HW 1.05); snout relatively sharp, longer than eye diameter (SL/ED 1.23); canthus rostralis distinct; interorbital region flat; pineal ocellus absent; internasal distance slightly greater than interorbital distance; interorbital distance greater than upper eyelid width (IOS/UEW 1.12) and smaller than eye diameter (IOS/ED 0.83); distance between snout tip and nostril equal to distance between nostril and eye (SN/NE 1.00); loreal region slightly concave; tympanum indistinct; supratympanic fold distinct, narrow; vomerine teeth absent; tongue elongated oval, notched posteriorly; choanae small, round; single subgular vocal sac, vocal sac slit opening at bottom of mouth on each side.

Forelimbs slender, moderately long; relative lengths of fingers $I < II < IV < III$; tips of fingers dilated into discs; subarticular tubercles formula 1, 1, 2, 2, ones on first and second fingers and distal ones on third and fourth fingers large and distinct, proximal ones on third and fourth fingers small and indistinct; supernumerary tubercles indistinct; webbing between fingers absent; inner metacarpal (thenar) tubercle distinct, no distinct outer metacarpal tubercle; nuptial pads yellowish white, composed of tiny granules, present on bases of first and second fingers.

Hindlimbs slender, moderately long; tibiotarsal articulation reaching center of eye when hindlimbs pressed forward; relative length of toes $I < II < III < V < IV$; tips of toes dilated into discs, discs on toes smaller than on fingers; subarticular tubercles formula 1, 1, 2, 3, 2, all small but distinct; supernumerary tubercle absent; webbing between toes undeveloped, formula $I2-2\frac{1}{4}II1\frac{2}{3}-3III2\frac{1}{4}-3\frac{1}{2}IV3-2V$; inner metatarsal tubercle oval, relatively long, length approximately one third of length of first toe, outer metatarsal tubercle absent (Figure 7).

Dorsal skin relatively smooth with many tiny tubercles; ventral skin of head relatively smooth with indistinct tiny tubercles, skin of abdomen with flat granules, ventral skin of forelimbs smooth, ventral skin of hindlimbs smooth except for some flat granules on bases of ventral thigh; some protruding tubercles around vent; dermal calcars at heels absent.

Coloration of holotype in life. Dorsal surface light brownish yellow with some tiny black spots, a transverse brown stripe between eyes, a longitudinal curved brown stripe from behind eye to crotch on each side, a brown stripe on midline of posterior dorsum, some brown transverse bands on limbs; loreal region brown, upper lip yellowish white, a brown stripe from behind eye through tympanum to above shoulder, a grayish brown stripe from above shoulder to groin; ventral surface of head yellowish white with some dark spots, surface of abdomen light yellowish white, ventral surface of limbs flesh color (Figure 8).

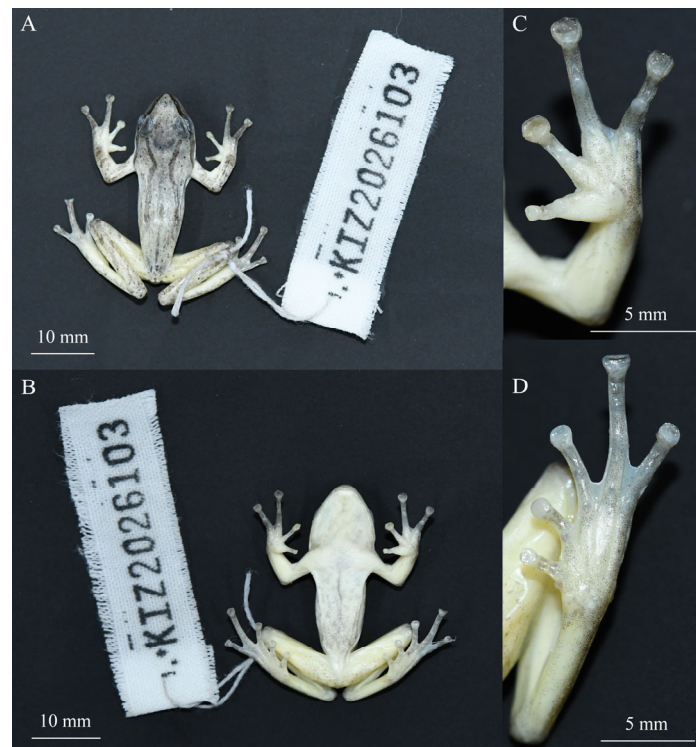


Figure 7. The holotype (KIZ2026103) of *Feihyla menghaiensis* sp. nov. in preservative. (A) Dorsal view; (B) ventral view; (C) close-up view of the left volar; (D) close-up view of the left plantar.



