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A New Species of *Rhacophorus* Kuhl and Van Hassalt, 1822 (Anura: Rhacophoridae) from Southeastern Yunnan, China

Shuo Liu[1], Mian Hou[2], Dingqi Rao[3]* and Song Li[1,4]*

[1]*Kunming Natural History Museum of Zoology, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan 650223, China*
[2]*College of Continuing (Online) Education, Sichuan Normal University, Chengdu, Sichuan 610066, China*
[3]*Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan 650201, China*
[4]*Yunnan Key Laboratory of Biodiversity Information, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan 650201, China*

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Key words
16S rRNA, Malipo, Morphology, Taxonomy, Wenshan

ABSTRACT

We describe a new species of *Rhacophorus* based on morphological and molecular evidence of specimens collected from Malipo County, Wenshan Prefecture, Yunnan Province, China. The new species is morphologically most similar and phylogenetically closely related to *R. laoshan*, but it can be distinguished from the latter by the following morphological characteristics: Relatively greater eye diameter, relatively greater transverse diameter of third-finger disc, tibiotarsal articulation reaching or beyond nostril when hindlimb is adpressed forward, and nuptial pad absent in adult males. In addition, the new species differs from *R. laoshan* by 3.3 % in 16S rRNA sequences.

INTRODUCTION

Frogs of the genus *Rhacophorus* are generically called flying frogs or parachuting frogs, which consisting of 45 species, distributed in India, Bhutan, Myanmar, China, Japan, Thailand, Laos, Cambodia, Vietnam, Malaysia, Indonesia, and Philippines (Li *et al*., 2022; Frost, 2023).

Rhacophorus laoshan is a species which was described based on morphology only from Guangxi Cenwanglaoshan National Nature Reserve, Tianlin County, Baise City, Guangxi Autonomous Region, China (Mo *et al*., 2008). The phylogenetic position of this species had not been resolved since there had been no available molecular data of this species, until Yuan *et al*. (2022) investigated the molecular phylogenetic status of this species using the gene sequence of a specimen collected from its type locality. Currently, *R. laoshan* is recorded from Guangxi Autonomous Region, and Hunan and Yunnan provinces, China (Mo *et al*., 2008; Wu *et al*., 2019; Song *et al*., 2022).

During the field survey in Malipo County, southwestern Yunnan, China (Fig. 1), we collected 13 specimens of *Rhacophorus* resembling *R. laoshan* in dorsal skin smooth, dermal ridges present on forearms and above vent, dermal calcars present at heels, anterior and posterior surface of thighs orange red in color, and tympanum distinct and large. However, they can be distinguished from *R. laoshan* in morphological and molecular characteristics. Therefore, we describe these specimens as a new species of *Rhacophorus*.

MATERIALS AND METHODS

Sampling

Specimens were collected, euthanized, and then fixed in 75% ethanol for long term storage. Some of them were photographed before euthanasia. Liver tissue samples were preserved in analytical pure ethanol for molecular analysis. All specimens were deposited at Kunming Institute of Zoology, Chinese Academy of Sciences (KIZ).

* Corresponding author: lis@mail.kiz.ac.cn, raodq@mail.kiz.ac.cn
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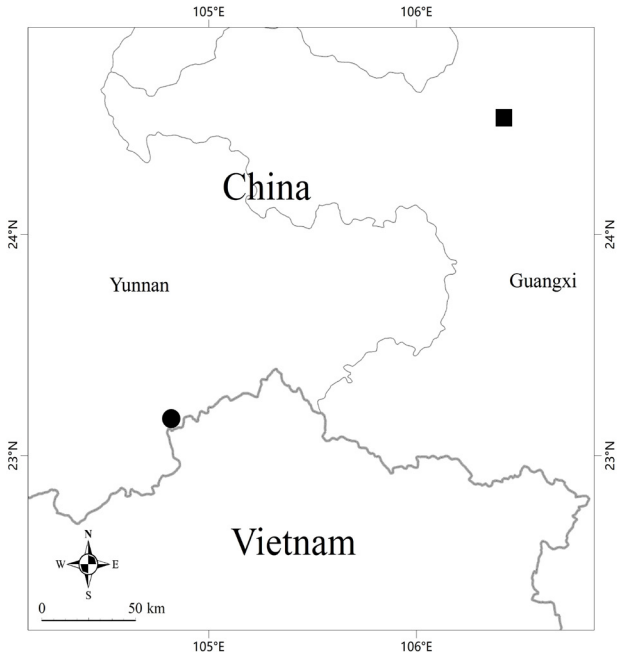

Fig. 1. Map showing the type locality of *Rhacophorus hujianshengi* sp. nov. in Malipo County, southeastern Yunnan, China (black dot) and the type locality of *R. laoshan* in Tianlin County, northwestern Guangxi, China (black square).

Morphology

Measurements were taken with a digital caliper to the nearest 0.1 mm. Morphological terminology followed Mo *et al*. (2008): SVL, snout-vent length; HL, head length, from tips of snout to the commissure of jaws; HWJ, head width at the commissure of jaws; HWG, maximum width of head; SL, snout length, from tip of snout to the anterior corner of eye; INS, internarial space; IOS, interorbital space, the smallest space between the inner edge of upper eyelid; UEW, width of upper eyelid; ED, diameter of eye; TD, horizontal diameter of tympanum; LAHL, length of lower arm and hand; HAL, hand length; HLL, hindlimb length; TL, tibia length; FTL, length of foot and tarsus; FL, foot length; TFDD, third-finger disc transverse diameter. Note that since the head width defined by Mo *et al*. (2008) is measured at the commissure of the jaws, while most others measured at the widest region of the head, we use both methods here to measure the width of the head and use HWJ to correspond to the head width defined by Mo *et al*. (2008) and HWG to correspond to that defined by most others.

