



Research article

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A New Species of the Genus *Zhangixalus* (Amphibia: Rhacophoridae) from Vietnam

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Abstract. We describe a new species of the genus *Zhangixalus* Li, Jiang, Ren & Jiang, 2019 from Ha Giang Province, Vietnam based on morphological and molecular data. In the molecular phylogenetic analyses, the new species is nested in the *Zhangixalus duboisi* (Ohler, Marquis, Swan & Grosjean, 2000) group, where it is sister to *Z. duboisi* with a genetic distance of 2.51%. The new species, *Zhangixalus franki* sp. nov., differs from its congeners by a combination of the following morphological characters: size large, SVL 77.9–85.8 mm in males; finger webbing formula I1–II0–III½–0IV; dorsal surface of head and body green with dark brown spots; ventral surface grey or dark grey, white stripe along edge of jaw, insertion of limbs, and along lateral ridges of fore and hind limbs and flank, separating upper green part from lower grey part. The new species occurs in evergreen montane tropical forests at an elevation of ca 1300 m a.s.l. The new discovery brings the total number of known species in the genus *Zhangixalus* to 38 and the species number reported from Vietnam to nine.

Keywords. New species, *Zhangixalus franki* sp. nov., molecular phylogeny, taxonomy, Vietnam.

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Introduction

The family Rhacophoridae represents one of the most diverse anuran families, with 427 currently recognized species in the world (Frost 2020). Representatives of the family are distributed in Sub-Saharan Africa and southern Asia from Sri Lanka, Nepal and India, eastwards to Japan and southwards to Indonesia and the Philippines (Frost 2020). The genus *Zhangixalus* Li, Jiang, Ren & Jiang, 2019 was recently split from *Rhacophorus* by Jiang *et al.* (2019). *Zhangixalus* currently contains 37 species with a distribution range of from northeastern India, Nepal, Bhutan, southern China, Myanmar, northern Thailand, Laos, northern Vietnam, Taiwan and Japan, southwards to Indonesia, Brunei and Malaysia (Frost 2020). In Vietnam, eight species have been recorded (Frost 2020), namely *Z. dennysi* (Blanford, 1881), *Z. dorsovireidis* (Bourret, 1937), *Z. duboisi* (Ohler, Marquis, Swan & Grosjean, 2000), *Z. dugritei* (David, 1872), *Z. feae* (Boulenger, 1893), *Z. hungfuensis* (Liu & Hu, 1961), *Z. nigropunctatus* (Liu, Hu & Yang, 1962), and *Z. pachyproctus* (Yu, Hui, Hou, Wu, Rao & Yang, 2019).

During our recent field work in Ha Giang Province in 2019, we collected a series of treefrogs that morphologically resembled *Z. burmanus* (Andersson, 1939), a species that is known from western Yunnan of China, northern Myanmar, and northeastern India (Sengupta & Ahmed 2017). However, the newly collected specimens from Vietnam are notably larger in size than any known specimen of *Z. burmanus*. In addition, the specimens from Ha Giang Province differ from *Z. burmanus* by having ventral surface grey or dark grey; flank with a white stripe, separating upper green part from lower cream part. Furthermore, the interspecific uncorrected genetic distances (16S rRNA gene) between the new species from Ha Giang Province and other analyzed congeners varied from 2.51% to 11.57%, which is higher in this study than those among recognized taxa of *Zhangixalus* (i.e. 1.17% between *Z. duboisi* and *Z. omeimontis* (Stejneger, 1924) or 2.40% between *Z. minimus* and *Z. puerensis*). The combination of morphological and molecular data indicate the population from Ha Giang to represent an independent evolutionary lineage that could not be assigned to any known species of *Zhangixalus*. Herein, we describe the *Zhangixalus* from Ha Giang Province, Vietnam as a new species.

Material and methods

Sampling

Field surveys were conducted from 1 to 15 June 2019 by T.T. Nguyen in Quan Ba District, Ha Giang Province, northern Vietnam. Geographic coordinates and elevations were obtained using a Garmin GPSMAP 76CSX (WGS84 datum). After photographing the frogs alive, they were euthanized in a closed vessel with a piece of cotton wool containing ethyl acetate (Simmons 2002), fixed in 80% ethanol for five hours, and then transferred to 70% ethanol for permanent storage. Liver tissue samples were preserved separately in 70% ethanol prior to fixation. Specimens were subsequently deposited in the collections of the Vietnam National Museum of Nature (VNMN) and Institute of Ecology and Biological Resources (IEBR), Hanoi, Vietnam.

