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Species

A new species of *Nanorana* Günther, 1896 (Anura: Dicroglossidae) from western Yunnan, China

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ABSTRACT

A new species of *Nanorana* is described based on five specimens collected from Yongde County, Lincang City, Yunnan Province, China. Phylogenetically, the new species formed a distinct clade closely related to *N. maculosa* and *N. chayuensis*. Morphologically, the new species is most similar to *N. maculosa*, however, it can be distinguished from the latter by having relatively larger eyes, relatively smaller interorbital distance, relatively smoother and tighter skin, and incurved webbing between toes. This study brings the total number of recognized species of the genus *Nanorana* to 34, of which 25 occur in China.

Keywords: mitochondrion gene, morphology, phylogeny, spiny frog, taxonomy

1. INTRODUCTION

The genus *Nanorana* is a widespread, complex, and diverse taxon in the family Dicroglossidae (Qi et al., 2019; Frost, 2025). It is widely distributed in Pakistan, India, Nepal, Bhutan, China, Myanmar, Thailand, Laos, and Vietnam (Ohler and Dubois, 2006; Che et al., 2009, 2010; Li et al., 2010; Fei et al., 2012; Qi et al., 2019; Liu et al., 2021; Tang et al., 2023; Hofmann et al., 2024; Frost, 2025). This genus currently contains 33 species, of which 24 species were recorded in China (Frost, 2025).

The piebald spiny frog *Nanorana maculosa* is a relatively characteristic species in the genus *Nanorana*. The dorsal surface of adults of this species is dark brown, covered with irregular reticular patterns composed of yellowish-green small dots (Liu et al., 1960). This species was initially described from Jingdong County, Yunnan Province, China, and currently, it is considered to be distributed in Jingdong County and nearby Shuangbai and Xinping counties (Yang and Rao, 2008; Fei et al., 2009, 2010, 2012; AmphibiaChina, 2025; Frost, 2025).

During our recent field work in Yunnan Province, China, some specimens that closely resemble *Nanorana maculosa* were collected from Yongde County, Lincang City. Morphological and molecular analysis indicated that these specimens belong to an unnamed species closely related to *N. maculosa*. Herein, we describe and illustrate this new species.

2. MATERIAL AND METHODS

Specimens were collected by hand at night, after being photographed, euthanized, fixed, and then preserved in approximately 75% ethanol. Tissue samples were taken

from the liver and stored in absolute ethanol. All specimens were deposited at Kunming Natural History Museum of Zoology, Kunming Institute of Zoology, Chinese Academy of Sciences (KIZ).

Genomic DNA was extracted from liver tissues. Two mitochondrion genes, namely 12S ribosomal RNA (12S) gene and 16S ribosomal RNA (16S) gene, were amplified. Primers used for 12S were FS01: 5'-AACGCTAAGATGAACCCTAAAAAGTTCT-3' and R16: 5'-ATAGTGGGGTATCTAATCCCAGTTTGTTTT-3' and for 16S were L3975: 5'-CGCCTGTTTACCAAAAACAT-3' and H4551: 5'-CCGGTCTGAACTCAGATCACGT-3' (Qi et al., 2019). Amplification and sequencing were completed by Sangon Biotech (Shanghai) Co., Ltd. All new sequences were deposited in GenBank (Table 1). *Chrysopaa sternosignata* and *Quasipaa spinosa* were used as outgroups according to Hofmann et al. (2023, 2024). The sequences of congeners and out groups were obtained from GenBank.

Table 1. Information of samples used in the molecular analysis of this study.

Species name	Locality	Specimen voucher	12S	16S
Nanorana ouyangorum sp. nov.	Yongde, Yunnan, China	KIZ2025015	PX331600	PX331595
Nanorana ouyangorum sp. nov.	Yongde, Yunnan, China	KIZ2025016	PX331601	PX331596
Nanorana ouyangorum sp. nov.	Yongde, Yunnan, China	KIZ2025017	PX331602	PX331597
Nanorana ouyangorum sp. nov.	Yongde, Yunnan, China	KIZ2025018	PX331603	PX331598
Nanorana ouyangorum sp. nov.	Yongde, Yunnan, China	KIZ2025019	PX331604	PX331599
Nanorana aenea	Sa Pa, Lao Cai, Vietnam	ROM 37984	EU979693	EU979830
Nanorana aenea	Sa Pa, Lao Cai, Vietnam	MNHN 1999.5818	AY880456	AY880443
Nanorana bangdaensis	Basu, Xizang, China	CIB QZ2021033	OR583515	OR583594
Nanorana bangdaensis	Basu, Xizang, China	CIB QZ2021255	OR583537	OR583616
Nanorana blanfordii	Yatung, Xizang, China	SYNU-1507011	MH315954	MH315963
Nanorana chayuensis	Zayü, Xizang, China	SYNU-XZ54	EU979710	DQ118508
Nanorana chayuensis	Zayü, Xizang, China	SYNU-XZ64	EU979709	DQ118509
Nanorana chayuensis	Zayü, Xizang, China	SYNU-XZ67	EU979708	DQ118510
Nanorana chayuensis	Zayü, Xizang, China	SYNU-1608005	MH315953	MH315962
Nanorana conaensis	Cona, Xizang, China	KIZ-YP152	EU979703	EU979834
Nanorana hazarensis	Kotli Sattian, Pakistan	WLM:AH299173	—	MW898167
Nanorana laojunshanensis	Lijiang, Yunnan, China	CIB YN201909226	OR583492	OR583566
Nanorana laojunshanensis	Dali, Yunnan, China	CIB XZ2022260	OR583501	OR583580
Nanorana liebigii	Janakpur, Nepal	A17_12_NME	MN011989	MN012104
Nanorana maculosa	Jingdong, Yunnan, China	YNU-HU2002308	EU979706	EU979835
Nanorana maculosa	Jingdong, Yunnan, China	YNU-HU2002322	EU979707	DQ118512
Nanorana medogensis	Medôg, Xizang, China	SYNU-XZ35	EU979705	DQ118506
Nanorana medogensis	Medôg, Xizang, China	SYNU-XZ75	EU979704	DQ118507
Nanorana minica	Himachal Pradesh, India	WT008	—	OQ079488
Nanorana parkeri	Nyalam, Xizang, China	CIB XJ2021063	OR583538	OR583617
Nanorana parkeri	Namling, Xizang, China	CIB XJ2021230	OR583541	OR583621
Nanorana phrynoides	Yimen, Yunnan, China	YNU-HU20024012	EU979686	EU979825
Nanorana pleskei	Zöiga, Sichuan, China	SCUM045856WD	EU979720	EU979841
Nanorana pleskei	Kangding, Sichuan, China	CIB QZ2021194	OR583471	OR583546
Nanorana polunini	Pangum, Nepal	K1553	—	KR827957
Nanorana quadranus	An, Sichuan, China	SCUM20030031GP	EU979694	EU979831
Nanorana quadranus	Maowen, Sichuan, China	SCUM20045195CJ	EU979695	DQ118514
Nanorana rarica	Nepal	A1961/13_NME	MN012062	MN012202