Molecular analyses

Total genomic DNA was extracted from liver tissue samples which were digested with proteinase K, and then purified by standard phenol-chloroform separation and ethanol precipitation (Sambrook *et al*., 1989). The fragment encoding partial 16S ribosomal RNA (16S) was amplified via the polymerase chain reaction (PCR) using the primers L2188: 5'–AAAGTGGGCCTAAAAGCAGCCA–3' (Matsui *et al*., 2006) and 16H1: 5'–CTCCGGTCTGAACTCAGATCACGTAGG–3' (Hedges, 1994). The product was purified and sequenced by Tsingke Biotechnology (Beijing) Co., Ltd. All new sequences were deposited in GenBank. Species of the genus *Zhangixalus* were selected as outgroups respectively according to Li *et al*. (2022). Homologous and outgroup sequences were obtained from GenBank (Table I). The technical method for sequences alignment was the same as that in Liu *et al*. (2022), and the computation methods for genetic distance calculation, best substitution model selection, Bayesian inference and maximum likelihood phylogenetic analyses were the same as those in Liu *et al*. (2021).

RESULTS

The obtained sequence alignment is 916 bp in length. Bayesian inference and maximum likelihood analyses showed similar results, the specimens from Malipo County formed a distinct lineage which is sister to *Rhacophorus laoshan* with strong support by Bayesian inference and moderate support by maximum likelihood analyses (Fig. 2). The uncorrected genetic p-distances between the sequences of the specimens from Malipo County and the sequence of *R. laoshan* is 3.3 %, which is approximate to that (3.5 %) between *R. modestus* and *R. poecilonotus* and that (3.6 %) between *R. orlovi* and *R. spelaeus* (Table II).

Rhacophorus hujianshengi sp. nov.
(https://zoobank.org/AE9FF9EF-D7A0-4C11-B9E4-DA7968F1C721)
(Figs. 3–6)

Type material

Holotype: KIZ2020012, adult male, collected on 22 July 2020 by Shuo Liu from Yunling Village, Xiajinchang Township, Malipo County, Wenshan Prefecture, Yunnan Province, China (23°9'19"N, 104°50'15"E, ca 1500 m a.s.l.).

Paratypes: KIZ2020001-KIZ2020004 and KIZ2020013, five adult males, KIZ2020005–KIZ2020011, seven adult females, collection information the same as the holotype.

Etymology

The species name, *hujianshengi*, is a patronym honoring the retired professor of Yunnan University, Dr. Jiansheng Hu. We name the new species after Dr. Hu in recognition of his contributions to the zoological research in Yunnan Province, China. We suggest "Malipo Tree Frog" as its English name, and "麻栗坡树蛙" (Pinyin: má lì pō shù wā) as its Chinese name.

Table I. Samples used in molecular analyses of this study.