Molecular data and phylogenetic analyses

We used the protocols of Kuraishi *et al.* (2013), modified by Nguyen *et al.* (2015), for DNA extraction, amplification, and sequencing. Fragments of the mitochondrial DNA gene 16S rRNA were amplified using the primers from Kuraishi *et al.* (2013).

Table 1. Samples of *Zhangixalus* Li, Jiang, Ren & Jiang, 2019 and other species used for DNA analysis in this study.

No.	Scientific name	Voucher	Locality	Genbank No.	Source
1.	<i>Buergeria buergeri</i>	IABHU 41011	Hiroshima, Japan	AB127977	Sano <i>et al.</i> 2004
2.	<i>Zhangixalus burmanus</i>	Rao6239	Xizang, China	JX219422	Li <i>et al.</i> 2012b
3.	<i>Z. chenfui</i>	SCUM 060404L	Sichuan, China	EU215534	Li <i>et al.</i> 2008
4.	<i>Z. chenfui</i>	RAO ZT 0806013	Yunnan, China	JX219431	Li <i>et al.</i> 2012b
5.	<i>Z. dennysi</i>	Li06	Hunan, China	JX219433	Li <i>et al.</i> 2012b
6.	<i>Z. dennysi</i>	SCUM 060401L	Guangdong, China	EU215545	Li <i>et al.</i> 2008
7.	<i>Z. dorsovireidis</i>	ROM38015	Lao Cai, Vietnam	JX219423	Li <i>et al.</i> 2012b
8.	<i>Z. dorsovireidis</i>	Rao060821199	Yunnan, China	JX219426	Li <i>et al.</i> 2012b
9.	<i>Z. duboisi</i>	ROM38771	Lao Cai, Vietnam	JX219413	Li <i>et al.</i> 2012b
10.	<i>Z. duboisi</i>	VNMN 4103	Lao Cai, Vietnam	LC010581	Nguyen <i>et al.</i> 2017
11.	<i>Z. dugritei</i>	LJT 060559	Sichuan, China	JN688875	Li <i>et al.</i> 2012a
12.	<i>Z. dugritei</i>	SCUM 051017 L 11	Sichuan, China	EU215540	Li <i>et al.</i> 2012a
13.	<i>Z. feae</i>	VNMN 3462	Sa Pa, Vietnam	LC010588	Nguyen <i>et al.</i> 2017
14.	<i>Z. feae</i>	SCUM 050642W	Yunnan, China	EU215544	Nguyen <i>et al.</i> 2017
15.	<i>Z. hongchibaensis</i>	CIB 097687	Chongqing, China	JN688883	Li <i>et al.</i> 2012a
16.	<i>Z. hongchibaensis</i>	CIB 097696	Chongqing, China	JN688882	Li <i>et al.</i> 2012a
17.	<i>Z. hungfuensis</i>	SCUM 060425L	Sichuan, China	EU215538	Li <i>et al.</i> 2008
18.	<i>Z. hungfuensis</i>	SCUM Li 01	Sichuan, China	JN688878	Li <i>et al.</i> 2012a
19.	<i>Z. minimus</i>	KIZ 061214YP	Guangxi, China	EU215539	Li <i>et al.</i> 2008
20.	<i>Z. minimus</i>	KUHE:70049	China	LC386569	Matsui <i>et al.</i> 2019
21.	<i>Z. nigropunctatus</i>	Rao3494	Yunnan, China	JX219429	Li <i>et al.</i> 2012b
22.	<i>Z. nigropunctatus</i>	GZ070658	Guizhou, China	JX219430	Li <i>et al.</i> 2012b
23.	<i>Z. omeimontis</i>	Li02	Sichuan, China	JX219420	Li <i>et al.</i> 2012b
24.	<i>Z. smaragdinus</i>	KIZ 06241Rao	Tibet, China	JX219411	Li <i>et al.</i> 2012a
25.	<i>Z. pinglongensis</i>	NHMG201002011	Guangxi, China	KU170684	Mo <i>et al.</i> 2016
26.	<i>Z. pinglongensis</i>	NHMG201002003	Guangxi, China	KU170683	Mo <i>et al.</i> 2016
27.	<i>Z. puerensis</i>	SCUM 060649L	Yunnan, China	EU215542	Li <i>et al.</i> 2012a
28.	<i>Z. puerensis</i>	ROM 37996	Lao Cai, Vietnam	JN688891	Li <i>et al.</i> 2012a
29.	<i>Z. schlegelii</i>	KUHE 44531	Okayama, Japan	LC369670	Matsui <i>et al.</i> 2019
30.	<i>Z. schlegelii</i>	Genbank	Hiroshima, Japan	AB202078	Sano <i>et al.</i> 2005
31.	<i>Z. pachyproctus</i>	VNMN 1534	Bac Giang, Vietnam	LC010592	Nguyen <i>et al.</i> 2017
32.	<i>Z. pachyproctus</i>	VNMN 4113	Nghe An, Vietnam	LC010593	Nguyen <i>et al.</i> 2017
33.	<i>Z. taroensis (burmanus)</i>	SCUM 060614L	Yunnan, China	EU215537	Li <i>et al.</i> 2008
34.	<i>Z. wui</i>	CIB 097685	Hubei, China	JN688881	Li <i>et al.</i> 2012a
35.	<i>Z. wui</i>	CIB 097690	Hubei, China	JN688880	Li <i>et al.</i> 2012a
36.	<i>Z. yaoshanensis</i>	NHMG150408	Guangxi, China	MG322122	Chen <i>et al.</i> 2018
37.	<i>Z. yaoshanensis</i>	NHMG150404	Guangxi, China	MG322121	Chen <i>et al.</i> 2018
38.	<i>Z. zhoukaiyae</i>	AHU-RhaDb-120428	Anhui, China	KU601502	Pan <i>et al.</i> 2017
39.	<i>Z. zhoukaiyae</i>	RhaDb-150420-02	Anhui, China	KU601501	Pan <i>et al.</i> 2017
40.	<i>Rhacophorus annamensis</i>	VNMN 07225	Ninh Thuan, Vietnam	LC548739	This study