Nanorana rostandi	Kyirong, Xizang, China	SYNU-1507058	MH315955	MH315964
Nanorana sichuanensis	Huili, Sichuan, China	SCUM20030091GP	EU979685	EU979824
Nanorana taihangnica	Jiyuan, Henan, China	KIZ-HN0709001	EU979724	EU979842
Nanorana taihangnica	Jiyuan, Henan, China	KIZ-HN0709002	EU979725	EU979843
Nanorana unculuanus	Jingdong, Yunnan, China	YNU-HU2002502601	EU979699	DQ118490
Nanorana unculuanus	Jingdong, Yunnan, China	YNU-HU2002502702	EU979700	DQ118491
Nanorana ventripunctata	Shangri-La, Yunnan, China	CIB 5334210020	OR583482	OR583557
Nanorana ventripunctata	Shangri-La, Yunnan, China	SCUM045887WD	EU979717	DQ118501
Nanorana vicina	Murree, Pakistan	WLM:NV289171	—	MW898174
Nanorana yunnanensis	Yongde, Yunnan, China	YNU-HU20011102	EU979691	EU979829
Nanorana zhaoermii	Lhünzê, Xizang, China	SYNU-1706049	MH315947	MH315956
Nanorana zhaoermii	Lhünzê, Xizang, China	SYNU-1706058	MH315948	MH315957
Nanorana xuelinensis	Lancang, Yunnan, China	KIZL2019012	MZ410625	MZ410628
Nanorana xuelinensis	Lancang, Yunnan, China	KIZL2019013	MZ410624	MZ410627
Chrysopaa sternosignata	Paghman, Afghanistan	CUHC 9561	—	OP173778
Quasipaa spinosa	Jiujiang, Jiangxi, China	KIZ-JX0709001	EU979646	EU979798

DNA sequences were aligned in MEGA 11.0.13 (Tamura et al., 2021) using MUSCLE (Edgar, 2004) with the default parameters. Uncorrected pairwise distances (p-distance) between species were calculated in MEGA 11.0.13. The best substitution model GTR + F + I + G4 was selected in ModelFinder (Kalyaanamoorthy et al., 2017) using the corrected Akaike Information Criterion. Bayesian inference and maximum likelihood analysis were used to construct the phylogenetic tree using the selected substitution model. Bayesian phylogenetic inference was performed in MrBayes 3.2.6 (Ronquist et al., 2012), and the Markov chains were run for one million generations and sampled every 100 generations. Maximum likelihood analysis was performed in IQ-TREE 1.6.12 (Nguyen et al., 2015) and the nodal supports were estimated by 1,000 ultrafast bootstrap replicates.

All measurements were taken using digital calipers to the nearest 0.1 mm. Measurement methods followed Qi et al., (2019). The morphometrics and character terminology include SVL: Snout-vent length, from the tip of the snout to the vent; HL: head length, from the posterior corner of the mandible to the tip of the snout; HW: head width, at the greatest cranial width; SL: snout length, from the tip of the snout to the anterior corner of the eye; ID: internasal distance, the distance between the nostrils; IOD: interorbital distance, the least distance between the upper eyelids; UEW: upper eyelid width, the maximum width of the upper eyelid; EHD: eye horizontal diameter; SND: snout to nostril distance, the distance from the tip of the snout to the nostril; END: eye to nostril distance, the distance from the anterior corner of the eye to the nostril; LAL: length of lower arm, from the proximal end of the outer metacarpal tubercle to the elbow joint; HAL: hand length, from the proximal end of the outer metacarpal tubercle to the tip of the finger III; FML: femur length; TIL: tibia length; TFL: length of tarsus and foot, from the proximal end of the tarsus to the tip of the toe IV; FL: foot length, from the proximal end of the inner metatarsal tubercle to the tip of the toe IV.

To compare morphological characteristics between the newly collected specimens and the closely related species, we examined 15 topotypic specimens of *Nanorana maculosa* preserved in KIZ.

3. RESULTS

Bayesian inference and maximum likelihood analyses yielded similar results; only the Bayesian tree was shown with the Bayesian posterior probabilities and maximum likelihood bootstrap values labeled next to the nodes (Figure 1). The newly collected specimens from Lincang City formed a distinct clade closely related to *Nanorana maculosa* and *N. chayuensis* with strong support; however, the genealogical relationships among the novel clade, *N. maculosa*, and *N. chayuensis* were not resolved.

The uncorrected p-distance between the novel clade and *Nanorana maculosa* was 2.1% and the uncorrected p-distance between the novel clade and *N. chayuensis* was 3.1% in 12S gene, both greater than that (2.0%) between *N. maculosa* and *N. chayuensis* (Table 2). The uncorrected p-distance between the novel clade and *N. maculosa* was 1.2% and the uncorrected p-distance between the novel clade and

