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A new species of *Zhangixalus* (Anura, Rhacophoridae) from Yunnan, China

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Abstract

We described herein *Zhangixalus yunnanensis* **sp. nov.**, a new treefrog species from central and western Yunnan, China, which had previously been confused with *Z. nigropunctatus*, based on morphological and molecular evidence. Our phylogenetic analyses revealed that the new species is sister to the clade of *Z. nigropunctatus* and *Z. melanoleucus* with strong support (100% and 73% for BI and ML, respectively). Our morphological analysis suggested that *Z. yunnanensis* **sp. nov.** is distinctly different from all known congeners by the combination of the following morphological characters: black blotches on body flank and hind-limb, medium body size (SVL31.3–36.0 mm in males and 47.6–48.6 mm in females), head wider than long, iris yellowish-brown, dorsum uniformly green, vocal sac external, throat black, webbing greyish and fingers webbed one third and toes webbed half. Additionally, we revealed that the specimens ROM 38011 (Sa Pa, Vietnam) and VNMN 4099 (Son La, Vietnam) are neither *Z. dorsoviridis* nor *Z. nigropunctatus*, but probably represent one or two cryptic species of *Zhangixalus* pending further morphological and molecular data. Including the new species described herein, the genus *Zhangixalus* currently comprises 42 species, 30 of which are distributed in China with 11 species known from Yunnan. Amongst different zoogeographic regions in Yunnan, south-eastern Yunnan has the highest diversity of *Zhangixalus*, followed by western Yunnan and southern Yunnan. More studies are required to clarify the species diversity of this genus based on multiple lines of evidence (e.g. morphological and molecular data).

Key Words

cryptic species, treefrog, Zhangixalus, Zhangixalus nigropunctatus

Introduction

The treefrog genus *Zhangixalus* Li, Jiang, Ren & Jiang, 2019, recently removed from *Rhacophorus* Kuhl & Van Hasselt, 1822 *sensu lato* and recognised as a distinct genus, is characterised by medium-to-large body size, absence of dermal folds along limbs, absence of tarsal projections and usual green dorsal colouration (Jiang et al. 2019). The genus is distributed widely in South, East and Southeast Asia and currently contains 41 species (Frost 2023). In China, 29 species of *Zhangixalus* have been

recorded to date, 10 of which are distributed in Yunnan: Z. burmanus (Andersson, 1939), Z. dorsoviridis (Bourret, 1937), Z. duboisi (Ohler, Marquis, Swan & Grosjean, 2000), Z. feae (Boulenger, 1893), Z. franki Ninh, Nguyen, Orlov, Nguyen & Ziegler, 2020, Z. nigropunctatus (Liu, Hu & Yang, 1962), Z. omeimontis (Stejneger, 1924), Z. pachyproctus Yu, Hui, Hou, Wu, Rao & Yang, 2019, Z. puerensis (He, 1999) and Z. smaragdinus (Blyth, 1852) (AmphibiaChina 2023).

Amongst others, the taxonomy of *Zhangixalus ni*gropunctatus is confusing. This species was originally

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described from western Guizhou, China (Weining and Shuicheng) by Liu et al. (1962) and, afterwards, was recorded from Yunnan (Longling, Longchuan, Yingjiang, Qiaojia, Pingbian, Jinping) (Yang 1991; Zhao and Yang 1997; Fei 1999; Fei et al. 2005, 2009, 2010; Yang and Rao 2008; Yu et al. 2008), Hunan, and Anhui (Fei 1999; Fei et al. 2005, 2009, 2010). Orlov et al. (2001) considered that Z. nigropunctatus is closely allied with Z. dorsoviridis based on the descriptions of the former and, therefore, noted that Z. nigropunctatus was possibly a synonym of Z. dorsoviridis. Yu et al. (2009) revealed that Z. nigropunctatus from Pingbian did not group together with topotypes of Z. nigropunctatus, indicating that misidentification may be involved within Z. nigropunctatus. Li et al. (2012a) revealed that both Z. nigropunctatus and Z. dorsoviridis did not form a monophyletic group. They found that Z. nigropunctatus from the type locality and Longling formed clade II with a sample of Z. dorsoviridis (ROM 38011) from Sa Pa, northern Vietnam and this clade was closely related to Z. chenfui (Liu, 1945). Meanwhile, Z. nigropunctatus from Jinping and Pingbian grouped into clade I with another sample of Z. dorsoviridis from Sa Pa (ROM 38015; see fig. 1 of Li et al. (2012a)). Based on morphological evidence, Li et al. (2012a) referred clade II to true Z. nigropunctatus and referred clade I to Z. dorsoviridis. This finding implies that previous records of Z. nigropunctatus from Jinping and Pingbian belong to Z. dorsoviridis (Zhang et al. 2011) and that Z. nigropunctatus is distributed in northern Vietnam. However, Mo et al. (2016) revealed that the sample ROM 38011 was distantly related to Z. nigropunctatus and it was sister to Z. pinglongensis (Mo, Chen, Liao & Zhou, 2016), indicating that the record of Z. nigropunctatus in northern Vietnam may be misidentified. In addition, Pan et al. (2017) revealed that Z. nigropunctatus recorded from the Dabie Mountains of Anhui Province represents an independent species and named it as Z. zhoukaiyae (Pan, Zhang & Zhang, 2017).

During our recent field surveys in central Yunnan, China, specimens of a treefrog species resembling Zhangixalus nigropunctatus were collected from Xinping County. Amphibian diversity in Yunnan is still poorly understood. In recent years, a number of new amphibian species have been reported from Yunnan, China (e.g. Gan et al. (2020); Du et al. (2022); Wang et al. (2022a); Tang et al. (2023a, 2023b)). Considering the taxonomic history of Z. nigropunctatus and the status of amphibian diversity in Yunnan, we sequenced our newly-collected treefrogs from Yunnan to confirm their identity. Our phylogenetic analyses recovered these specimens as a distinct lineage and are sister to the clade composed of Z. nigropunctatus and Z. melanoleucus Brakels, Nguyen, Pawangkhanant, Idiiatullina, Lorphengsy, Suwannapoom & Poyarkov, 2023. Furthermore, our morphological examination suggested that this lineage differs from Z. nigropunctatus, Z. melanoleucus and other members of Zhangixalus by a series of characters. Herein, we officially describe this lineage as a new species of Zhangixalus.

Materials and methods

Sampling

This study was carried out in accordance with the ethical guidelines issued by the Ethics Committee of Guangxi Normal University. Field surveys were conducted in July 2019 and April 2020 at Xinping County, Yunnan, China (Fig. 1) and a total of nine treefrog specimens were collected during the surveys. Specimens were photographed, euthanised, fixed and then stored in 75% ethanol. Liver tissues were preserved in 99% ethanol. Specimens were deposited at Guangxi Normal University (**GXNU**).

Phylogenetic analysis

Total genomic DNA was extracted from liver tissues stored in 99% ethanol. A fragment encoding mitochondrial 12S rRNA, tRNA^{val} and 16S rRNA genes was amplified and sequenced using the primers and protocols of Yu et al. (2019). Seven samples were newly sequenced and all new sequences have been deposited in GenBank under Accession Nos. PP177446 and PP187265–PP187270 (Table 1). Additionally, 43 homologous sequences of other *Zhangixalus* species and outgroups were obtained from GenBank (Table 1). *Theloderma albopunctatum* (Liu & Hu, 1962), *Rhacophorus rhodopus* Liu & Hu, 1960 and *Leptomantis gauni* (Inger, 1966) were included in the data as outgroups.