Taxon	Voucher No.	Locality	GenBank No.
Rhacophorus annamensis	VNMN 4090	Dak Nong, Nam Nung, Vietnam	LC010566
Rhacophorus baluensis	FM235958	Sabah, Malaysia	KC961089
Rhacophorus bengkuluensis	UTA A-62770	Lampung, Sumatra, Indonesia	KM212948
Rhacophorus bipunctatus	PUCZM/IX/SL360	Mizoram, India	MH087073
Rhacophorus borneensis	BORN:22410	Maliau Basin, Sabah, Malaysia	AB781693
Rhacophorus calcaneus	VNMN 4093	Dak Lac, Chu Yang Sin, Vietnam	LC010573
Rhacophorus catamitus	ENS 14726	Sumatra, Indonesia	KX398877
Rhacophorus exechopygus	VNMN 4107	Kon Ka Kinh, Gia Lai, Vietnam	LC010585
Rhacophorus helenae	AMS R 173230	Binh Thuan, Vietnam	JQ288087
Rhacophorus hoabinhensis	IEBR A.2016.18	Hoa Binh, Vietnam	LC331096
Rhacophorus hoabinhensis	VNMN A.2016.16	Hoa Binh, Vietnam	LC331097
Rhacophorus indonesiensis	MZB:Amp:23619	Indonesia	AB983367
Rhacophorus kio	VNMN 4110	Kon Ka Kinh, Gia Lai, Vietnam	LC010589
Rhacophorus laoshan	1705014	Tianlin, Guangxi, China	MW149528
Rhacophorus lateralis	SDB.2010.330	Karnataka, Bygoor, India	KC571277
Rhacophorus malabaricus	Rmal-In	Madikeri, India	AB530549
Rhacophorus margaritifer	ENS 16162	Java, Indonesia	KX398889
Rhacophorus modestus	ENS 16853	Sumatra, Indonesia	KX398904
Rhacophorus napoensis	GXNU YU000171	Napo, Guangxi, China	ON217796
Rhacophorus nigropalmatus	Rao081203	Malaysia	JX219438
Rhacophorus norhayatiae	NNRn	Johor, Endau Rompin, Malaysia	AB728191
Rhacophorus orlovi	VNMN 3067	Huong Son, Ha Tinh, Vietnam	LC010598
Rhacophorus orlovi	VNMN 4114	Xuan Lien, Thanh Hoa, Vietnam	LC010597
Rhacophorus pardalis	FMNH273243	Sarawak, Bintulu, Malaysia	JX219454
Rhacophorus poecilonotus	ENS 16480	Sumatra, Indonesia	KX398920
Rhacophorus pseudomalabaricus	SDB.2011.1010	Kerala, Kadalar, India	KC593855
Rhacophorus reinwardtii	ENS 16447 (UTA)	Sumatra, Bandung, Indonesia	KY886335
Rhacophorus rhodopus	SCUM 060692L	Mengyang, Yunnan, China	EU215531
Rhacophorus robertingeri	VNMN 4123	Kon Ka Kinh, Gia Lai, Vietnam	LC010613
Rhacophorus robertingeri	VNMN 3446	Kon Plong, Kon Tum, Vietnam	LC010615
Rhacophorus spelaeus	IEBR A.2011.1	Khammouan, Lao	LC331095
Rhacophorus translineatus	Rao6237	Medog, Tibet, China	JX219449
Rhacophorus tuberculatus	KIZ014154	Medog, Tibet, China	MW111522
Rhacophorus tuberculatus	Rao6254	Medog, Tibet, China	JX219436
Rhacophorus vampyrus	VNMN 4125	Hon Ba, Khanh Hoa, Vietnam	LC010616
Rhacophorus hujianshengi sp. nov.	KIZ2020001	Malipo, Yunnan, China	OR449731
Rhacophorus hujianshengi sp. nov.	KIZ2020002	Malipo, Yunnan, China	OR449732
Rhacophorus hujianshengi sp. nov.	KIZ2020003	Malipo, Yunnan, China	OR449733
Rhacophorus hujianshengi sp. nov.	KIZ2020004	Malipo, Yunnan, China	OR449734
Rhacophorus hujianshengi sp. nov.	KIZ2020005	Malipo, Yunnan, China	OR449735
Rhacophorus hujianshengi sp. nov.	KIZ2020006	Malipo, Yunnan, China	OR449736
Rhacophorus hujianshengi sp. nov.	KIZ2020007	Malipo, Yunnan, China	OR449737
Rhacophorus hujianshengi sp. nov.	KIZ2020008	Malipo, Yunnan, China	OR449738
Rhacophorus hujianshengi sp. nov.	KIZ2020009	Malipo, Yunnan, China	OR449739
Rhacophorus hujianshengi sp. nov.	KIZ2020010	Malipo, Yunnan, China	OR449740
Rhacophorus hujianshengi sp. nov.	KIZ2020011	Malipo, Yunnan, China	OR449741
Zhangixalus dennysi	SCUM 060401L	Shaoguan, Guangdong, China	EU215545
Zhangixalus dugritei	SCUM 051001L	Baoxing, Sichuan, China	EU215541