Figure 8. The holotype (KIZ2026103) of *Feihyla menghaiensis* sp. nov. in life. (A) Dorsal view; (B) ventral view; (C) left view; (D) right view.

Variation. The male paratypes are very similar to the holotype in morphometric characteristics, the female paratype is also similar to the holotype in morphometric characteristics except that it has a larger body size (Table 4). The coloration of the paratypes is also

very similar to that of the holotype except that the background color is darker and the stripes on dorsum and limbs are more indistinct in some individuals (Figure 9).

Table 4. Measurements (in mm) of the type specimens of *Feihyla menghaiensis* sp. nov. For abbreviations, see Section 2.

	KIZ2026103	KIZ2026099	KIZ2026100	KIZ2026101	KIZ2026102	KIZ2026104	KIZ2026105	KIZ2026106	KIZ2026107
	Holotype	Paratype	Paratype	Paratype	Paratype	Paratype	Paratype	Paratype	Paratype
	Male	Male	Male	Male	Male	Male	Male	Male	Female
SVL	29.9	29.2	28.0	27.4	29.0	28.1	29.5	29.4	36.7
HL	9.8	9.3	9.3	9.2	9.4	9.5	9.6	9.4	12.0
HW	9.3	8.6	8.5	8.4	8.9	8.9	8.7	8.6	11.0
SL	4.3	4.1	4.0	4.4	4.4	4.3	4.4	4.3	5.3
SN	2.1	2.0	2.0	2.2	2.2	2.1	2.2	2.1	2.6
NE	2.1	2.0	2.0	2.2	2.2	2.1	2.2	2.1	2.6
INS	3.2	3.1	3.0	3.0	3.1	3.2	3.2	3.1	3.7
IOS	2.9	2.8	2.8	2.7	2.8	3.0	3.0	2.9	3.4
UEW	2.6	2.6	2.4	2.3	2.7	2.6	2.6	2.4	2.8
ED	3.5	3.4	3.3	3.3	3.5	3.5	3.5	3.4	3.8
LAHL	15.1	14.2	14.4	14.1	14.6	15.1	14.3	15.3	18.4
HAL	9.5	8.6	8.7	8.6	8.8	9.6	9.0	9.2	11.4
HLL	47.5	44.2	45.3	44.4	45.3	45.3	45.5	46.7	58.7
TL	14.7	13.5	13.8	13.2	14.0	13.8	13.8	14.0	18.0
TFL	21.8	20.8	20.3	20.1	21.8	21.4	21.3	21.3	27.0
FL	14.0	13.1	13.2	12.9	13.9	13.4	13.2	13.8	16.9



Figure 9. The paratypes of *Feihyla menghaiensis* sp. nov. in life. (A) The male KIZ2026101; (B) the male KIZ2026102; (C) the male KIZ2026106; (D) the female KIZ2026107.

Comparisons. *Feihyla menghaiensis* sp. nov. differs from *F. palpebralis* by head length being greater than head width (vs. head being as long as broad) in females, distance between snout tip and nostril being equal to distance between nostril and eye (vs. nostril being closer to snout tip than to eye) in females, interorbital distance being greater than width of upper eyelid (vs. interorbital distance being as broad as upper eyelid) in females, webbing between fingers being absent (vs. rudimentary webbing being present between outer two fingers) in females, and toes being approximately one third webbed (vs. toes being approximately half webbed) in females.

Feihyla menghaiensis sp. nov. differs from *F. fuhua* by distance between snout tip and nostril being equal to distance between nostril and eye (vs. nostril being closer to eye than to snout tip) in males, internasal distance being greater than interorbital distance (vs. internasal distance being smaller than or equal to interorbital distance) in males, having a smaller ratio of interorbital distance to eye diameter (IOS/ED 0.80–0.86 vs. 0.94–1.03) in males, and tibiotarsal articulation reaching center of eye when hindlimbs pressed forward (vs. tibiotarsal articulation reaching anterior corner of eye) in males.