Sequences were aligned using MUSCLE with default parameters in MEGA v.7.0 (Kumar et al. 2016). Uncorrected pairwise distances (using 16S rRNA sequences) between species were calculated in MEGA v.7.0. The best substitution model was selected using the corrected Akaike Information Criterion (AICc) in jModelTest v.2.1.10 (Darriba et al. 2012). Bayesian Inference was performed in MrBayes v.3.2.6 (Ronquist et al. 2012) under the selected substitution model (GTR + I + G). Two runs were performed simultaneously with four Markov chains starting from a random tree. The chains were run for 3,000,000 generations and sampled every 100 generations. The first 25% of the sampled tree was discarded as burn-in after the standard deviation of split frequencies of the two runs was less than 0.01. The remaining trees were then used to create a consensus tree and to estimate Bayesian posterior probabilities (BPPs). In addition, a Maximum Likelihood (ML) analysis was conducted in raxmlGUI v.2.0 (Edler et al. 2021) with 1000 rapid bootstrap replicates. The node was considered strongly supported with BPP ≥ 0.95 and bootstrap value ≥ 70 (Huelsenbeck and Hillis 1993; Leaché and Reeder 2002).

Morphology

Morphometric data were taken using electronic digital calipers to the nearest 0.1 mm. Morphological terminology



Figure 1. Known distribution sites of *Zhangixalus yunnanensis* sp. nov. in Yunnan, China. The red star represents the type locality of the new species. The map was generated using ArcMap v.10.2 (ESRI Inc.).

followed Yu et al. (2019). Measurements included: snoutvent length (SVL, from tip of snout to vent); head length (HL, from tip of snout to rear of jaws); head width (HW, width of head at its widest point); snout length (SL, from tip of snout to anterior corner of eye); internarial distance (IND, distance between nares); interorbital distance (IOD, minimum distance between upper eyelids); upper eyelid width (UEW, maximum width of upper eyelid); eye diameter (ED, diameter of exposed portion of eyeball); distance between nostril and eye (DNE, from nostril to anterior border of eye); tympanum diameter (TD, the greater of tympanum vertical and horizontal diameters); forearm and hand length (FHL, from elbow to tip of third finger); tibia length (TL, distance from knee to heel); foot length (FL, from proximal end of inner metatarsal tubercle to tip of fourth toe); and length of foot and tarsus (TFL, from tibiotarsal joint to tip of fourth toe). Webbing formula followed Myers and Duellman (1982).

Comparative morphological data of other *Zhangixalus* species were taken from their original descriptions or re-descriptions (Boulenger 1892; Stejneger 1907; Inger 1947; Liu et al. 1962; Lue et al. 1994, 1995; He 1999; Ohler et al. 2000; Harvey et al. 2002; Matsui and Panha 2006; Rao et al. 2006; Bordoloi et al. 2007; Chou et al. 2007; Fei et al. 2009, 2010; Zhang et al. 2011; Li et al. 2012b; Mo et al. 2016; Liu et al. 2017; Pan et al. 2017; Yu et al. 2019; Nguyen et al. 2020; Ninh et al. 2020; Brakels et al. 2023). Multivariate principal component analysis (PCA) was conducted in SPSS v.17.0 (SPSS Inc.), based

on the correlation matrix of size-standardised measurements (all measurements divided by SVL) of adult males. Scatter plots of the scores of the first two factors of PCA were used to examine the differentiation between the new species and its closest relatives revealed by phylogenetic analyses (*Z. nigropunctatus* and *Z. melanoleucus*). Differences in quantitative characters of adult males between these three species were also evaluated with t-tests in SPSS. In these analyses, Levene's test was also performed for each character to test for equality of variances. Specimens of *Z. nigropunctatus* were collected from the type locality and data for *Z. melanoleucus* were retrieved from Brakels et al. (2023).

Results

Phylogenetic relationship

The obtained sequence alignment was 1971 bp. Phylogenetic analyses revealed that the specimens from Xinping, Yunnan form a distinct clade with two individuals previously identified as *Z. nigropunctatus* (KIZ Rao3494 and KIZ Rao3496; Li et al. (2012a)) from Longling, Yunnan. This clade was recovered as sister to the clade consisting of *Z. nigropunctatus* from the type locality and *Z. melanoleucus* with strong support (Fig. 2). The genetic distances between the novel lineage and other *Zhangixalus* species ranged from 4.8% to 13.9% in 16S rRNA sequences and the distances Table 1. Species used for phylogenetic analyses in this study (Z. = Zhangixalus).

Species	Voucher No.	Locality	Accession No.
Theloderma albopunctatum	ROM 30246	Vietnam	AF458148
Rhacophorus rhodopus	SCUM 060692L	Mengyang, Yunnan, China	EU215531
Leptomantis gauni	FMNH 273928	Sarawak, Malaysia	JX219456
Zhangixalus dulitensis	BORNEENSIS09087	Borneo, Malaysia	AB847123
Z. pachyproctus	KIZ 090148	Puer, Yunnan, China	MN613222
Z. smaragdinus	KIZ 20160298	Yingjiang, Yunnan, China	MN613219
Z. dennysi	ROM 30249	Vietnam	AF458139
Z. feae	SCUM 050642W	Hekou, Yunnan, China	EU215544
Z. chenfui	SCUM 060404L	Mt. Omei, Sichuan, China	EU215534
Z. jodiae	VNMN 07122	Vietnam	LC545595
Zhangixalus sp.	ROM 38011	Sa Pa, Lao Cai, Vietnam	JX219427
	VNMN 4099	Son La, Vietnam	LC010577
Z. dorsoviridis	ROM 38015	Sa Pa, Lao Cai, Vietnam	JX219423
	Rao060821200	Jinping, Yunnan, China	JX219424
	YN080446	Pingbian, Yunnan, China	JX219425
	Rao060821199	Pingbian, Yunnan, China	JX219426
	KIZ 060821287	Jinping, Yunnan, China	EF564563
Z. yaoshanensis	NHMG150408	Jinxiu, Guangxi, China	MG322122
Z. pinglongensis	NHMG201002011	Shiwandashan, Guangxi, China	KU170684
Z. nigropunctatus	KIZ07061001	Weining, Guizhou, China	EU924623
	GZ070658	Weining, Guizhou, China	JX219430
	SCUM 070657L	Weining, Guizhou, China	EU215533
	GXNU YU000361	Weining, Guizhou, China	PP187265
	GXNU YU000362	Weining, Guizhou, China	PP187266
	GXNU YU000363	Weining, Guizhou, China	PP187267
Z. yunnanensis sp. nov.	GXNU YU20160340	Xinping, Yunnan, China	PP187268
	GXNU YU20160268	Xinping, Yunnan, China	PP187269
	GXNU YU20160267	Xinping, Yunnan, China	PP187270
	Rao3494	Longling, Yunnan, China	JX219429
	Rao3496	Longling, Yunnan, China	JX219428
Z. moltrechti	SCUM 061106L	Lianhuachi, Taiwan, China	EU215543
Z. schlegelii		Hiroshima, Japan	AB202078
Z. arboreus	TTU-R-11748	Japan	AF458142
Z. puerensis	SCUM 060649L	Puer, Yunnan, China	EU215542
Z. dugritei	SCUM 051001L	Baoxing, Sichuan, China	EU215541
Z. hui	Li01	Zhaojue, Sichuan, China	JN688878
Z. hongchibaensis	CIB 097687	Wuxi, Chongqing, China	JN688883
Z. hungfuensis	SCUM 060425L	Wenchuan, Sichuan, China	EU215538
Z. minimus	KIZ 061214YP	Mt. Dayao, Guangxi, China	EU215539
Z. burmanus	SCUM 060614L	Mt. Gaoligong, Yunnan, China	EU215537
Z. franki	VNMN 011687	Ha Giang, Vietnam	LC548746
Z. duboisi	SCUM 061104L	Pingbian, Yunnan, China	EU215536
Z. omeimontis	SCUM 0606137L	Pengxian, Sichuan, China	EU215535
Z. zhoukaiyae	AHU-RhaDB-120428	Jinzhai, Anhui, China	KU601502
	HM05293	Anhui, China	PP177446
Z. lishuiensis	YPX47792	Lishui, Zhejiang, China	KY653720
Z. melanoleucus	BEI 01010	Phou Samsoum Mt., Xiengkhoang, Laos	OQ305233
	ZMMU A-7781	Phou Samsoum Mt., Xiengkhoang, Laos	OQ305234
	BEI 01011	Phou Samsoum Mt., Xiengkhoang, Laos	OQ305235
	AUP 02507	Phou Samsoum Mt., Xiengkhoang, Laos	0Q305236