Table II. Uncorrected pairwise distances (%) among species of *Rhacophorus* calculated from 16S rRNA 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	27	28	29	30	31
1 *Rhacophorus hujianshengi* sp. nov.																															
2 *R. annamensis*	10.6																														
3 *R. baluensis*	11.6	10.6																													
4 *R. bengkuluensis*	10.0	11.1	11.8																												
5 *R. bipunctatus*	12.6	10.4	10.1	10.3																											
6 *R. borneensis*	12.8	9.9	8.7	7.8	9.0																										
7 *R. calcaneus*	9.7	10.9	10.6	10.6	10.0	10.2																									
8 *R. catamitus*	9.6	12.2	12.3	8.5	11.4	10.5	10.4																								
9 *R. exechopygus*	13.0	7.2	11.8	11.1	9.5	10.4	10.0	13.2																							
10 *R. helenae*	9.7	11.1	8.5	8.7	7.8	5.1	11.3	10.4	12.0																						
11 *R. hoabinhensis*	6.3	9.5	10.4	10.7	13.4	13.1	9.3	10.1	13.2	9.7																					
12 *R. indonesiensis*	10.4	13.1	11.6	9.9	10.8	10.5	9.6	9.6	11.5	11.4	11.2																				
13 *R. kio*	11.1	11.1	11.3	10.4	9.0	6.2	11.5	12.9	11.3	5.3	11.8	12.0																			
14 *R. laoshan*	3.3	10.2	10.9	10.9	12.8	13.0	8.1	9.7	11.9	9.7	6.2	9.4	11.1																		
15 *R. lateralis*	11.3	13.8	12.7	11.1	12.3	9.8	11.8	12.2	14.3	11.5	12.0	11.8	12.7	11.8																	
16 *R. malabaricus*	14.0	12.5	13.4	11.4	14.0	12.7	12.0	11.3	13.7	12.0	12.9	11.6	13.9	13.4	12.1																
17 *R. margaritifer*	9.4	11.3	11.7	4.2	10.6	8.4	9.9	8.3	12.7	8.8	9.9	9.6	10.4	10.2	12.3	11.0															
18 *R. modestus*	8.6	9.4	9.9	6.9	8.8	7.7	9.5	7.7	10.4	8.2	8.8	9.1	10.4	7.7	11.5	8.1	5.8														
19 *R. napoensis*	12.6	10.2	10.0	9.4	7.6	6.1	10.7	11.1	10.7	6.7	13.1	9.8	7.7	12.5	11.8	12.4	9.5	7.9													
20 *R. nigropalmatus*	13.8	9.9	11.6	9.9	14.4	12.9	11.3	11.5	10.2	10.4	14.3	11.4	12.5	13.7	11.7	15.1	9.6	7.6	13.7												
21 *R. norhayatiae*	12.8	9.0	10.4	9.0	9.7	5.1	10.2	10.8	11.1	7.1	13.8	11.4	7.1	12.9	11.3	13.3	7.9	7.9	7.2	14.0											
22 *R. orlovi*	9.6	11.3	12.4	10.3	10.8	10.8	10.8	11.0	12.8	11.9	9.0	11.9	11.5	9.0	12.4	12.0	11.7	10.3	10.9	11.5	10.5										
23 *R. pardalis*	15.5	12.0	13.8	11.7	15.4	14.4	12.5	12.6	14.3	12.7	15.9	13.6	14.3	15.5	12.5	14.4	11.6	11.7	14.9	16.4	15.0	14.5									
24 *R. poecilonotus*	8.8	10.4	9.3	8.2	8.3	7.2	8.3	8.7	10.2	8.4	10.2	7.9	10.4	7.9	11.1	8.7	6.2	3.5	7.8	8.6	7.2	11.5	10.3								
25 *R. pseudomalabaricus*	9.0	11.5	12.1	9.9	10.8	9.2	10.9	10.2	11.8	10.0	10.1	10.8	11.8	10.1	10.2	6.1	10.0	7.9	10.2	11.7	9.0	10.6	11.5	8.7							
26 *R. reinwardtii*	9.4	10.4	9.9	9.5	7.0	4.9	10.4	10.6	10.2	6.2	8.9	11.2	9.0	9.1	11.3	11.0	8.7	8.2	4.8	10.4	3.8	11.0	12.1	7.5	9.7						
27 *R. rhodopus*	14.3	11.1	11.7	10.3	9.6	8.0	11.3	11.0	11.1	9.5	14.3	10.5	10.8	13.9	11.9	13.6	10.0	8.8	7.4	13.4	7.6	11.9	15.9	9.0	10.2	6.6					
28 *R. robertingeri*	7.8	11.4	12.1	10.9	11.5	9.6	9.6	10.6	13.1	11.7	10.3	11.1	11.0	8.0	12.8	13.2	10.9	9.7	10.6	11.8	10.0	10.2	14.0	8.8	10.2	11.0	11.9				
29 *R. spelaeus*	9.0	11.6	12.0	9.9	11.3	10.6	10.2	11.8	13.0	11.3	9.2	11.2	11.8	8.8	12.6	12.2	10.6	10.1	11.3	12.2	11.3	3.6	14.5	10.8	10.3	12.0	12.4	9.9			
30 *R. translineatus*	12.6	9.2	10.0	10.1	11.5	11.5	8.8	10.8	9.2	10.6	12.0	9.8	11.8	12.1	12.3	13.8	9.6	7.8	11.7	13.1	12.4	10.5	14.6	7.9	10.6	10.2	11.1	10.5	10.6		
31 *R. tuberculatus*	9.8	12.5	11.2	11.3	11.7	12.1	10.5	11.6	13.3	9.8	11.3	11.5	10.3	9.8	12.8	14.5	11.0	10.9	11.3	14.2	11.8	7.4	14.7	9.0	10.2	9.6	13.5	10.2	9.0	12.7	
32 *R. vampyrus*	11.9	12.8	12.6	13.5	14.0	10.9	13.0	14.0	14.2	12.8	13.1	14.2	13.2	12.1	13.2	15.0	14.8	12.5	12.6	13.7	13.2	14.3	13.9	13.0	14.6	13.0	15.5	13.6	13.5	12.3	14.1

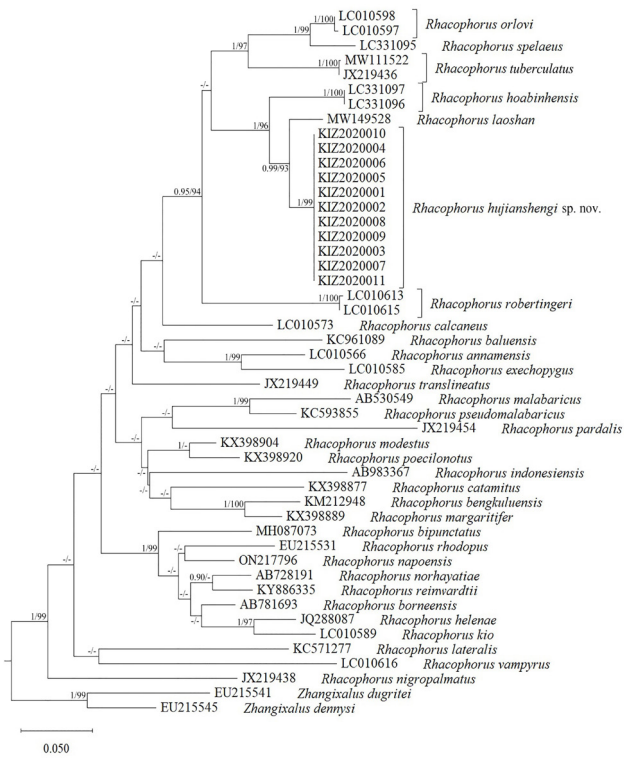

Fig. 2. Maximum likelihood phylogenetic tree of the genus *Rhacophorus* inferred from 16S rRNA sequences. Numbers before slashes indicate Bayesian posterior probabilities (≥ 0.90 remain) and numbers after slashes indicate ultrafast bootstrap support for maximum likelihood analyses (≥ 90 remain).