Feihyla menghaiensis sp. nov. differs from *Feihyla wenshanensis* by distance between snout tip and nostril being equal to distance between nostril and eye (vs. nostril being closer to eye than to snout tip) in males, and tibiotarsal articulation reaching center of eye when hindlimbs pressed forward (vs. tibiotarsal articulation reaching anterior corner of eye) in males.

Feihyla menghaiensis sp. nov. differs from *Feihyla zhaotongensis* by having a smaller body size (SVL 27.4–29.9 mm vs. 32.0–33.5 mm) in males, distance between snout tip and nostril being equal to distance between nostril and eye (vs. nostril being closer to snout tip than to eye) in males, internasal distance being greater than interorbital distance (vs. internasal distance being smaller than interorbital distance) in males, and relatively longer hindlimbs (HLL/SVL 1.51–1.62 vs. 1.39–1.45) in males.

Feihyla menghaiensis sp. nov. differs from *Feihyla changningensis* sp. nov. by having a larger body size (SVL 27.4–29.9 mm vs. 24.6–26.9 mm in males and 36.7 mm vs. 32.5 mm in females), distance between snout tip and nostril being equal to distance between nostril and eye (vs. nostril being closer to snout tip than to eye), a greater ratio of internasal distance to interorbital distance (INS/IOS 1.07–1.11 vs. 0.96–1.00 in males and 1.09 vs. 1.00 in females), and nuptial pads present on bases of first and second fingers (vs. nuptial pad present on base of first finger) in males (Figure 10).

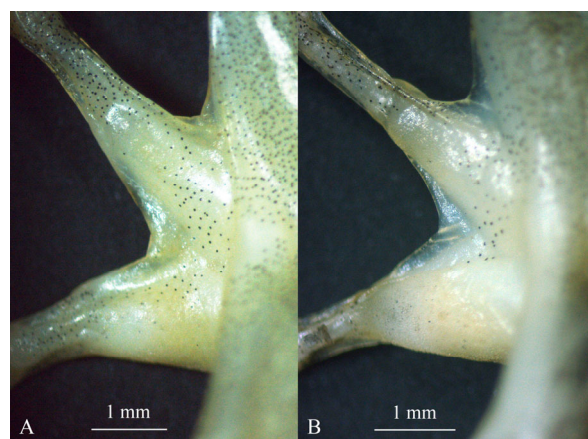


Figure 10. Comparison of the nuptial pads between the two new species. (A) The holotype (KIZ2026096) of *Feihyla changningensis* sp. nov.; (B) the holotype (KIZ2026103) of *Feihyla menghaiensis* sp. nov.

Etymology. The name refers to Menghai County, the locality where the new species was found. We propose “Menghai Jelly-nest Treefrog” for the common English name and “勐海费树蛙” for the common Chinese name of the new species.

Distribution. This species is currently known to be only distributed in Menghai County, Xishuangbanna Prefecture, Yunnan Province, China.

Natural history notes. The specimens of *Feihyla menghaiensis* sp. nov. were collected beside a pond at night. There were many grasses and trees around the pond. Males were calling on the leaves of the trees. Some jelly-nests of eggs were found on the leaves or small branches of plants (Figure 11). *Nidirana lini* (Chou, 1999) was found inhabiting the same pond.



Figure 11. The habitat and the jelly-nests of eggs of *Feihyla menghaiensis* sp. nov.

4. Discussion

Recently, Liu et al. [3] described two species of *Feihyla* sensu stricto that were previously confused with *F. fuhua* from southeastern and northeastern Yunnan Province,

China, respectively, and proposed that the records of *F. fuhua* from the regions of Yunnan except Honghe and Wenshan prefectures and Zhaotong City should be verified. In this study, we found that the specimens of *Feihyla* sensu stricto from western and southwestern Yunnan differ in morphology from each other and from the three known species of *Feihyla* sensu stricto in China (Table 5) and demonstrated that these specimens belong to two distinct species, which are formally described. Thus far, it is confirmed that five species of *Feihyla* sensu stricto are distributed in China, namely *F. fuhua* from southern Yunnan, *F. wenshanensis* from southeastern Yunnan, *F. zhaotongensis* from northeastern Yunnan, *Feihyla changningensis* sp. nov. from western Yunnan, and *Feihyla menghaiensis* sp. nov. from southwestern Yunnan (Figure 12). Although we have not collected specimens of *Feihyla* sensu stricto from central Yunnan, we speculate it is likely that species of *Feihyla* sensu stricto are also distributed in central Yunnan. More field surveys may discover potential populations from central Yunnan, and more studies will address remaining issues within this group.