between the novel lineage, *Z. nigropunctatus* and *Z. melan-oleucus* ranged from 5.1%–5.5% (Suppl. material 1).

Additionally, KIZ 060821287 and ROM 38011, two samples previously identified as *Z. nigropunctatus* by Yu et al. (2008) and Li et al. (2012a), respectively, were not grouped together with topotypes of *Z. nigropunctatus*. KIZ 060821287 was nested within the clade of *Z. dorsoviridis* from the type locality (Sa Pa, Lao Cai, Vietnam) and Yunnan and this clade was sister to the clade of *Z. lishuiensis* and *Z. zhoukaiyae*. ROM 38011 was sister to the lineage of nominal *Z. dorsoviridis* from Son La, Vietnam (VNMN 4099) and the two together were sister to the clade of Z. yaoshanensis (Liu & Hu, 1962) and Z. pinglongensis with strong support (98% for BI and 83% for ML; Fig. 2).

Morphometric analysis

Morphometric data are summarised in Table 2. Three measurements (FHL, TFL and FL) were not included in PCA analysis because they were not available for *Z. melanoleucus*. We retained the first two principal components that accounted for 66.99% of the total variance (Table 3). Loadings for PC1, which accounted for 45.21% of the

Table 2. Measurements of *Zhangixalus yunnanensis* sp. nov. (1–9), *Z. nigropunctatus* (10–17) and *Z. melanoleucus* (18–21). Specimens of *Z. nigropunctatus* were collected from the type locality (Weining, Guizhou) and data of *Z. melanoleucus* were obtained from Brakels et al. (2023).

ID	Voucher no.	Sex	SVL	HL	HW	SL	IND	IOD	UEW	ED	TD	DNE	FHL	TL	TFL	FL
1	GXNU YU20160267	Μ	31.3	9.7	11.4	4.9	4.0	4.0	2.8	3.5	1.9	2.2	17.3	13.3	21.8	14.9
2	GXNU YU20160268	Μ	34.0	11.1	12.6	5.3	4.0	4.0	3.2	4.0	2.1	2.2	16.9	13.0	21.6	14.8
3	GXNU YU20160269	Μ	35.4	11.5	12.5	5.5	4.1	4.1	3.4	4.1	2.1	2.2	18.7	14.5	23.2	15.7
4	GXNU YU20160335	F	48.6	15.1	18.0	7.2	5.6	5.6	4.2	4.9	2.9	3.0	25.5	20.0	32.5	22.5
5	GXNU YU20160336	Μ	35.3	11.2	13.6	5.4	4.3	4.3	3.5	4.3	2.3	2.2	18.3	14.3	23.5	15.5
6	GXNU YU20160337	Μ	35.6	11.6	12.9	5.3	4.2	4.2	3.2	4.3	2.2	2.1	18.3	13.9	22.4	15.1
7	GXNU YU20160338	Μ	34.4	11.3	12.9	5.3	4.3	4.5	3.3	4.1	2.2	2.3	18.1	14.9	22.9	15.6
8	GXNU YU20160340	Μ	36.0	11.8	13.9	5.5	4.3	4.3	3.6	4.3	2.3	2.2	18.5	14.3	23.5	16.2
9	GXNU YU20160355	F	47.6	14.4	17.2	6.8	5.8	5.7	4.1	5.0	2.9	2.9	25.5	20.5	33.2	23.3
10	KIZ25362	F	39.4	13.2	13.7	5.9	4.2	5.0	3.1	4.1	2.7	2.8	22.1	17.1	27.7	19.0
11	KIZ25367	F	43.7	13.0	13.8	6.1	4.2	4.8	3.0	4.3	2.8	2.8	22.7	17.1	27.5	20.0
12	KIZ25369	Μ	31.7	11.2	11.4	4.8	3.2	3.7	2.4	3.7	2.2	2.3	16.4	12.8	20.6	14.7
13	KIZ25370	Μ	33.6	11.5	12.0	5.1	3.4	3.9	2.7	3.9	2.3	2.3	17.4	12.9	21.4	15.8
14	KIZ25372	Μ	34.8	11.7	12.5	5.1	3.7	4.1	2.6	4.0	2.4	2.4	17.1	13.5	21.6	15.6
15	KIZ25373	Μ	34.1	11.9	12.1	4.9	3.7	4.0	2.7	3.9	2.5	2.3	18.6	13.4	21.9	15.2
16	KIZ25374	Μ	34.7	11.7	11.8	5.3	3.3	3.8	2.9	3.6	2.3	2.5	16.8	13.3	21.1	15.4
17	KIZ25375	Μ	33.2	11.8	11.3	5.1	3.4	4.0	2.7	3.7	2.2	2.2	16.5	11.9	20.4	14.3
18	BEI 01010	Μ	35.0	13.6	13.3	6.1	4.3	4.7	3.2	4.6	2.3	2.4	-	14.7	-	-
19	BEI 01011	Μ	34.4	12.6	12.2	5.8	4.2	4.1	3.0	4.2	1.9	2.0	-	14.0	-	-
20	ZMMU A-7781	Μ	36.3	13.3	13.4	6.1	4.4	4.8	3.1	4.7	2.2	2.6	-	14.3	-	-
21	AUP 02507	Μ	34.4	12.0	12.0	5.4	4.2	4.2	3.1	4.1	1.9	2.4	-	13.8	-	-



Figure 2. Bayesian phylogram of *Zhangixalus* inferred from mitochondrial 12S-tRNA-16S sequences. Numbers above and below branches are Bayesian posterior probabilities and ML bootstrap values (only values above 50% are shown), respectively.