Diagnosis

Body size moderate, SVL 31.9–36.1 mm in adult males and 48.6–52.7 mm in adult females; snout rounded; nostril closer to tip of snout than to eye; internasal space smaller than interorbital space, interorbital space larger than width of upper eyelid; tympanum distinct, approximately half of eye diameter; vomerine teeth well developed; dorsal skin smooth; distinct dermal calcars present at heels; coloration in life very variable; single subgular vocal sac present in adult males, and nuptial pad absent in adult males.

Description of holotype

Adult male, body size moderate (SVL 36.1 mm); head width approximately equal to head length (HWG/HL 99.3 %); snout rounded, longer than diameter of eye (SL/ED 133.3 %); nostril closer to tip of snout than to eye; internasal space smaller than interorbital space (INS/IOS 90.5 %), interorbital space larger than width of upper eyelid (IOS/UEW 135.5 %); canthus rostralis well developed; loreal region concave, sloped towards lip; interorbital region flat; pineal ocellus absent; tympanum distinct, approximately half of eye diameter (TD/ED 52.1 %); supratympanic fold distinct; vomerine teeth well developed, in oblique ridges, widely separated; tongue cordiform, notably notched posteriorly; choanae oval; single subgular vocal sac, vocal sac opening at bottom of mouth on either side.

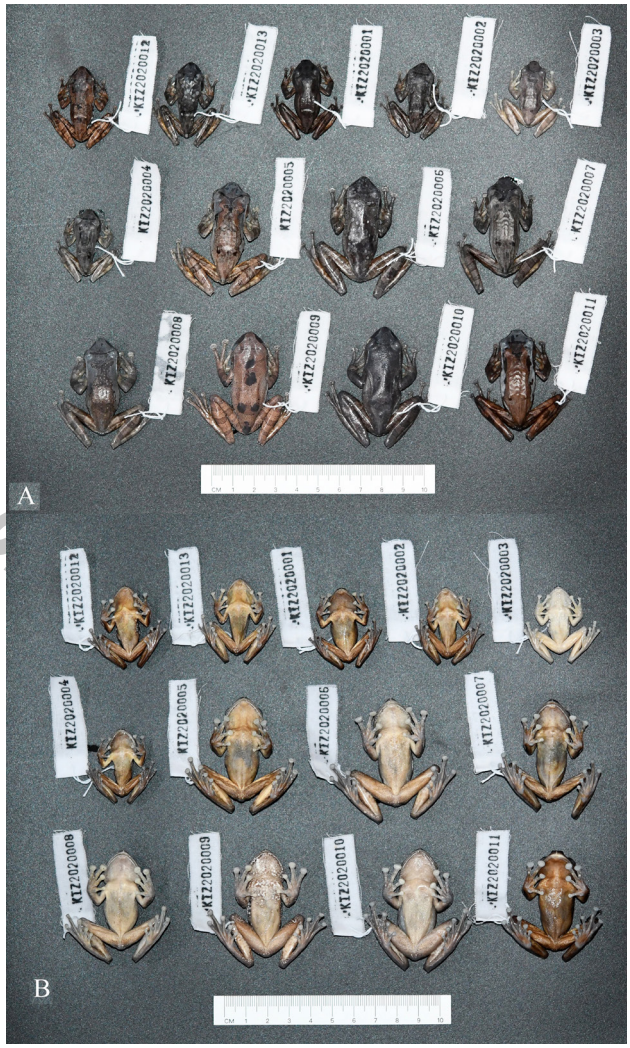

Fig. 3. Type series of *Rhacophorus hujianshengi* sp. nov. in preservative. A, dorsal view; B, ventral view.

Forelimbs slender, relative lengths of fingers I < II < IV < III; subarticular tubercles formula 1, 1, 2, 2, subarticular tubercles on first and second fingers and distal ones on third and fourth fingers large, while proximal ones on third and fourth fingers small; small supernumerary tubercles below the base of finger present; tips of fingers dilated into well developed, broad discs with circumferential groove; transverse diameter of third finger disc slightly

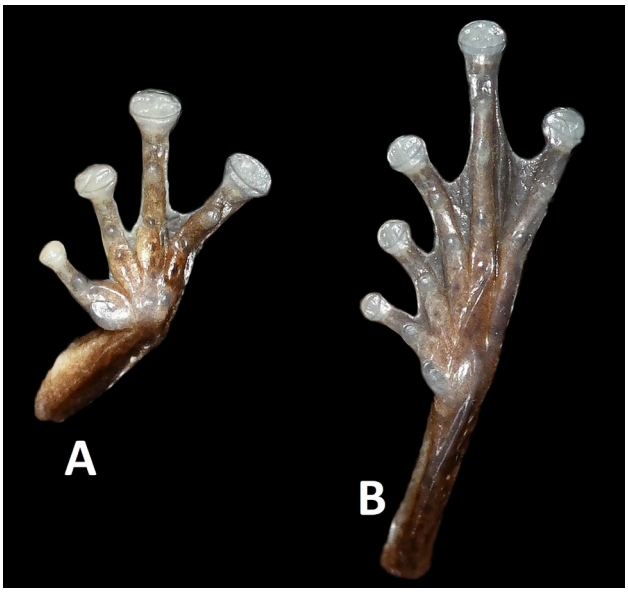

Fig. 4. Close-up views of the hand and foot of the holotype (KIZ2020012) of *Rhacophorus hujianshengi* sp. nov. in preservative. A, ventral view of the left hand; B, ventral view of the left foot.