Rao and Yang [4] and Fei et al. [6,31] recorded *Feihyla* sensu stricto from Longling County, Baoshan City, Yunnan Province, China. Fei et al. [6,31] did not provide morphological data for specimens from Longling County, and Rao and Yang [4] provided brief morphological data for six specimens from Longling County. According to Rao and Yang [4], the specimens from Longling County have a relatively small body size, with internasal distance being smaller than interorbital distance, which are consistent with the characteristics of *Feihyla changningensis* sp. nov. In addition, considering the proximity between Longling County and Changning County, we speculate that the specimens of *Feihyla* sensu stricto from Longling and Changning counties are likely conspecific. In the future, collecting fresh specimens from Longling County and obtaining their molecular data will verify this speculation.

Table 5. Morphological comparisons among *Feihyla changningensis* sp. nov., *Feihyla menghaiensis* sp. nov., *F. fuhua*, *F. wenshanensis*, and *F. zhaotongensis*.

	<i>Feihyla changningensis</i> sp. nov. Males n = 7	<i>Feihyla menghaiensis</i> sp. nov. Males n = 8	<i>Feihyla fuhua</i> Males n = 4	<i>Feihyla wenshanensis</i> Males n = 6	<i>Feihyla zhaotongensis</i> Males n = 2
SVL (mm)	24.6–26.9	27.4–29.9	24.4–30.0	26.1–29.0	32.0–33.5
HL/SVL	0.33–0.35	0.32–0.34	0.34–0.36	0.33–0.35	0.32–0.33
HL/HW	1.12–1.14	1.05–1.10	1.11–1.18	1.09–1.15	1.05–1.07
SL/HL	0.42–0.49	0.43–0.48	0.44–0.46	0.42–0.45	0.45
SN/NE	0.75–0.89	1.00	1.05	1.05–1.21	0.85–0.88
ED/HL	0.35–0.36	0.35–0.37	0.32–0.34	0.37–0.40	0.38–0.39
INS/HW	0.33–0.35	0.34–0.37	0.35–0.38	0.33–0.36	0.31–0.32
IOS/HW	0.33–0.36	0.31–0.34	0.35–0.38	0.29–0.33	0.33
INS/IOS	0.96–1.00	1.07–1.11	0.97–1.00	1.04–1.24	0.94–0.97
IOS/ED	0.84–0.90	0.80–0.86	0.94–1.03	0.64–0.79	0.81–0.83
UEW/HW	0.28–0.32	0.27–0.30	0.24–0.28	0.25–0.28	0.27
LAHL/SVL	0.48–0.52	0.48–0.54	0.49–0.53	0.47–0.49	0.48–0.50
HAL/SVL	0.30–0.33	0.29–0.34	0.31–0.34	0.29–0.30	0.30–0.32
HLL/SVL	1.48–1.66	1.51–1.62	1.53–1.68	1.42–1.54	1.39–1.45
TL/SVL	0.46–0.52	0.46–0.49	0.48–0.55	0.47–0.51	0.46–0.47
TFL/SVL	0.69–0.75	0.71–0.76	0.68–0.75	0.70–0.72	0.67–0.71
FL/HLL	0.28–0.30	0.29–0.31	0.28–0.29	0.29–0.30	0.31