total variance, were most heavily loaded on internarial distance (IND) and Loadings for PC2, which accounted for 21.78%, were most heavily loaded on head length (HL). Differentiation was found along both the PC1 and PC2 axis between the specimens from Xinping and Z. nigropunctatus from the type locality and differentiation was also found along the PC2 axis between the specimens from Xinping and Z. melanoleucus (Fig. 3). The results of PCA analysis revealed distinct morphometric differences in head length and internarial distance between the specimens from Xinping and Z. nigropunctatus from the type locality, as well as in head length between the specimens from Xinping and Z. melanoleucus. Moreover, the t-tests demonstrated that male specimens from Xinping differed significantly (p < 0.05) from male topotypes of Z. nigropunctatus in HL, HW, IND, UEW, TD, DNE and TFL (Table 4) and differed significantly (p < 0.05) from Z. melanoleucus in HL, SL, UEW and ED (Table 5). Additionally, the new lineage is distinguishable from its congeners by body size and the combination of texture and colouration pattern. Therefore, on the basis of the above molecular and morphological evidence, we officially describe them as a new species of the genus Zhangixalus.

Table 3. Factor loadings of first two principal components of 10 size-adjusted male morphometric characteristics of *Zhangixalus yunnanensis* sp. nov., *Z. nigropunctatus* and *Z. melanoleucus*.

Character	PC1	PC2
Eigenvalue	4.521	2.178
% variation	45.214%	21.778%
HL (head length)	0.124	0.898
HW (head width)	0.708	-0.185
SL (snout length)	0.698	0.527
IND (internarial distance)	0.926	-0.208
IOD (interorbital distance)	0.778	0.402
UEW (width of upper eyelid)	0.713	-0.571
ED (eye diameter)	0.801	0.363
TD (tympanum diameter)	-0.577	0.192
DNE	-0.323	0.596
TL (tibia length)	0.685	-0.072



Figure 3. Scatterplot of principal components 1 and 2 of size-adjusted male morphometric data of *Z. yunnanensis* sp. nov., *Z. nigropunctatus* and *Z. melanoleucus*.

Taxonomic account

Zhangixalus yunnanensis sp. nov.

https://zoobank.org/742755AC-423C-4080-8568-8E7D34700F36 Figs 4–6

Yunnan tree Frog/ Yún Nán Shù Wā (云南树蛙)

Type material. *Holotype:* GXNU YU20160340, adult male, collected on 20 April 2020 by Guohua Yu from Mopan Mountain, Xinping County, Yunnan Province, China (23°56'06"N, 102°01'18"E, 2443 m a.s.l.).

Paratypes: GXNU YU20160267–20160269, three adult males, collected on 14 July 2019 from the type locality by Guohua Yu; GXNU YU20160336–20160338,

Table 4. Summary statistics of male specimens (mean \pm standard deviation) and results of the *t*-test between the *Z. yunnanensis* sp. nov. (n = 7) and *Z. nigropunctatus* (n = 6) from the type locality. The *t*-test was performed on the size-adjusted data, except SVL. * = p < 0.05, ** = p < 0.01.

Character	Mean \pm SD (n = 7)	Mean \pm SD (n = 6)	Leven	e's test	t-test		
	Z. yunnanensis sp. nov.	Z. nigropunctatus	F	p-value	t	p-value	
SVL	34.6 ± 1.6	33.7 ± 1.2	0.370	0.555	1.129	0.283	
HL	0.323 ± 0.0068	0.346 ± 0.0082	0.793	0.392	-5.420	0.000**	
HW	0.371 ± 0.0122	0.352 ± 0.0092	0.388	0.546	3.144	0.009**	
SL	0.154 ± 0.0026	0.150 ± 0.0039	2.043	0.181	2.117	0.058	
IND	0.121 ± 0.0043	0.102 ± 0.0047	0.016	0.902	7.350	0.000**	
IOD	0.122 ± 0.0056	0.116 ± 0.0037	1.862	0.200	1.956	0.076	
UEW	0.095 ± 0.0041	0.079 ± 0.0034	0.297	0.597	7.468	0.000**	
ED	0.118 ± 0.0034	0.113 ± 0.0049	0.519	0.486	2.266	0.045*	
TD	0.062 ± 0.0021	0.069 ± 0.0026	0.010	0.921	-4.966	0.000**	
DNE	0.064 ± 0.0038	0.069 ± 0.0025	1.169	0.303	-3.008	0.012*	
FHL	0.522 ± 0.0171	0.509 ± 0.0226	0.925	0.357	1.146	0.276	
TL	0.406 ± 0.0182	0.385 ± 0.1508	0.556	0.472	2.246	0.046*	
FL	0.446 ± 0.0164	0450 ± 0.0143	0.029	0.868	-0.517	0.615	
TFL	0.657 ± 0.0223	0.629 ± 0.0167	0.086	0.775	2.576	0.026*	

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Table 5. Summary statistics of male specimens (mean \pm standard deviation) and results of the *t*-test between the *Z. yunnanensis* sp. nov. (n = 7) and *Z. melanoleucus* (n = 4) from the type locality. The *t*-test was performed on the size-adjusted data, except SVL. * = p < 0.05, ** = p < 0.01.

Character	Mean ± SD (n = 7)	Mean \pm SD (n = 4)	Leven	e's test	t-test		
-	Z. yunnanensis sp. nov.	Z. melanoleucus	F	p-value	t	p-value	
SVL	34.6 ± 1.6	35.0 ± 0.9	0.856	0.379	-0.515	0.619	
HL	0.323 ± 0.0068	0.368 ± 0.0163	1.420	0.264	-6.502	0.000**	
HW	0.371 ± 0.0122	0.363 ± 0.0141	0.239	0.637	0.968	0.358	
SL	0.154 ± 0.0026	0.167 ± 0.0072	3.050	0.115	-4.491	0.002**	
IND	0.121 ± 0.0043	0.122 ± 0.0007	8.056	0.019	-0.773	0.467	
IOD	0.122 ± 0.0056	0.127 ± 0.0074	1.342	0.276	-1.359	0.207	
UEW	0.095 ± 0.0041	0.089 ± 0.0027	0.751	0.409	2.753	0.022*	
ED	0.118 ± 0.0034	0.126 ± 0.0058	4.303	0.068	-2.737	0.023*	
TD	0.062 ± 0.0021	0.059 ± 0.0050	6.278	0.034	1.211	0.300	
DNE	0.064 ± 0.0038	0.067 ± 0.0061	0.836	0.384	-1.107	0.297	
TL	0.406 ± 0.0182	0.406 ± 0.0110	1.215	0.299	0.057	0.956	

three adult males, collected on 20 April 2020 by Guohua Yu from the type locality; and two adult females (GXNU YU20160335 and YU20160355), collected on 20 April 2020 by Guohua Yu from the type locality.

Etymology. The species epithet is named for Yunnan, China, where the species was collected. We suggest the English common name "Yunnan tree frog" and the Chinese common name "Yún Nán Shù Wā (云南树蛙)".