N. chayuensis was 1.4% in 16S gene, both greater than that (0.9%) between *N. maculosa* and *N. chayuensis* (Table 3).

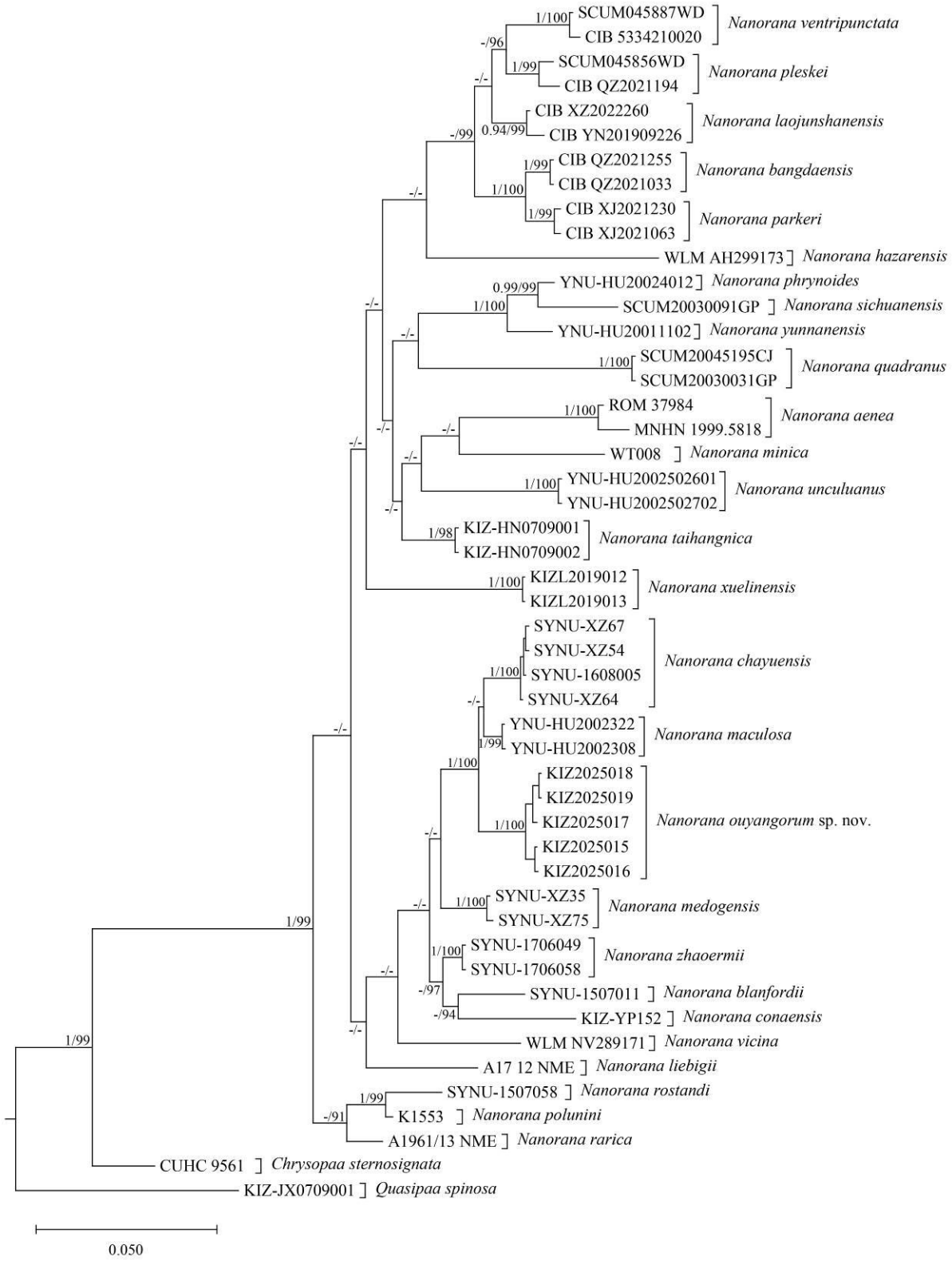

Figure 1. Bayesian phylogeny tree based on concatenated 12S and 16S fragments. Node numbers indicate Bayesian posterior probabilities/ultrafast bootstrap support for maximum likelihood analyses, "-" indicates values below 0.90/90.

Table 2. Uncorrected p-distances (%) calculated from 12S gene sequences.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1 *Nanorana ouyangorum* sp. nov.																						
2 *Nanorana aenea*	7.1																					
3 *Nanorana bangdaensis*	6.3	6.5																				
4 *Nanorana blanfordii*	6.2	7.7	7.0																			
5 *Nanorana chayuensis*	3.1	6.9	4.8	4.9																		
6 *Nanorana conaensis*	6.2	7.7	7.1	5.3	6.6																	
7 *Nanorana laojunshanensis*	6.0	6.1	4.5	6.2	4.9	7.7																
8 *Nanorana liebigii*	4.2	5.8	5.2	5.1	4.6	5.4	5.5															
9 *Nanorana maculosa*	2.1	7.2	4.7	4.8	2.0	5.8	5.5	3.9														
10 *Nanorana medogensis*	3.3	6.1	4.0	3.9	3.3	4.0	5.1	3.5	2.8													
11 *Nanorana parkeri*	6.4	7.0	1.5	6.7	5.1	7.4	4.7	4.7	5.1	4.7												
12 *Nanorana phrynoides*	7.7	6.6	8.4	7.0	7.1	8.3	7.3	6.4	7.1	6.0	8.5											
13 *Nanorana pleskei*	6.2	6.0	4.1	5.2	5.1	6.8	3.1	5.2	5.3	4.7	4.1	7.4										
14 *Nanorana quadranus*	8.5	7.3	8.0	7.8	7.2	8.1	8.3	6.4	7.9	7.0	8.3	7.9	7.6									
15 *Nanorana rarica*	6.5	6.8	6.5	6.1	5.8	6.3	6.2	5.5	5.5	4.8	6.2	6.4	6.1	7.1								
16 *Nanorana rostandi*	6.6	6.2	5.9	6.3	5.5	7.5	5.5	5.6	5.8	5.4	6.0	7.3	5.6	7.6	3.6							
17 *Nanorana sichuanensis*	10.3	9.1	8.7	8.8	8.1	11.4	7.8	9.1	9.3	9.0	8.7	4.0	8.2	9.9	8.6	9.6						
18 *Nanorana taihangnica*	5.7	5.3	5.8	5.3	5.2	6.3	5.1	3.4	4.9	4.0	5.4	5.4	4.5	5.7	5.1	5.3	8.1					
19 *Nanorana unculuanus*	6.2	5.5	6.7	4.8	5.4	6.8	5.4	5.1	4.9	4.0	6.7	5.6	5.4	7.1	4.4	4.8	8.9	3.7				
20 *Nanorana ventripunctata*	6.6	8.3	5.8	6.6	6.8	8.7	4.6	6.8	6.8	6.4	5.8	8.3	3.6	8.9	7.9	7.8	9.7	6.9	6.6			
21 *Nanorana xuelinensis*	5.6	6.2	5.8	5.6	6.0	6.6	6.7	3.6	5.4	4.6	6.4	6.9	6.1	7.1	5.7	5.8	9.6	3.9	6.1	7.6		
22 *Nanorana yunnanensis*	6.7	4.5	6.1	6.2	5.2	8.1	5.3	5.7	5.7	5.6	6.2	1.9	5.2	7.4	5.7	5.7	2.2	4.9	5.7	6.9	6.5	
23 *Nanorana zhaoermii*	3.3	6.0	4.6	3.2	3.2	3.5	4.5	3.0	2.1	1.4	4.5	5.9	3.6	6.3	3.9	4.4	7.8	3.5	3.2	5.4	4.5	5.8

Table 3. Uncorrected p-distances (%) calculated from 16S gene sequences.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
1 *Nanorana ouyangorum* sp. nov.																										
2 *Nanorana aenea*	4.7																									
3 *Nanorana bangdaensis*	3.4	4.1																								
4 *Nanorana blanfordii*	3.2	4.1	2.6																							
5 *Nanorana chayuensis*	1.4	4.7	3.3	2.7																						
6 *Nanorana conaensis*	4.0	4.6	3.4	2.8	3.7																					
7 *Nanorana hazarensis*	6.3	7.2	6.5	6.3	6.5	7.2																				
8 *Nanorana laojunshanensis*	3.9	4.0	1.9	2.3	3.8	3.7	5.6																			
9 *Nanorana liebigii*	4.4	5.2	4.3	4.7	4.7	5.6	8.1	4.0																		
10 *Nanorana maculosa*	1.2	4.4	2.8	2.8	0.9	4.2	6.4	2.9	4.3																	
11 *Nanorana medogensis*	3.0	4.9	3.4	3.2	2.9	4.4	7.1	3.7	4.4	2.6																
12 *Nanorana minica*	6.5	4.8	4.5	5.6	6.5	6.3	7.9	4.5	6.5	5.8	5.9															
13 *Nanorana parkeri*	4.4	4.5	1.2	2.9	4.0	3.3	6.6	1.7	5.2	3.5	4.5	5.5														
14 *Nanorana phrynoides*	5.3	4.2	4.4	4.7	5.2	4.0	8.1	4.1	4.9	5.3	5.5	7.0	4.1													
15 *Nanorana pleskei*	4.4	4.1	2.7	2.8	4.0	4.1	6.2	1.6	4.7	3.7	4.2	4.8	2.7	4.7												
16 *Nanorana polunini*	4.6	6.0	4.6	3.8	4.2	4.4	8.1	4.2	6.6	4.2	3.9	6.9	5.0	6.6	5.1											
17 *Nanorana quadranus*	6.0	5.9	5.4	4.6	6.1	5.7	9.5	5.1	7.2	6.2	5.5	7.9	5.3	5.1	6.0	6.4										
18 *Nanorana rarica*	3.8	4.4	3.4	2.8	3.1	3.1	6.8	3.1	4.6	3.1	3.3	5.6	3.7	4.0	3.9	1.5	5.4									