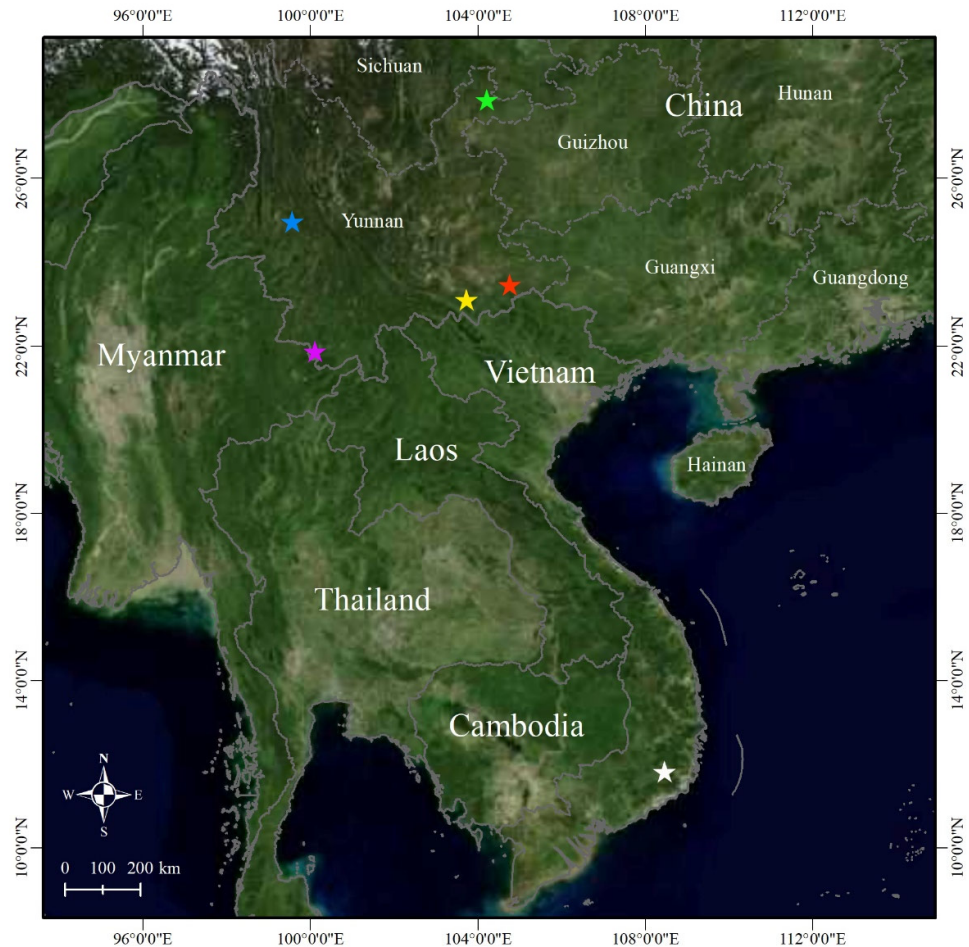


Figure 12. Map showing the type localities of *Feihyla changningensis* sp. nov. in Changning County, Baoshan City, Yunnan Province, China (purple star), *Feihyla menghaiensis* sp. nov. in Menghai County, Xishuangbanna Prefecture, Yunnan Province, China (blue star), *F. fuhua* in Pingbian County, Honghe Prefecture, Yunnan Province, China (yellow star), *F. palpebralis* in Lam Dong Province, Vietnam (white star), *F. wenshanensis* in Xichou County, Wenshan Prefecture, Yunnan Province, China (red star), and *F. zhaotongensis* in Yiliang County, Zhaotong City, Yunnan Province, China (green star).

Using mitochondrial genes for taxonomic research is currently a common and widespread practice. Especially for studies on the taxonomy of amphibians, most are based only on the 16S gene, including this study. However, relying solely on the mitochondrial genes for phylogenetic analysis undoubtedly has some drawbacks, as they are only maternal. In the future, the use of nuclear genes should be strengthened to obtain more reliable systematic results and verify the validity of many current species.

We estimate that the population sizes of the two new species are relatively large. For each species, we encountered many individuals, heard many calls, and found some egg clusters in one night. The collection sites of these two species are not within nature reserves; although there are still some habitats suitable for their survival and reproduction in those areas, the surrounding environment is greatly affected by human activities. Further field surveys are needed to determine their distribution range and threat status.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/taxonomy6040063/s1>, Table S1: Measurements (in mm) of examined specimens of *Feihyla fuhua*, *F. wenshanensis*, and *F. zhaotongensis*.

Author Contributions: Conceptualization, D.R.; methodology, D.R.; validation, Q.M., Z.H., Q.W., and P.Z.; formal analysis, M.H. and S.L.; investigation, S.L., Z.G., Q.M., Z.H., Q.W., and P.Z.; resources, Z.G., Q.M., Z.H., Q.W., and P.Z.; data curation, S.L.; writing—original draft preparation, S.L.; writing—review and editing, M.H., D.R., and W.Z. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study on animals was approved by the Ethics Committee of Kunming Institute of Zoology, Chinese Academy of Sciences on 17 March 2025 (protocol codes: IACUCPA-2025-03-122).

Data Availability Statement: All data are presented in this article and in GenBank (<https://www.ncbi.nlm.nih.gov> (accessed on 8 September 2026)).

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