Diagnosis. The new treefrog species is assigned to *Zhangixalus* by the presence of intercalary cartilage between terminal and penultimate phalanges of digits, Y-shaped distal end of terminal phalanx, tips of digits expanded into large discs bearing circum-marginal grooves, and vomerine teeth present, dermal folds along limbs not significant, tarsal projections absent, green dorsa colouration and medium body size (Jiang et al. 2019). Phylogenetically, the new species is nested within the genus *Zhangixalus* with strong support (100% for BI and 73% for ML).

Zhangixalus yunnanensis sp. nov. can be distinguished from its congeners by a combination of the following characters: 1) body size medium (SVL 31.3–36.0 mm [34.6 \pm 1.6, n = 7] in males and 47.6–48.6 mm [48.1 \pm 0.71, n = 2] in females); 2) head wider than long; 3) iris yellowish-brown; 4) tibiotarsal articulation reaching posterior corner of eye; 5) IND/SVL 11.6%–12. 8% (12.1% \pm 0.0043, n =7) in males; 6) HL/SVL 31.0%–32.8% (32.3% \pm 0.0068, n = 7) in males; 7) UEW/SVL 8.9%–10.00% (9.5% \pm 0.0041, n = 7) in males; 8) dorsum uniformly green; 9) black blotches in axilla, groin and posterior part of thigh; 10) vocal sac external, single; 11) throat black; 12) webbing greyish; and 13) fingers webbing formula I2–2II2–3III2–2IV and toes webbing formula I2–2II1.5–2.5III2–3IV3–1.5V.

Description of holotype. Adult male, body robust, size small (SVL 36.0 mm); HL (11.8 mm) 84.9% of HW (13.9 mm); snout rounded, sloping in profile, protruding beyond margin of lower jaw in ventral view; snout (SL 5.5 mm) longer than eye (ED 4.3 mm); canthus rostralis blunt; lore region oblique, slightly concave; nostril oval, slightly protuberant, located at the middle between snout tip and eye; IND (4.3 mm) equal to IOD and wider than

UEW (3.6 mm); pineal spot absent; pupil oval, horizontal; tympanum distinct (TD 2.3 mm), rounded, slightly greater than half of ED, nearly equal to the distance between eye and nostril (DNE 2.2 mm); supratympanic fold distinct, curves from posterior edge of eye to insertion of arm; vomerine teeth in two oblique series touching inner front edge of choanae, separated by space almost equal to length of each series; choanae oval; tongue attached anteriorly and notched posteriorly; single external vocal sac, with a sac slit opening on floor of mouth at each corner.

Relative length of fingers I \leq II \leq IV \leq III; tips of all fingers expanded into discs with circum-marginal grooves; nuptial pad present on first finger; fingers webbed one third, webbing formula I2–2II2–3III2–2IV; lateral fringe on free edge of all fingers; subarticular tubercles prominent and rounded, formula 1, 1, 2, 2; supernumerary tubercles present; inner metacarpal tubercle large, ovoid, outer metacarpal tubercle smaller in size, flattened; white dermal fringe along outer edge of forearm present, not well developed.

Hind limbs relatively short, heels do not meet when legs positioned at right angle to body; tibiotarsal articulation reaching posterior margin of eye; relative length of toes I < II < V < III < IV; tibia (TL 14.3 mm) 39.7% of body size, shorter than foot (FL16.2 mm); tips of toes expanded into discs with circum-marginal grooves, smaller than finger discs; toes webbed, webbing formula I2–2II1.5–2.5III2–3IV3–1.5V; subarticular tubercles prominent and rounded, formula 1, 1, 2, 3, 2; supernumerary tubercles present; inner metatarsal tubercle oval, prominent; outer metatarsal tubercle absent; white dermal fringe along outer edge of tibia, tarsus and fifth toe.

Dorsal surface of body and head smooth; dorsolateral folds absent; throat smooth; chest, belly and ventral surface of thigh granular; a few white warts around vent.

Colouration in life. Iris yellowish-brown with dark wash; dorsal surface green; side of head and tympanic region green; lower part of flanks cream mottled with greyish-brown; throat black; venter and chest creamwhite mottled with yolk yellow; limbs dorsally green and ventrally light yellow; anterior and posterior of thigh light yellow; large black blotches in axilla, groin and posterior



Figure 4. Views of the holotype (GXNU YU20160340) in life and in preservative. Photos by Guohua Yu.



Figure 5. Views of vomerine teeth, hand and foot of the holotype. Photos by Guohua Yu.

part of thigh; white fringes along outer edge from elbow to the fourth finger and from tibiotarsal articulation to the fifth toe; webbing between fingers and toes grey; dorsal surface of discs greyish-brown.

Colouration in preservative. Dorsal surface violet; lower part of flanks and ventral surface of body and limbs white.

Sexual dimorphism. The new species is sexually dimorphic with females being distinctly larger than males (Table 2) and having no vocal sac and nuptial pad. Males have external single subgular vocal sac and light yellow nuptial pad on base of finger I.

Morphological variation. The colour pattern varied amongst individuals. The paratype GXNU YU20160267 has no black blotches on both anterior and posterior parts of the thigh (Fig. 6a, b). The holotype (GXNU YU20160340) and two paratypes (GXNU YU20160267 and GXNU YU20160338) have no black blotches on tibia and tarsal, paratypes GXNU YU20160268 and GXNU YU20160269 have black blotches on tarsal, but have none on tibia and the other four paratypes (GXNU YU20160355, YU20160336, YU20160335 and YU20160337) have black blotches on both tibia and tarsal. The two specimens from Longling have no black blotches on tibia and tarsal. Dorsal surfaces are uniformly green in all types with the exception of GXNU YU20160335, which has small yellow spots scattered on the dorsal surface of the body and limbs (Fig. 6c). In addition, colouration of the new species in life can vary as day period and microhabitat change; dorsum is lighter nocturnally than during the day, with dorsal surfaces appearing light to dark green.

Distribution and ecology. The new species is currently known from the type locality (Mt. Mopan, Xinping, Yunnan) and Longling, Yunnan, China. Breeding was observed from April to July at the type locality. In April, adult males assembled and called on ground beside permanent pools (Fig. 7a) and the eggs were laid in white foam nests deposited in mud near the pool (Fig. 7b); in mid-July, sparse calls of this species were heard. *Zhangixalus puerensis* and *Hyla anectans* (Jerdon, 1870) were found at the same site. The Jinping and Pingbian popula**Comparison.** Phylogenetically, the new species is closely related to *Zhangixalus nigropunctatus* and *Z. melanoleucus. Zhangixalus yunnanensis* sp. nov. can be distinguished from *Z. nigropunctatus*, with which the new species has previously been confused, by yellowish-brown iris (vs. yellowish-gold; Fig. 8 and Table 6), head obviously wider than long (vs. head width nearly equal to head length), tibiotarsal articulation reaching posterior corner of eye (vs. reaching posterior or edge of tympanum), wider internarial space (mean IND/SVL in males $12.1\% \pm 0.0043$ [11.6%-12.8%, n = 7] vs. $10.2\% \pm 0.0047$ [9.51%-10.85%, n = 6]), longer hind-limb (mean TFL/SVL in males $65.7\% \pm 0.0223$ [62.92%-69.65%, n = 7] vs. $62.9\% \pm 0.0167$



Figure 6. Views of the paratype GXNU YU20160267 in preservative (**a**, **b**) and the paratype GXNU YU20160335 in life. Photos by Guohua Yu.