19 *Nanorana rostandi*	3.9	5.2	3.7	4.4	4.4	3.6	7.4	3.3	5.3	3.9	3.6	6.3	4.0	5.2	4.4	1.5	4.9	2.2								
20 *Nanorana sichuanensis*	5.4	4.4	4.9	4.9	5.7	4.1	7.8	4.2	5.6	5.8	6.1	7.6	4.2	0.9	4.4	7.1	4.8	4.4	5.4							
21 *Nanorana taihangnica*	4.3	3.9	2.5	3.4	4.5	3.6	6.0	2.9	4.6	4.2	4.4	4.3	2.9	4.0	3.0	5.1	6.5	3.6	4.3	4.5						
22 *Nanorana unculuanus*	6.6	4.8	5.7	5.8	6.0	6.5	8.3	5.6	6.8	6.1	6.7	7.6	5.8	5.9	5.7	8.1	7.0	6.0	6.1	5.9	5.4					
23 *Nanorana ventripunctata*	4.0	4.6	1.9	2.6	3.6	3.7	6.4	1.2	4.2	3.5	3.9	5.2	1.7	3.7	1.8	4.9	4.9	3.7	3.7	3.9	3.7	5.5				
24 *Nanorana vicina*	4.2	5.8	4.4	4.4	4.2	4.8	7.9	4.5	5.3	4.0	4.5	5.4	5.3	6.3	4.4	6.1	7.6	4.6	5.3	6.5	5.1	7.8	4.1			
25 *Nanorana xuelinensis*	6.2	5.9	5.3	6.0	6.0	5.6	9.0	4.8	6.6	5.6	5.3	7.6	5.0	5.6	5.8	6.8	6.6	5.7	5.2	6.2	5.5	7.6	5.6	7.1		
26 *Nanorana yunnanensis*	4.7	4.7	4.8	4.7	5.4	4.4	7.7	4.5	5.6	5.4	5.8	6.5	4.8	2.2	4.8	6.3	5.1	4.2	4.9	2.1	4.4	5.8	4.2	6.1	6.2	
27 *Nanorana zhaoermii*	2.4	4.6	3.2	1.5	2.2	3.1	6.2	3.3	4.6	2.4	2.8	6.1	3.5	4.9	3.4	3.7	4.5	2.7	4.5	4.7	4.0	6.3	3.2	3.9	5.7	4.7

***Nanorana ouyangorum* sp. nov.**

(Figures 2–4, 7A, 7B, 8A, 8B)

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Holotype

KIZ2025015, adult male, collected on 23 August 2025 from Jueba Village, Wumulong Township, Yongde County, Lincang City, Yunnan Province, China (24°11'54″N, 99°37′50″E; 2500m asl).

Paratypes

KIZ2025016, adult male; and KIZ2025017–KIZ2025019, three adult females; the same collection information as the holotype.

Specimens examined

Nanorana maculosa (n = 15): KIZ 91065–KIZ 91071, KIZ 91074, KIZ 91075, KIZ 91113, males, Jinping, Jingdong, Pu'er, Yunnan, China; KIZ 91112, KIZ 91115, KIZ 91117, KIZ 91118, KIZ 581254, females, Jinping, Jingdong, Pu'er, Yunnan, China.

Diagnosis

Body size moderate, SVL 72.7–82.2 in adults; skin relatively smooth, dorsal tubercles with no spines; tympanum invisible; dorsal surface dark purple, covered with irregular reticular patterns composed of light yellow spots; forelimbs strongly hypertrophied, keratinized spines present on chest, inner metacarpal tubercle, fingers I to III, and internal vocal sac present in adult males.

Description of holotype

Adult male, habitus stout, SVL 74.9 mm; head slightly broader than long (HW/HL 1.08); snout blunt and rounded in dorsal and lateral views, length greater than eye horizontal diameter (SL/EHD 1.30); canthus rostralis obtuse; tympanum invisible; supratympanic fold distinct, from posterior corner of eye to posterior corner of mandible; eye relatively large (EHD/HL 0.30), pupil slightly rhombic; choanae oval; vomerine teeth distinct; tongue oval, deeply notched posteriorly; vocal sac openings small.

Forelimbs short, strongly hypertrophied; relative finger length III > IV > II ≈ I; finger tips rounded, not dilated; webbing between fingers absent; subarticular tubercles oval, formula 1, 1, 2, 2, distal ones on fingers II and III small and indistinct, others large and distinct; supernumerary tubercles absent; three metacarpal tubercles, inner one large, middle one small, outer one elongate; dorsal surfaces of inner metacarpal tubercle and fingers I, inner surface of finger II, and inner surface of base of finger III with keratinized nuptial spines.

Hindlimbs long and stout; relative toe length IV > III ≈ V > II > I; tips of toes rounded, not dilated; webbing between toes developed, all reached toe tips, slightly incurved between toes I and II and between toes II and III, obviously incurved between toes III and IV and between toes IV and V; lateroventral groove on outer sides of toes I and V present; subarticular tubercles distinct, oval, formula 1, 1, 2, 3, 2, proximal ones large and distal ones small; supernumerary tubercles absent; inner metatarsal tubercles elongate; outer metatarsal tubercle absent; tarsal fold absent.

Dorsal skin of head and limbs smooth with no or a few tubercles; dorsal skin of body slightly rough with some tubercles; ventral skin smooth; chest with two oval clusters of keratinized spines, far apart from each other.