Fig. 5. Adult males of *Rhacophorus hujianshengi* sp. nov. in life. A, B, C, the holotype (KIZ2020012); D, E, F, the paratype (KIZ2020013).

Fig. 6. Adult females of *Rhacophorus hujianshengi* sp. nov. in life. A, B, C, the paratype (KIZ2020009); D, E, F, the paratype (KIZ2020011).

larger than tympanum diameter (TFDD/TD 104.0 %); webbing between fingers underdeveloped, formula I$1^{1/2}$-$1^{2/3}$II1-2III$1^{1/2}$-$1^{1/3}$IV; one large oval inner metacarpal (thenar) tubercle; two small outer metacarpal tubercles.

Hindlimbs slender, tibiotarsal articulation beyond nostril when hindlimbs pressed forward; relative length of toes I < II < III < V < IV; subarticular tubercles large, formula 1, 1, 2, 3, 2; supernumerary tubercle below the base of toe absent; one oval inner metatarsal tubercle, outer metatarsal tubercle absent; discs on toes smaller than those on fingers; webbing between toes relatively underdeveloped, formula I2/3-2$^{1/3}$II1/2-$1^{1/2}$III1/2-2IV$1^{1/2}$-1/2V.

Skin smooth dorsally and laterally; skin on throat smooth, on abdomen and ventral thighs with flat granules; weak tubercles and protuberances on outer edges of lower arms and tarsus; dermal calcars at heels distinct, moderately developed; small tubercles forming weak transverse skin fold above vent.

Color of holotype in life

Dorsum reddish brown with some irregular dark brown and black stripes and spots; dorsal surface of limbs reddish brown with distinct dark brown transverse bands; upper part of iris yellowish brown, lower part of iris reddish brown, pupil black; some irregular yellowish white spots on upper jaw and flanks; anterior and posterior surfaces of thighs orange red; inner surface of tarsus and foot light orange; webbings dark gray; throat region brownish white, chest yellowish white, belly and ventral surface of limbs pinkish gray.

Color of holotype in preservative

Dorsum and dorsal surfaces of limbs reddish brown, dark stripes and spots on dorsum and transverse bands on dorsal surfaces of limbs still distinct; pupil turned to white, iris turned to black; yellowish white spots on upper jaw and flanks turned to white; ventral surface turned to brownish yellow.

Male secondary sexual characteristics

Single subgular vocal sac present, nuptial pad absent.

Variations

Morphometric variations are small (Table III), but the variations of coloration in life are quite large. Dorsum orange, chocolate colored, gray, or brown with some irregular dark strips or spots; many small yellowish green spots present on the dorsum in some individuals; green marking presents from dorsal surface of snout bifurcating through upper eyelids and shoulders, and ending on sides of sacrum in some individuals; no spots on upper jaw and flanks in some individuals; and some dark edged white spots on present on throat and chest region as well as posterior surface of thighs in some individuals.

Distribution

The new species was currently known only from its type locality, Xiajinchang Township, Malipo County, Wenshan Prefecture, Yunnan Province, China (Fig. 1). It is speculated that it may be found in northern Vietnam.

Table III. Measurements (mm) of *Rhacophorus hujianshengi* sp. nov.

	KIZ 2020012 Male holotype	KIZ 2020013 Male paratype	KIZ 2020001 Male paratype	KIZ 2020002 Male paratype	KIZ 2020003 Male paratype	KIZ 2020004 Male paratype	KIZ 2020005 Female paratype	KIZ 2020006 Female paratype	KIZ 2020007 Female paratype	KIZ 2020008 Female paratype	KIZ 2020009 Female paratype	KIZ 2020010 Female paratype	KIZ 2020011 Female paratype
SVL	36.1	36.0	35.9	34.6	31.9	33.3	50.8	50.0	48.9	48.6	49.5	52.7	49.8
HL	13.7	13.9	13.8	13.3	12.3	12.9	18.2	17.8	18.3	17.6	17.4	18.5	17.9
HWJ	12.9	13.1	13.0	12.8	12.1	12.6	17.1	17.2	17.1	16.8	17.2	17.9	17.1
HWG	13.6	14.0	13.7	13.5	12.3	12.9	18.2	18.1	17.9	17.6	17.6	18.3	18.2
SL	6.4	6.1	6.5	6.3	5.5	5.8	8.0	8.3	8.6	8.4	7.8	8.3	8.2
INS	3.8	3.8	3.8	3.7	3.5	3.6	5.0	4.9	5.1	4.9	5.0	5.3	5.1
IOS	4.2	4.2	4.3	4.4	3.8	4.1	5.8	5.7	6.0	6.2	5.9	6.1	6.0
UEW	3.1	3.2	3.4	3.4	3.0	3.1	4.4	4.3	3.8	4.0	3.7	4.3	4.1
ED	4.8	4.9	5.0	4.7	4.2	4.5	6.0	5.8	5.6	5.6	5.6	5.6	5.7
TD	2.5	2.5	2.5	2.3	2.1	2.2	3.3	3.3	3.4	3.1	3.3	3.6	3.6
LAHL	18.6	18.4	18.7	18.3	16.9	17.0	24.2	26.2	24.7	23.5	25.1	26.5	25.0
HAL	11.1	11.2	12.0	11.3	10.8	10.5	15.1	16.4	15.6	15.0	15.5	16.0	15.3
HLL	61.5	61.6	59.8	58.9	54.5	56.6	78.9	85.5	78.6	74.0	79.2	86.0	83.3
TL	19.5	19.6	18.9	19.0	18.2	18.5	25.2	26.6	25.1	23.9	25.6	27.9	26.6
FTL	26.0	26.1	26.0	25.1	23.5	23.6	34.4	36.9	34.2	32.3	33.7	36.9	35.4
FL	16.1	16.3	16.0	15.9	14.2	14.4	21.3	23.2	21.2	19.8	20.7	22.5	21.6
TFDD	2.6	2.6	2.7	2.6	2.2	2.3	3.8	3.9	3.6	3.5	3.7	4.0	3.8