Figure 7. Habitat of the new species at the type locality (a) and foam nests of the new species (b). Photos by Guohua Yu.

Table 6. Morphological comparison between the new species and congeners of *Zhangixalus*. Characters are: (1) dorsum colour: 0 = solid green or green with a few small light spots, 1 = green mottled with brown, 2 = green mottled with black, 3 = brownish; (2) black blotches in axilla, groin and thigh: 0 = present, 1 = absent; (3) vocal sac: 0 = external, 1 = internal, 2 = absent; (4) throat: 0 = blackish, 1 = not blackish; (5) development of finger web: 0 = webbed one third, 1 = webbed half, 2 = entirely webbed, 3 = rudimentary; (6) development of toe web: 0 = webbed half, 1 = entirely webbed, 2 = webbed 2/3, 3 = webbed 1/3. "?" means unknown.

Species Body size		/ size	1	2	3	4	(5)	6	Webbing color	Iris color
	Male	Female	-							
Z. yunnanensis sp. nov.	31.3–36.0	47.6-48.6	0	0	0	0	0	0	greyish	yellowish-brown
Z. achantharrhena	36.5-40.6	47.1	2	1	0	1	1	2	mottled red and green	dull red edged with silvery white
Z. chenfui	33–41	46–55	0	1	0	1	0	0	pale brownish-yellow	reddish-orange
Z. dorsoviridis	31.3-42.4	37.9–42.8	0	0	1	1	0	0	greyish-brown	orange red
Z. dulitensis	38.4–39.7	?	1	1	?	1	2	1	webbing between outer two toes red	reddish
Z. hungfuensis	30.8–36.8	45.5	0	1	0	1	0	0	greyish	greenish
Z. jarujini	33.7–40.0	41.5-46.1	3	1	1	1	0	1	reddish-orange	yellow
Z. jodiae	34.8–39.8	?	0	0	0	1	0	0	cream with black blotches	silver
Z. leucofasciatus	35-48.2	?	0	1	?	1	2	1	greyish black or greyish-purple	yellowish
Z. lishuiensis	34.2-35.8	45.9	0	1	1	1	0	0	golden yellow	yellow
Z. melanoleucus	34.4-36.3	53.7	0	0	0	0	0	2	cream with black blotches	reddish-orange
Z. minimus	21.3-33.0	31.7–38.3	0	1	0	0	3	3	grey mottled with dark blotches	yellowish-gold
Z. moltrechti	36.3-42.4	44.7–54.6	0	0	0	1	1	1	orange red spotted with black	red or red brown
Z. nigropunctatus	32.0-37.0	44.0-45.0	0	0	0	0	3	0	greyish	yellowish-gold
Z. pinglongensis	32.0-38.5	?	0	0	0	1	1	1	tangerine	silver
Z. puerensis	35.5-41.1	52.0-55.2	1	0	0	0	0	0	grey mottled with dark blotches	yellowish-gold
Z. schlegelii	32–43	43–53	0	0	1	1	1	2	purple	yellowish-gold
Z. suffry	38.5-52.9	31.5-61.0	0	1	?	1	2	1	orange red	yellowish-gold
Z. taipeianus	30.4-37.9	39.1-43.0	0	1	0	1	3	1	yellow	yellowish-gold
Z. wui	35.2-38.2	48.6	1	0	1	1	3	3	greyish-brown	yellowish-gold
Z. yaoshanensis	31.6-36.4	49.2-51.1	0	1	0	1	3	1	greyish	pale yellowish-gold
Z. zhoukaiyae	27.9-37.1	42.1-44.7	0	0	0	1	1	2	greyish	golden-yellow
Z. amamiensis	45–56	65–76	0	0	1	1	0	1	purple	greenish
Z. arboreus	42–60	59–82	0	0	1	1	1	1	purple	reddish
Z. arvalis	39.0-46.4	59.5-64	0	1	0	1	1	0	pink	yellow
Z. aurantiventris	47.8–53.9	?	0	1	1	1	1	2	orange-red	pale yellow
Z. burmanus	54–72	66–82	1	0	1	1	0	2	dull brown	greenish
Z. dennysi	68–92	83–109	0	1	1	1	1	1	brown	yellowish-gold
Z. duboisi	>61.5	?	1	0	2	1	1	2	black and white	dark gold
Z. dugritei	41.5-45.4	57.7-64.3	1	0	0	1	1	2	black and grey	yellowish-brown
Z. feae	86-111	68–116	0	1	1	1	2	1	green	green-gold
Z. franki	77.9–85.8	?	1	1	?	1	2	1	grey	bronze
Z. hongchibaensis	46.5-49.7	55.3	1	0	1	1	3	3	grey	yellowish-brown
Z. hui	40-45.4	?	1	0	0	1	3	3	black and grey	reddish-brown
Z. omeimontis	52.0-65.5	70.0–79.5	1	0	1	1	1	1	yellowish	yellowish-gold
Z. owstoni	42-51	?	0	0	1	1	1	2	yellow	yellowish-gold mottled with silver
Z. pachyproctus	73.4–78.2	102.4	0	1	1	1	2	1	pale blue black	bronze
Z. prasinatus	44.8-58.5	63.9–66.9	0	0	0	1	1	1	purple	yellowish-gold
Z. prominanus	50.5-51.3	?	1	1	?	1	1	1	red	reddish
Z. smaragdinus	76.3–79.6	?	0	1	1	1	2	1	blue black	yellowish-gold
Z. viridis	41–54	52–68	0	0	1	1	1	2	red	yellow
Z. yinggelingensis	43.0-43.4	?	0	0	?	1	0	0	red	silver



Figure 8. Irises of the new species (a. Photo by Guohua Yu), Z. nigropunctatus (b. Photo by Guohua Yu) and Z. melanoleucus (c. reproduced from Brakels et al. (2023)).

[60.8%-65.0%, n = 6]), shorter head (mean HL/SVL in males $32.3\% \pm 0.0068$ [31.0%-32.8%, n = 7] vs. $34.6\% \pm$ 0.0082 [33.6%-35.5%, n = 6]), wider upper eyelid (mean UEW/SVL in males 9.5% ± 0.0041 [8.9%-10.0%, n = 7] vs. 7.9% \pm 0.0034 [7.5%–8.4%, n = 6]), wider head (mean HW/SVL 37.1% ± 0.0122 [35.3%-38.6%, n = 7] vs. $35.2\% \pm 0.0092$ [34.0%–36.0%, n = 6]), smaller tympanum (mean TD/SVL 6.2% ± 0.0021 [5.9%-6.5%, n = 7] vs. $6.9\% \pm 0.0026$ [6.6%–7.3%, n = 6]) and smaller DNE (mean DNE/SVL 6.4% ± 0.0038 [5.9%-7.0%, n = 7] vs. $6.9\% \pm 0.0025$ [6.7%–7.3%, n = 6]) (Tables 1, 4; Fig. 3); and from Z. melanoleucus by yellowish-brown iris (vs. reddish-orange; Fig. 8), shorter head (mean HL/ SVL in males $32.3\% \pm 0.0068$ [1.0%-32.8%, n = 7] vs. $36.8\% \pm 0.0163$ [34.9%–38.9%, n = 4]), shorter snout (mean SL/SVL in males $15.4\% \pm 0.0026$ [14.9%–15.7%, n = 7] vs. 16.7% ± 0.0072 [15.7%–17.4%, n = 4]), wider upper eyelid (mean UEW/SVL in males $9.5\% \pm 0.0041$ [8.9%-10.0%, n = 7] vs. $8.9\% \pm 0.0027$ [8.5%-9.1%,n = 4]) and smaller eye (mean ED/SVL 11.8% ± 0.0034 [11.2%-12.2%, n=7] vs. $12.6\% \pm 0.0058$ [11.9%-13.1%, n=7]n = 4]) (Fig. 3; Table 5).