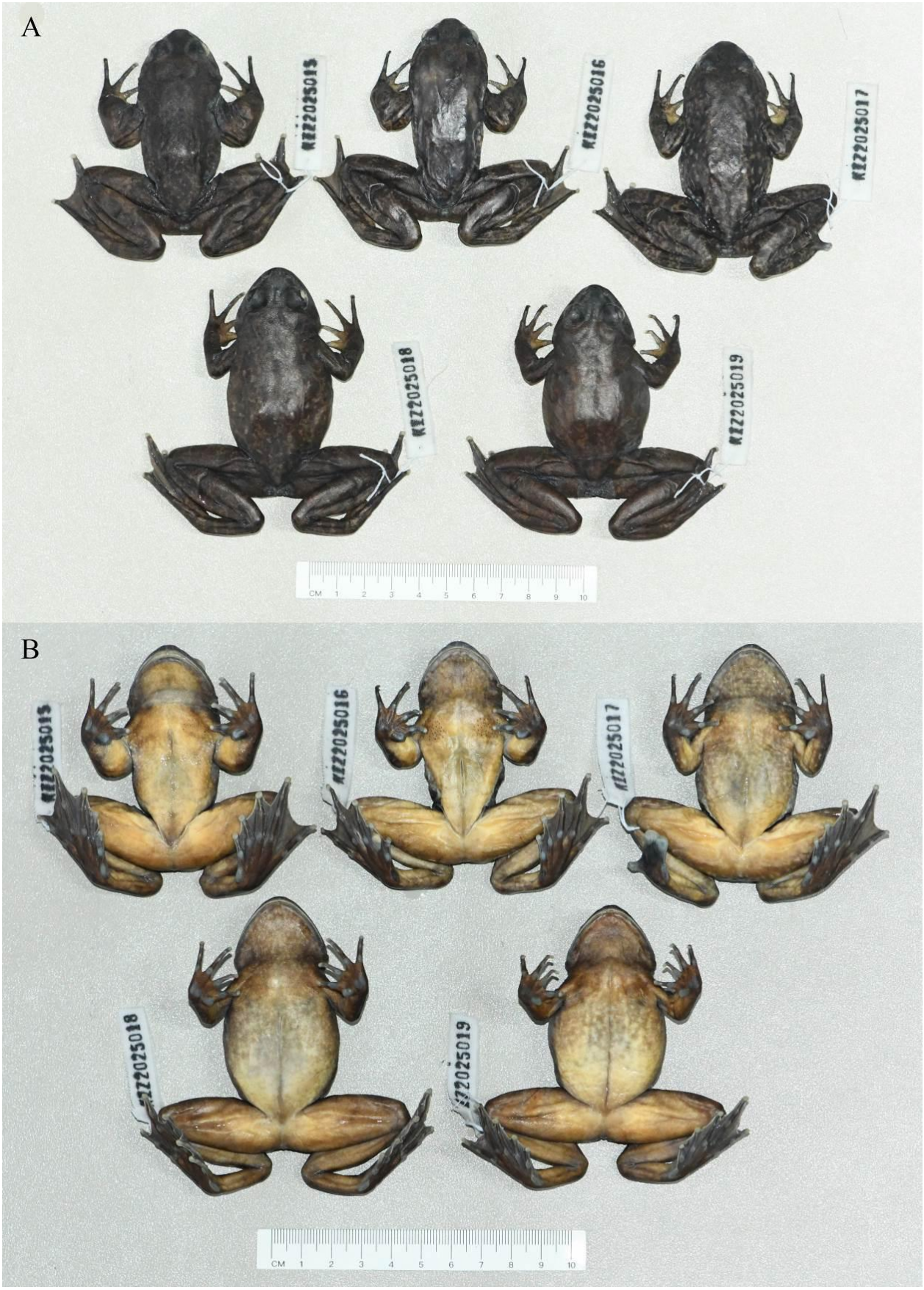

Figure 2. The type specimens of *Nanorana ouyangorum* sp. nov. in preservative. A, Dorsal view; B, ventral view.

Figure 3. The holotype (KIZ2025015) of *Nanorana ouyangorum* sp. nov. in life. A, Dorsal view; B, lateral view; C, ventral view; D, close-up view of the left hand.

Coloration of holotype in life

Dorsal surface of head dark purple with dense small light yellow spots, an indistinct dark transversal stripe between eyes; dorsal surface of body dark purple with many irregular large light yellow spots; dorsal surface of limbs dark purple, light yellow transversal bands indistinct on forelimbs and distinct on hindlimbs; ventral surface of head dark pink; dorsal surface of body and limbs pink; iris yellowish-green with a longitudinal black stripe through pupil.

Variations

Morphological measurements of the type specimens are provided in Table 4. The paratypes quite resemble the holotype except that the forelimbs are not hypertrophied and there are no nuptial spines on chest, inner metacarpal tubercle, and fingers in females.

Etymology

The specific epithet *ouyangorum*, a plural noun in the genitive case, acknowledges the discoverers of the new species. We suggest "Ouyang's spiny frog" as its English common name and "欧阳棘蛙" as its Chinese common name.

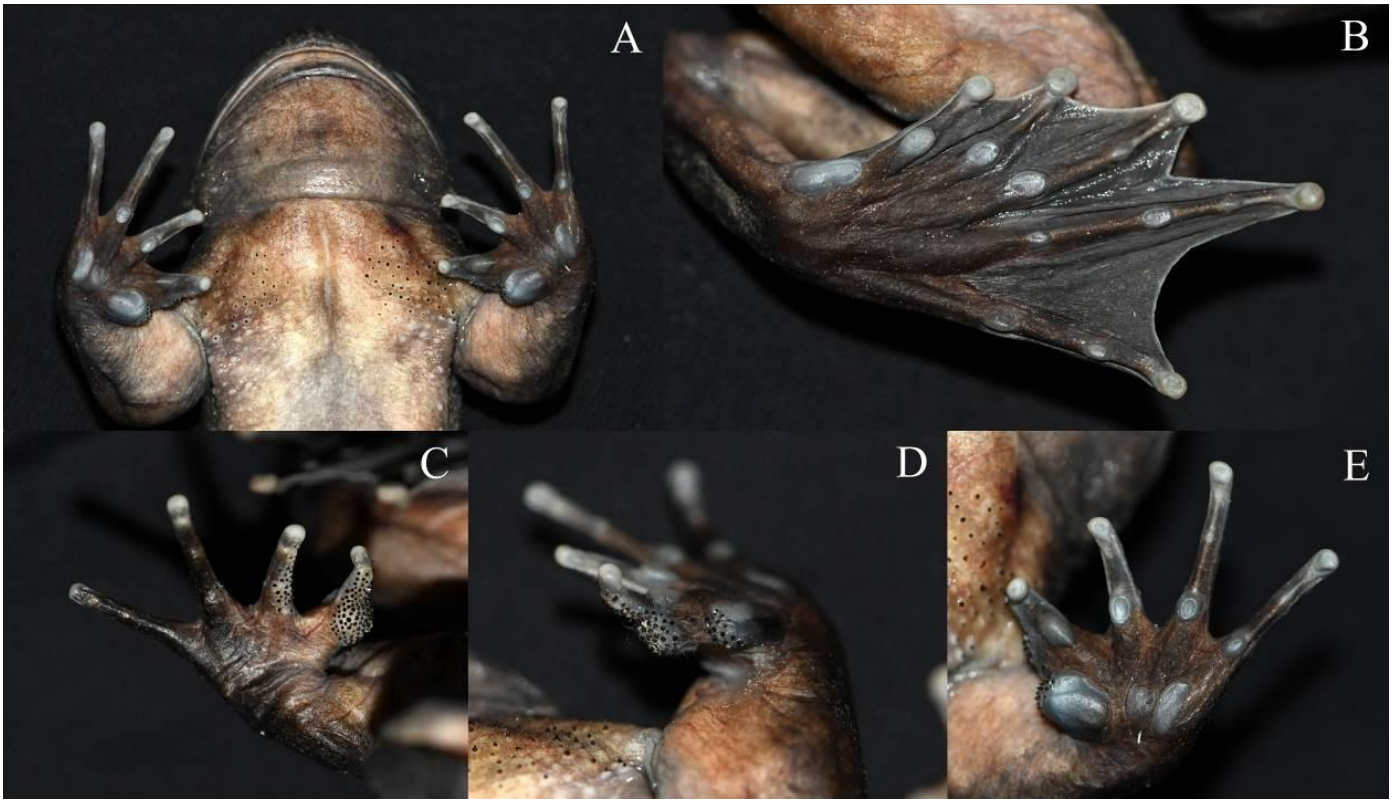

Figure 4. Close-up views of the holotype (KIZ2025015) of *Nanorana ouyangorum* sp. nov. in preservative. A, chest; B, ventral side of the left foot; C, dorsal side of the left hand; D, lateral side of the left hand; E, ventral side of the left hand.