Abbreviations are defined in “Materials and Methods”.

Ecology

Specimens of the new species were found on leaves approximately 1–2 m above ground in the karst forest, there are many grasses and trees and some caves in the forest (Fig. 7). Males were heard calling with seven to 10 calls making up a chorus. No eggs and tadpoles of this new species were not found.

Fig. 7. Habitat at the type locality of *Rhacophorus hujianshengi* sp. nov.

Comparisons

Rather than comparing the new species to all extant species of *Rhacophorus*, we focus on the comparisons with the phylogenetically closely related taxa, and those for which no corresponding molecular data are currently available but morphologically closely resemble the new species. We do not make detailed comparisons with the phylogenetically distantly related taxa and those for which no corresponding molecular data are currently available but differ significantly in morphology from the new species. Data for compared species were taken from the original and subsequent descriptions (Anderson, 1871; Huang, 1983; Ziegler and Köhler, 2001; Mo *et al*., 2008, 2014; Orlov *et al*., 2001, 2010, 2012; Fei *et al*., 2012; Ostroshabov *et al*., 2013; Nguyen *et al*., 2017; Che *et al*., 2020; Liu *et al*., 2020, 2022; Kropachev *et al*., 2019, 2022).

For species of *Rhacophorus* for which molecular data are available, and that are phylogenetically closely related to the new species. *Rhacophorus hujianshengi* sp. nov. differs from *R. hoabinhensis* by vomerine teeth present vs. absent, and having relatively smaller head length in adult males (HL/SVL 38.0–38.7 % vs. 40.2–40.3 %), and relatively greater maximum width of head in adult males (HWG/HL 99.3–101.5 % vs. 92.4–96.0 %). *Rhacophorus hujianshengi* sp. nov. differs from *R. orlovi* by tibiotarsal articulation reaching or beyond nostril when hindlimb is adpressed forward in adult males vs. reaching between eye and nostril, and having distinct dermal calcars at heels vs. no distinct dermal calcars at heels, and relatively less developed webbings between fingers ($I1^{1/2}$-$1^{2/3}II1$-$2III1^{1/2}$-$1^{1/3}IV$ vs. $I1^{1/4}$-$1^{1/4}II3/4$-$1^{1/2}III1^{1/4}$-3/4IV). *Rhacophorus hujianshengi* sp. nov. differs from *R. robertingeri* by having rounded snout vs. pointed, moderately developed dermal calcars at heels vs. highly developed, and relatively less developed webbings between fingers ($I1^{1/2}$-$1^{2/3}II1$-$2III1^{1/2}$-$1^{1/3}IV$ vs. $I1^{1/2}$-$1^{1/2}II1/2$-$1^{1/3}III1/2$-1/2IV). *Rhacophorus hujianshengi* sp. nov. differs from *R. spelaeus* by having relatively smaller body size in adult males (SVL 31.9–36.1 mm vs. 38.9–43.0 mm), distinct dermal calcars at heels vs. no dermal calcars at heels, and relatively less developed webbings between fingers ($I1^{1/2}$-$1^{2/3}II1$-$2III1^{1/2}$-$1^{1/3}IV$ vs. I1-1II1/2-$1^{1/2}III1$-1/2IV). *Rhacophorus hujianshengi* sp. nov. differs from *R. tuberculatus* by tibiotarsal articulation reaching or beyond nostril when hindlimb is adpressed forward in adult males vs. reaching anterior corner of eye, and having well developed vomerine teeth vs. underdeveloped vomerine teeth, and relatively less developed webbings between fingers ($I1^{1/2}$-$1^{2/3}II1$-$2III1^{1/2}$-$1^{1/3}IV$ vs. I1-1II0-1III1-1/2IV). *Rhacophorus hujianshengi* sp. nov. is phylogenetically sister to and most similar in morphology characteristic and coloration to *R. laoshan*, however, *Rhacophorus hujianshengi* sp. *nov.* can be differentiated from *R. laoshan* by tibiotarsal articulation reaching or beyond nostril when hindlimb is adpressed forward in adult males vs. reaching middle of eye, nuptial pad absent in adult males vs. nuptial pad present on the base of first finger, and having relatively smaller head width at the commissure of jaws in adult males (HWJ < HL vs. HWJ > HL), relatively greater eye diameter in adult males (ED/SVL 13.2–13.9 % vs. 12.3–13.0 %), and relatively greater transverse diameter of third-finger disc in adult males (TFDD/SVL 6.9–7.5 % vs. 6.3–6.8 %).