In body size, besides Zhangixalus nigropunctatus and Z. melanoleucus, the new species is relatively similar to Z. achantharrhena (Harvey, Pemberton & Smith, 2002), Z. chenfui, Z. dorsoviridis, Z. dulitensis (Boulenger, 1892), Z. hungfuensis (Liu & Hu, 1961), Z. jarujini (Matsui & Panha, 2006), Z. jodiae (Nguyen, Ninh, Orlov, Nguyen & Ziegler, 2020), Z. leucofasciatus (Liu & Hu, 1962), Z. lishuiensis (Liu, Wang & Jiang, 2017), Z. minimus (Rao, Wilkinsonand & Liu, 2006), Z. moltrechti (Boulenger, 1908), Z. pinglongensis, Z. puerensis, Z. schlegelii (Günther, 1858), Z. suffry (Bordoloi, Bortamuli & Ohler, 2007), Z. taipeianus (Liang & Wang, 1978), Z. wui (Li, Liu, Chen, Wu, Murphy, Zhao, Wang & Zhang, 2012), Z. yaoshanensis and Z. zhoukaiyae (Table 4). The new species can be easily distinguished from Z. achantharrhena, Z. dulitensis, Z. jarujini, Z. puerensis and Z. wui by dorsum uniformly green (vs. green with black and white spots in Z. achantharrhena, yellowish-green with a few purplish dots on head and back and a purplish line round snout in Z. dulitensis, brownish with dark marking in Z. jarujini, green with many reddish-brown blotches edged with dark brown in Z. puerensis and dark yellowish-brown to light green with numerous light-brown spots in Z. wui); from Z. achantharrhena, Z. chenfui, Z. dulitensis, Z. hungfuensis, Z. jarujini, Z. leucofasciatus, Z. lishuiensis, Z. minimus, Z. suffry, Z. taipeianus and Z. yaoshanensis by having black blotches in axilla, groin and posterior part of thigh (vs. absent); from Z. dorsoviridis, Z. jarujini, Z. lishuiensis, Z. schlegelii and Z. wui by vocal sac external (vs. internal); from Z. achantharrhena, Z. chenfui, Z. dorsoviridis, Z. dulitensis, Z. hungfuensis, Z. jarujini, Z. jodiae, Z. leucofasciatus, Z. lishuiensis, Z. moltrechti, Z. pinglongensis, Z. schlegelii, Z. suffry, Z. taipeianus, Z. wui, Z. yaoshanensis and Z. zhoukaiyae by throat black (vs. bright yellow in Z. achantharrhena, purplish flesh in Z. chenfui, yellow in Z. dorsoviridis and Z. taipeianus, cream in Z. hungfuensis and Z. jarujini, greyish in Z. jodiae and Z. yaoshanensis, white in Z. dulitensis, Z. leucofasciatus, Z. lishuiensis, Z. moltrechti and Z. zhoukaiyae, white with slightly grey background in Z. pinglongensis, cream-white in Z. schlegelii and Z. suffry and creamy-white with greyish-brown blotches in Z. wui); from Z. achantharrhena, Z. chenfui, Z. dulitensis, Z. jarujini, Z. jodiae, Z. lishuiensis, Z. minimus, Z. moltrechti, Z. pinglongensis, Z. puerensis, Z. schlegelii, Z. suffry and Z. taipeianus by webbing greyish (vs. having red colour in Z. achantharrhena, Z. dulitensis, Z. jarujini, Z. moltrechti, Z. pinglongensis and Z. suffry, pale brownish-yellow in Z. chenfui, mottled with black blotches in Z. jodiae, Z. minimus and Z. puerensis, purple in Z. schlegelii and yellow in Z. lishuiensis and Z. taipeianus); from Z. achantharrhena, Z. dulitensis, Z. leucofasciatus, Z. moltrechti, Z. suffry, Z. taipeianus and Z. zhoukaiyae by fingers webbed one third and toes webbed half (vs. fingers webbed half in Z. achantharrhena, Z. leucofasciatus and Z. zhoukaiyae, fingers entirely webbed in Z. dulitensis, fingers webbed half and toes webbed entirely in Z. moltrechti, fingers webbed entirely and toes webbed fully in Z. suffry and toes webbed entirely in Z. taipeianus); and from Z. achantharrhena, Z. chenfui, Z. dorsoviridis, Z. dulitensis, Z. jodiae, Z. moltrechti and Z. pinglongensis by iris yellowish-brown (vs. dull red, edged with silvery-white in Z. achantharrhena, orange-red in Z. chenfui and Z. dorsoviridis, reddish in Z. dulitensis, silver in Z. jodiae and Z. pinglongensis and red or reddish-brown in Z. moltrechti). The new species further differs from Z. hungfuensis and Z. wui by nuptial pad present on first finger (vs. present on fingers I and II), from Z. jodiae by lacking orange blotches in the groin, thigh and ventral side of the tibia (vs. present) and from Z. pinglongensis by black blotches on flank and hind-limb being fewer in number and discontinuous (vs. a number of black blotches united to be reticular).

The new species is distinguishable from Zhangixalus amamiensis (Inger, 1947), Z. arboreus (Okada & Kawano, 1924), Z. arvalis (Lue, Lai & Chen, 1995), Z. aurantiventris (Lue, Lai & Chen, 1994), Z. burmanus, Z. dennysi (Blanford, 1881), Z. duboisi, Z. dugritei (David, 1872), Z. feae, Z. franki, Z. hongchibaensis (Li, Liu Chen, Wu, Murphy, Zhao, Wang & Zhang, 2012), Z. hui (Liu, 1945), Z. omeimontis, Z. owstoni (Stejneger, 1907), Z. pachyproctus, Z. prasinatus (Mou, Risch & Lue, 1983), Z. prominanus (Smith, 1924), Z. smaragdinus, Z. viridis (Hallowell, 1861) and Z. vinggelingensis (Chou, Lau & Chan, 2007) by smaller body size (Table 4). Moreover, the new species can be easily distinguished from Z. burmanus, Z. duboisi, Z. dugritei, Z. hongchibaensis, Z. hui, Z. omeimontis and Z. prasinatus by dorsal surface uniformly green and no brown stripe along canthus rostralis and supratympanic fold (vs. dorsal surface green with brown blotches and a brown stripe along canthus rostralis and supratympanic fold or dorsum green with brown stripe along canthus rostralis and supratympanic fold); from Z. amamiensis, Z. arboreus, Z. aurantiventris, Z. burmanus, Z. dennysi, Z. feae, Z. hongchibaensis, Z. omeimontis, Z. owstoni, Z. pachyproctus, Z. smaragdinus, Z. viridis and Z. yinggelingensis by external vocal sac (vs. internal vocal sac); and from Z. arvalis, Z. aurantiventris, Z. dennysi, Z. feae, Z. franki, Z. pachyproctus, Z. prominanus and Z. smaragdinus by having black blotches in axilla, groin and anterior and posterior part of thighs (vs. absent).