Distribution

This species is currently known to be only distributed in Yongde County, Lincang City, Yunnan Province, China (Figure 5).

Natural history notes

The specimens of the new species were found in a mountain stream (Figure 6). Individuals were observed on stones that on the shore or protruding from water surface in the stream at night. They are alert and immediately jump into the water to hide in the underwater rock crevices when being disturbed. At the type locality, eight other species of amphibians were found, namely *Amolops tuberodepressus*, *Glyphoglossus yunnanensis*, *Leptobrachella niveimontis*, *Nanorana yunnanensis*, *Nidirana occidentalis*, *Rana chaochiaoensis*, *Tylototriton shanjing*, and *Zhangixalus puerensis*.

Comparisons

Nanorana ouyangorum sp. nov. differs from all other species of the genus except *N. maculosa* by its unique coloration: dorsal surface covered with irregular reticular patterns composed of light-yellow spots (Figure 7).

Nanorana ouyangorum sp. nov. further differs from *N. aenea*, *N. annandalii*, *N. ercepeae*, *N. gammii*, *N. liebigii*, *N. polunini*, *N. rarica*, *N. rostandi*, and *N. unculuanus* by the absence of dorsolateral folds (vs. presence).

Nanorana ouyangorum sp. nov. further differs from *N. arnoldi*, *N. feae*, *N. hazarensis*, *N. sichuanensis*, and *N. zhaoermii* by tympanum invisible (vs. visible).

Nanorana ouyangorum sp. nov. further differs from *N. bangdaensis*, *N. laojunshanensis*, *N. parkeri*, *N. pleskei*, and *N. ventripunctata* by the distinction of nuptial spines shape (large and conical spines vs. tiny and compact spines).

Nanorana ouyangorum sp. nov. further differs from *N. blanfordii*, *N. medogensis*, *N. xuelinensis* by nuptialspines present on finger I to finger III in adult males (vs. only on finger I and finger II).

Nanorana ouyangorum sp. nov. further differs from *N. conaensis* and *N. minica* by its larger body size.

Nanorana ouyangorum sp. nov. further differs from *N. kangxianensis*, *N. mokokchungensis*, *N. quadranus*, *N. taihangensis*, and *N. vicina* by the presence of nuptial spines in adult males (vs. absence).

Nanorana ouyangorum sp. nov. further differs from *N. phrynoides* and *N. yunnanensis* by dorsal surface with sparse smooth warty tubercles (vs. dorsal surface with dense warty tubercles bearing small black horny granules).

Nanorana ouyangorum sp. nov. further differs from *N. chayuensis* by tympanum invisible (vs. visible) and dorsal warty tubercles without horny granule (vs. dorsal warty tubercles with small black horny granules).

Nanorana ouyangorum sp. nov. is most similar to *N. maculosa* in morphology and coloration. However, *Nanorana ouyangorum* sp. nov. differs from *N. maculosa* by having relatively larger eyes (EHD/HL 0.29–0.31 vs. 0.22–0.25) and relatively smaller interorbital distance (IOD/EHD 0.57–0.68 vs. 0.75–1.00) (Table 5), loreal region vertical or slightly oblique outward and flat or slightly concave (vs. distinctly oblique outward and distinctly concave), supratympanic fold thin and distinctly oblique downward (vs. thick and slightly or moderate oblique downward), upper lip skin not thickened (vs. significantly thickened and somewhat keratinization), snout tip obtuse (vs. protuberant), skin tight or slightly loose (vs. quite loose), dorsal head skin smooth without tubercles (vs. rough with warty tubercles), webbing between toes incurved (vs. not incurved), and having a relatively longer toe IV (distal subarticular tubercles of toe IV slightly beyond tip of toe III vs. distal subarticular tubercles of toe IV approximately flush with tip of toe III) (Figure 8).

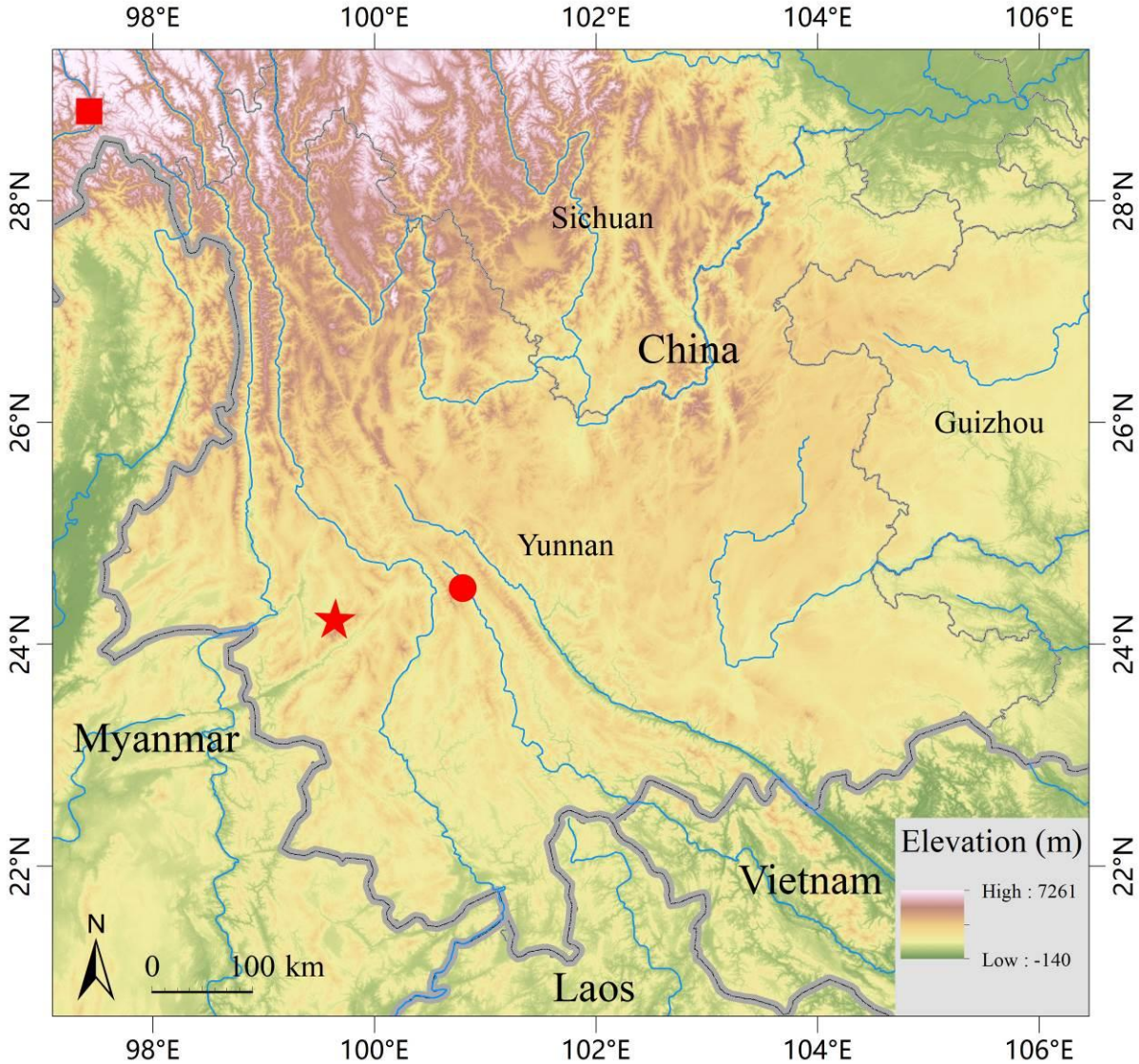

Figure 5. Map showing the type localities of *Nanorana ouyangorum* sp. nov. (red star), *N. maculosa* (red dot), and *N. chayuensis* (red square).