For species of *Rhacophorus* for which no molecular

data are available, and that superficially closely resemble the new species. *Rhacophorus hujianshengi* sp. nov. differs from *R. hoanglienensis* by having relatively smaller body size in adult males (SVL 31.9–36.1 mm vs. 41.2–55.9 mm), relatively greater tympanum diameter in adult males (TD/HL 17.1–18.2 % vs. 12.9–16.9 %), distinct tympanum vs. indistinct, rounded snout vs. pointed, and relatively less developed webbings between fingers (I1$^{1/2}$-1$^{2/3}$II1-2III1$^{1/2}$-1$^{1/3}$IV vs. I1-1$^{1/2}$II1/2-1$^{1/2}$III1-1/2IV). *Rhacophorus hujianshengi* sp. nov. differs from *R. larissae* by having relatively smaller body size in adult males (SVL 31.9–36.1 mm vs. 49.9 mm), relatively greater head length in adult males (HL/SVL 38.0–38.7 % vs. 34.9 %), relatively smaller maximum width of head in adult males (HWG/HL 99.3–101.5 % vs. 105.5 %), relatively greater eye diameter in adult males (ED/HL 34.1–36.2 % vs. 33.4 %), and orange red anterior and posterior surfaces of thighs vs. grayish brown. *Rhacophorus hujianshengi* sp. nov. differs from *R. trangdinhensis* by transverse diameter of third-finger disc larger than tympanum diameter vs. smaller than tympanum diameter, and having relatively greater head length in adult females (HL/SVL 35.1–37.4 % vs. 34.7 %), relatively greater eye diameter in adult females (ED/HL 30.3–33.0 % vs. 29.1 %), relatively smaller tympanum diameter in adult females (TD/HL 17.6–20.1 % vs. 21.1 %), relatively greater transverse diameter of third-finger disc in adult females (TFDD/ED 62.5–71.4 % vs. 47.1 %), distinct dermal calcars at heels vs. no dermal calcars at heels, and orange red anterior and posterior surfaces of thighs vs. gray to dark brown. *Rhacophorus hujianshengi* sp. nov. differs from *R. vanbanicus* by transverse diameter of third-finger disc larger than tympanum diameter vs. equal to tympanum diameter, and having relatively greater head length in adult males (HL/SVL 38.0–38.7 % vs. 32.5 %), relatively greater maximum width of head in adult males (HWG/HL 99.3–101.5 % vs. 85.8 %), relatively greater eye diameter in adult males (ED/HL 34.1–36.2 % vs. 29.2 %), and relatively greater tympanum diameter in adult males (TD/HL 17.1–18.2 % vs. 15.9 %). *Rhacophorus hujianshengi* sp. nov. differs from *R. viridimaculatus* by having relatively smaller body size (SVL 31.9–36.1 mm vs. 40.6–57.5 mm in adult males, 48.6–52.7 mm vs. 55.3 mm in adult females), relatively smaller tympanum diameter (TD/HL 17.1–18.2 % vs. 18.5–20.1 % in adult males, 17.6–20.1 % vs. 20.6 % in adult females), distinct dermal calcars at heels vs. no distinct dermal calcars at heels, and relatively less developed webbings between fingers (I1$^{1/2}$-1$^{2/3}$II1-2III1$^{1/2}$-1$^{1/3}$IV vs. I1-1II0-1III1-1/2IV).

DISCUSSION

Wu *et al*. (2019) recorded *Rhacophorus laoshan* from Xiaoxi National Nature Reserve in Hunan province, China, without reference to any voucher specimens. Geographically, *R. laoshan* is distributed in northwestern Guangxi, whereas Xiaoxi National Nature Reserve is located in northwestern Hunan, which were separated from each other by approximately a straight distance of 600 km. In addition, Hunan is beyond the distribution range *Rhacophorus* species. Gao *et al*. (2022) studied the species diversity and distribution of amphibians and reptiles in Hunan Province, China, and considered that *R. laoshan* could not be distributed in Hunan Province. We had checked the photos of the specimen that was identified as *R. laoshan* by Wu *et al*. (2019) from Xiaoxi National Nature Reserve, and found that it is a species of the genus *Polypedates* rather than *R. laoshan*. Therefore, we formally remove the record of *R. laoshan* from the herpetofauna of Hunan Province.

Song *et al*. (2022) reported *Rhacophorus laoshan* from Gulinqing Nature Reserve in Maguan County, Wenshan Prefecture, Yunnan Province, China. Morphologically, the specimens reported by Song *et al*. (2022) from Gulinqing Nature Reserve are similar to the new species described in this study. According to Song *et al*. (2022), the most obvious difference between the specimens from Gulinqing Nature Reserve and the new species is that nuptial pad is present on the first finger in the adult male specimen from Gulinqing Nature Reserve while absent in the adult males of the new species. However, from Figure 2A in Song *et al*. (2022), it is clear that there is no nuptial pad on the first finger. Moreover, Gulinqing Nature Reserve is close to the collection site of the new species and far from the type locality of *R. laoshan*. Therefore, we speculate that the specimens reported by Song *et al*. (2022) from Gulinqing Nature Reserve and the new species described in this study are likely to be conspecific. But given that Song *et al*. (2022) did not provide molecular data of the specimens from Gulinqing Nature Reserve, we respect their conclusions and tentatively consider *R. laoshan* is distributed in Gulinqing Nature Reserve.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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