Discussion

Zhangixalus nigropunctatus has been recorded widely in central and south-western China (e.g. Fei (1999); Fei et al. (2009, 2010)) and north-western Vietnam (Orlov et al. 2012; Li et al. 2012a). However, previous phylogenetic analyses revealed that records of this species actually involve multiple misidentified populations (Yu et al. 2009; Li et al. 2012a; Mo et al. 2016; Pan et al. 2017). In this study, based on molecular and morphological evidence, we revealed that the Xinping population represents a novel lineage of the genus Zhangixalus and previous records of Z. nigropunctatus from Longling, Yunnan belong to it. This result supports the viewpoint of Dufresnes and Litvinchuk (2022) that some populations assigned to Z. nigropunctatus in Yunnan represent a cryptic species and further improves our understanding of the taxonomy and distribution of Z. nigropunctatus complex.

With the Longling population transferred into Z. yunnanensis sp. nov., there are three records of Z. nigropunctatus left in Yunnan, China according to Yang and Rao (2008) and Fei et al. (2010), including Longchuan, Yingjiang and Qiaojia Counties (Fig. 9). Geographically, Longchuan and Yingjiang are very close to Longling, while Qiaojia is closer to the type locality of *Z. nigropunctatus* (Weining, Guizhou) than to the known distribution of the new species. Therefore, we presume that the populations of nominal *Z. nigropunctatus* in Longchuan and Yingjiang likely also belong to the new species and the Qiaojia population probably belongs to true *Z. nigropunctatus* pending further data.

In addition to the new species described here, we found that the taxonomy of the samples ROM 38011 and VNMN 4099 needs further investigation. The specimen ROM 38011 was collected from Sa Pa, Vietnam and initially identified as Z. dorsoviridis (Orlov et al. 2001). However, it obviously differs from other individuals of Z. dorsoviridis from Sa Pa (e.g. ROM 38015) by having a darkened vocal sac instead of yellow (Orlov et al. 2001). Li et al (2012a) found that phylogenetically ROM 38011 is closer to Z. nigropunctatus than to other samples of Z. dorsoviridis and transferred it into Z. nigropunctatus. Orlov et al. (2012) also listed Z. nigropunctatus as a member of Vietnamese rhacophorid frogs. However, Mo et al. (2016) recovered the specimen ROM 38011 as sister to Z. pinglongensis. The specimen VNMN 4099 was collected from Son La, Vietnam and was included as single representative of Z. dorsoviridis in Nguyen et al. (2014). In this study, we revealed that these two samples are sister to each other. Moreover, the clade formed by these two samples did not cluster together with the clade containing topotypes of Z. nigropunctatus or the clade containing the topotype of Z. dorsoviridis; instead, it is sister to the clade comprised of Z. pinglongensis and Z. yaoshanensis



Figure 9. Geographic distribution of Zhangixalus species in Yunnan, China. The map was generated using ArcMap v.10.2 (ESRI Inc.).

with strong support. These findings suggest that probably ROM 38011 and VNMN 4099 are neither *Z. nigropunctatus* nor *Z. dorsoviridis*, but potentially represent one or two distinct species pending further morphological and molecular data. Consequently, the presumption of Poyarkov et al. (2021) that records of *Z. nigropunctatus* from Vietnam are a misidentification with *Z. dorsoviridis* remains debatable. Geographically, the collection site of ROM 38011 (Sa Pa, Vietnam) is adjacent to Yunnan, China. Therefore, it could be expected that this potential cryptic species will also be found in Yunnan.

Including the new species described here, the genus Zhangixalus now contains 42 described species. Amongst these, 30 species are distributed in China and 11 species are known in Yunnan. Yunnan is a mountainous region with an extremely diverse topography and climate, which supports an extremely rich biodiversity and shapes different zoogeographic regions. Generally, six zoogeographic regions were recognised in Yunnan, namely North-western Hengduan Mountains (NHM), Western Hills of Yunnan (WHY), Tropical Hills of Southern Yunnan (THSY), South-eastern Hills of Yunnan (SHY), Northern and Central Yunnan Plateau (NCYP) and North-eastern Hills of Yunnan (NHY) and the three southern zoogeographic regions (WHY, THSY and SHY) located at the northern edge of tropic Asia have the highest overall diversity (Yang and Rao 2008; Wang et al. 2022b). Rhacophorids are conservative in their preferences to ecoregions since they primarily inhabit tropical and subtropical moist broadleaf forests ecoregions (Ellepola and Meegaskumbura 2023). Accordingly, in Yunnan, most members of the genus Zhangixalus are distributed in southern, south-eastern and western Yunnan, with the exceptions of Z. omeimontis and Z. nigropunctatus (Fig. 9). Amongst the three zoogeographic regions, south-eastern Yunnan has the highest diversity of Zhangixalus (six species, namely Z. franki, Z. duboisi, Z. puerensis, Z. dorsoviridis, Z. feae and Z. yunnanensis sp. nov.), followed by western Yunnan (five species including Z. smaragdinus, Z. burmanus, Z. puerensis, Z. yunnanensis sp. nov. and the doubtful records of Z. nigropunctatus in Longchuan and Yingjiang) and southern Yunnan (three species, namely Z. pachyproctus, Z. puerensis and Z. feae) in order (Fig. 9).

Recently, Dufresnes and Litvinchuk (2022) considered *Zhangixalus hui* as a synonym of *Z. dugritei*, noted that *Z. lishuiensis* is likely conspecific with *Z. zhoukaiyae* and *Z. duboisi* is likely conspecific with *Z. omeimontis* and suggested that *Z. schlegelii* covers additional cryptic species just based on genetic divergence at 16S sequences. In this study, we also revealed low genetic divergence between these sister species at the 16S rRNA gene (1.4% between *Z. lishuiensis* and *Z. zhoukaiyae*, 1.4% between *Z. duboisi* and *Z. omeimontis* and 0.4% between *Z. hui* and *Z. dugritei*; Suppl. material 1). However, we consider that the taxonomic rearrangements of Dufresnes and Litvinchuk (2022) should be treated with caution at the present time because they did not consider morphological data and other genetic factors (e.g. hybridisation and

incomplete lineage sorting) that possibly lead to the low divergence between these sister species. For instance, *Z. duboisi* has no vocal sac according to its original description (Ohler et al. 2000), but *Z. omeimontis* has an internal single subgular vocal sac (Fei et al. 2010). Therefore, we consider that more studies are required to resolve these taxonomic confusions and clarify the species diversity of the genus *Zhangixalus*, based on multiple lines of evidence (e.g. morphological and molecular data).

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Supplementary material 1

Genetic distances between *Zhangixalus* species estimated from 16S sequences

Authors: Yuanqiang Pan, Mian Hou, Guohua Yu, Shuo Liu Data type: xls

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