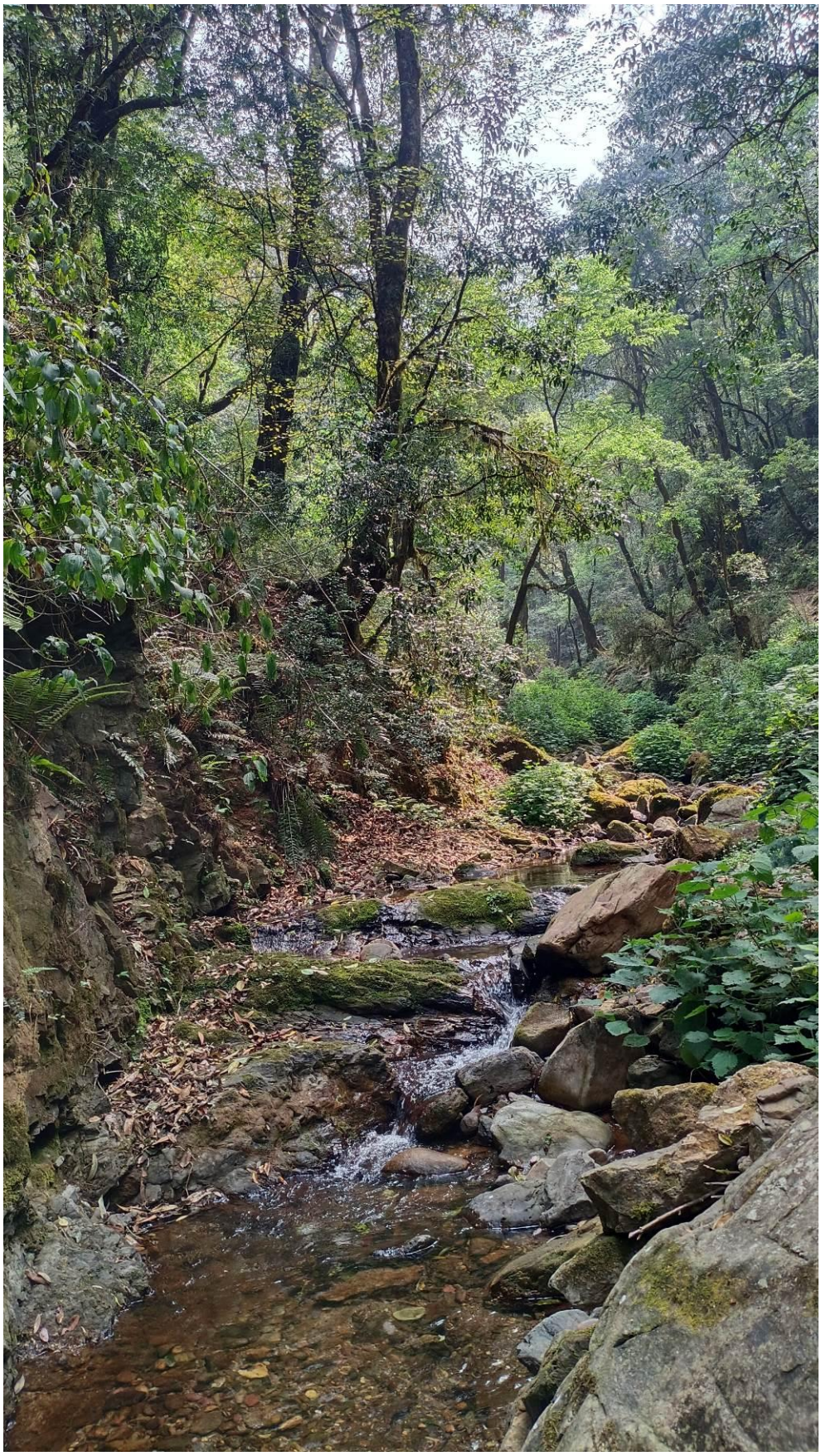

Figure 6. The habitat of *Nanorana ouyangorum* sp. nov. at the type locality.

Figure 7. Comparison of *Nanorana ouyangorum* sp. nov., *N. maculosa*, and *N. chayuensis* in life. A, male *Nanorana ouyangorum* sp. nov.; B, female *Nanorana ouyangorum* sp. nov., C, male *N. maculosa*; D, female *N. maculosa*; E, male *N. chayuensis*; F, female *N. chayuensis*.

Table 4. Measurements (in mm) of the type specimens of *Nanorana ouyangorum* sp. nov. For abbreviations, see the MATERIALS AND METHODS section.

	KIZ2025015 Holotype Male	KIZ2025016 Paratype Male	KIZ2025019 Paratype Female	KIZ2025017 Paratype Female	KIZ2025018 Paratype Female
SVL	74.9	72.7	75.9	76.4	82.2
HL	27	25.5	26.1	27.9	27.4
HW	29.1	27.6	29.3	30	30.6
SL	10.7	10.4	10.3	11.3	10.8
ID	7.9	7.5	7.5	8.3	7.9
IOD	4.9	5.3	4.6	5.3	4.9
UEW	6	5.4	5.6	5.6	6.1
EHD	8.2	7.8	7.9	8	8.6
SND	6.1	5.8	6.3	6.6	6.1
END	4.3	4.5	4.6	4.8	4.2
LAL	19.5	18.5	17.5	17.4	18.8
HAL	19.9	19.4	19.3	20.9	21
FML	43.1	41.7	41.7	43.7	43.3
TIL	43.4	42.1	41.8	44.4	43.2
TFL	61.4	60.8	60	64	63.5
FL	39.7	40.2	39.4	41.9	42.8

Table 5 Comparison between *Nanorana ouyangorum* sp. nov. and *N. maculosa*.

	Nanorana ouyangorum **sp. nov.**		*Nanorana maculosa*	
	Males (n=2)	**Females (n=3)**	**Males (n=10)**	**Females (n=5)**
HL/SVL	0.35-0.36	0.33-0.37	0.34-0.37	0.34-0.35
HW/HL	1.08	1.08-1.12	1.10-1.19	1.11-1.15
SL/HL	0.40-0.41	0.39-0.41	0.36-0.41	0.37-0.40
EHD/HL	0.30-0.31	0.29-0.31	0.23-0.25	0.22-0.25
IOD/EHD	0.60-0.68	0.57-0.66	0.84-0.93	0.75-1.00
LAL/SVL	0.25-0.26	0.23	0.25-0.27	0.21-0.25
HAL/SVL	0.27	0.25-0.27	0.26-0.28	0.24-0.28
FML/SVL	0.57-0.58	0.53-0.57	0.53-0.57	0.51-0.55
TIL/SVL	0.58	0.53-0.58	0.52-0.56	0.49-0.55
TFL/SVL	0.82-0.84	0.77-0.84	0.76-0.82	0.74-0.81
FL/SVL	0.53-0.55	0.52-0.55	0.53-0.58	0.52-0.55

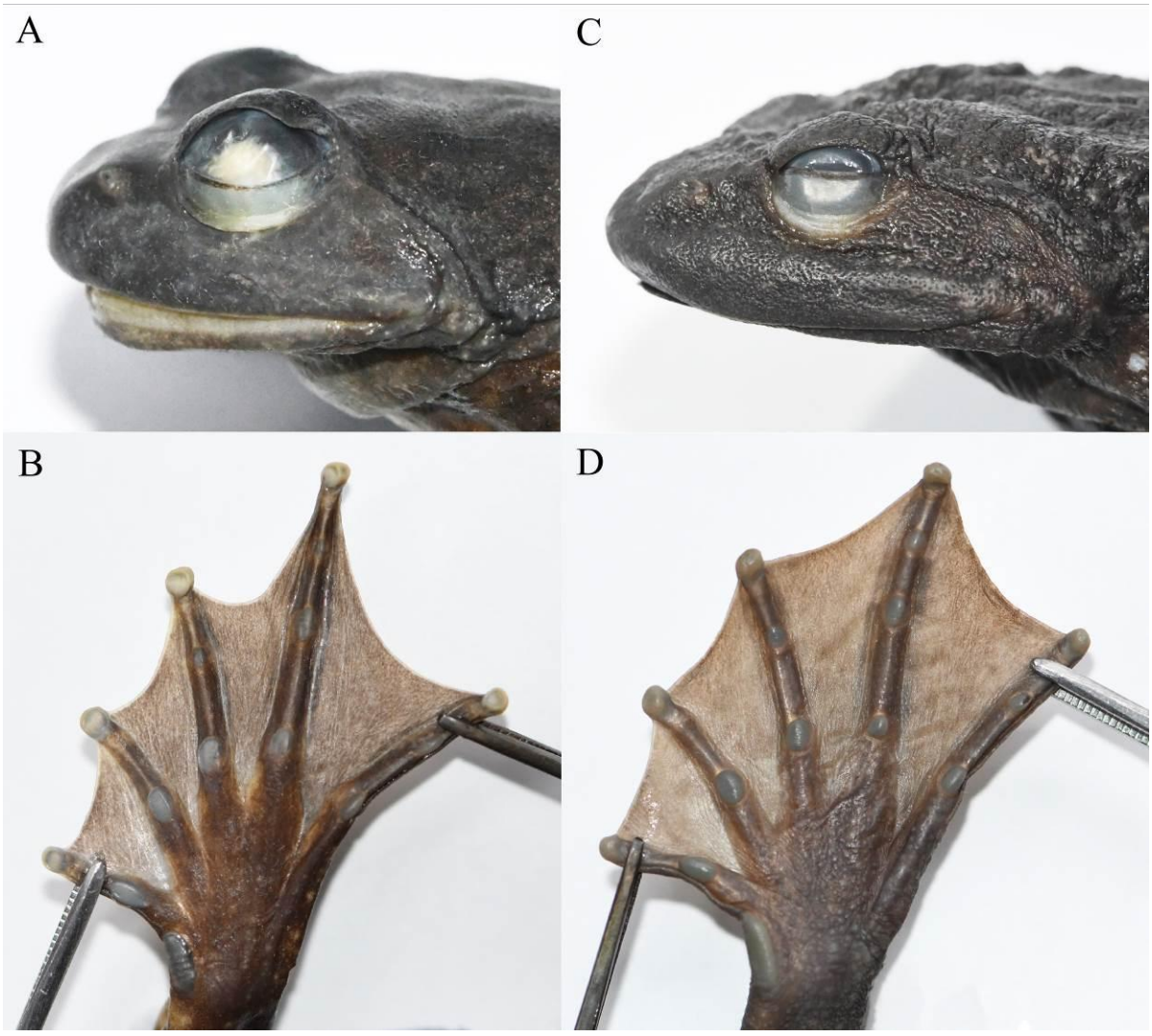

Figure 8 Comparison of the head and foot between *Nanorana ouyangorum* sp. nov. (A and B) and *N. maculosa* (C and D) in preservative.

4. DISCUSSION

In the original description, the type locality of *Nanorana maculosa* was written as Tiechang, Xinmin Township, Jingdong County, Yunnan Province (Liu et al., 1960). However, there has never been Xinmin Township in Jingdong County. Instead, there is a Xinmin Village in Jingdong County, which is under the jurisdiction of Jinping Town. Liu et al. (1960) also mentioned another place, Paozhuqing River, Xinmin Township, Jingdong County, Yunnan Province. Paozhuqing River does exist and is located in Xinmin Village. Therefore, it can be inferred that what Liu et al. (1960) referred to as Xinmin Township is actually Xinmin Village. Here, we clarify that the type locality of *N. maculosa* is in Xinmin Village, Jinping Town, Jingdong County, Yunnan Province, China.

The discovery site of *Nanorana ouyangorum* sp. nov. is similar in altitude to the distribution area of *Nanorana maculosa*, but their habitats are very different. Through our observation, *N. maculosa* lives in relatively large rivers or in deep water areas of streams, while *Nanorana ouyangorum* sp. nov. lives in relatively small, shallow streams. At night, *N. maculosa* usually lies at the bottom of the clear water, or only exposes the head out of the water near the shore, while *Nanorana ouyangorum* sp. nov. usually sits on stones near the stream. Their different habitats and habits are also consistent with their morphological characteristics. *Nanorana maculosa* has a relatively pointed snout, thickened upper lip skin, quite developed webbing between toes, and very loose body skin, which can better adapt to underwater life. On the contrary, compared with *N. maculosa*, the characteristics of *Nanorana ouyangorum* sp. nov. are more adapted to land life. Since their habits are different, it is speculated that their feeding habits should also be different. Subsequent studies can verify whether there are apparent differences in their diet compositions by collecting more specimens of these two species and analyzing their stomach contents.

5. CONCLUSION

Currently, *Nanorana ouyangorum* sp. nov. is known to be only distributed in Yongde County, Lincang City, Yunnan Province, China, and the population status of this species is unclear. Besides, this species is likely also distributed in the neighboring Gengma County of Lincang City. Therefore, the conservation status of this species has yet to be evaluated.

Authors contributions

SL: field survey, writing of manuscript, data analysis; JW: field survey, revising of manuscript; MH: experimental design, data analysis; DR: experimental design, revising of manuscript and proof reads.

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Conflict of Interest

The authors declare that they have no conflicts of interests, competing financial interests or personal relationships that could have influenced the work reported in this paper.

Informed consent

Not applicable.

Ethical approval & declaration

In this article, the animal regulations are followed as per the ethical committee guidelines of Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan 650201, China; the authors reported a new species of *Nanorana* Günther, 1896 (Anura: Dicroglossidae) from western Yunnan, China. The Animal ethical guidelines are followed in the study for species observation, identification & experimentation.

Data and materials availability

All data associated with this study are present in the paper.

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