Table 1. Continuation

No.	Scientific name	Voucher	Locality	Genbank No.	Source
41.	<i>R. annamensis</i>	HAO 01	Ninh Thuan, Vietnam	LC548740	This study
42.	<i>R. kio</i>	VN.2018.057	Kon Tum, Vietnam	LC548742	This study
43.	<i>R. kio</i>	VN.2018.082	Kon Tum, Vietnam	LC548741	This study
44.	<i>R. orlovi</i>	TQ.2018.20	Tuyen Quang, Vietnam	LC548743	This study
45.	<i>R. orlovi</i>	TQ.2018.56	Tuyen Quang, Vietnam	LC548744	This study
46.	<i>Zhangixalus franki</i> sp. nov	VNMN 011686	Ha Giang, Vietnam	LC548745	This study
47.	<i>Zhangixalus franki</i> sp. nov	VNMN 011687	Ha Giang, Vietnam	LC548746	This study

For the phylogenetic analyses, sequences of 21 species of *Zhangixalus* and three species of *Rhacophorus* from previous studies were used in combination with a sequence of *Buergeria buergeri* (Temminck & Schlegel, 1838) as an outgroup (Table 1).

Chromas Pro software (Technelysium Pty Ltd., Tewantin, Australia) was used to edit the sequences, which were aligned using MAFFT version 7 (Katoh & Standley 2013) with default settings. We then checked the initial alignments by eye and adjusted slightly. Phylogenetic trees were constructed by using maximum likelihood (ML) and Bayesian inference (BI). Prior to ML and BI analyses, we chose the optimum substitution models for all sequences using Kakusan 4 (Tanabe 2011) based on the Akaike information criterion (AIC). The best model selected for ML was the general time reversible model (GTR: Tavaré 1986) with a gamma shape parameter (G: 0.220 in ML and 0.248 in BI). The BI summarized two independent runs of four Markov Chains for 10 000 000 generations. A tree was sampled every 100 generations and a consensus topology was calculated for 70,000 trees after discarding the first 30 001 trees (burn-in 1 000 000). We checked parameter estimates and convergence using Tracer version 1.5 (Rambaut & Drummond 2009). The strength of nodal support in the ML tree was analyzed using non-parametric bootstrapping (MLBS) with 1,000 replicates. We regarded tree nodes in the ML tree with bootstrap values of 75% or greater as sufficiently resolved (Huelsenbeck & Hillis 1993), and nodes with a Bayesian Posterior Probability (BPP) of 95% or greater as significant in the BI analysis (Leaché & Reeder 2002). Pairwise comparisons of uncorrected sequence divergences (p-distance) were calculated for the 16S rRNA fragments only between species of the genus *Zhangixalus*.

Morphological characters

A total of 36 measurements was taken with a dial caliper to the nearest 0.1 mm following the methods in Nguyen *et al.* (2016). Abbreviations are as follows:

DAE	=	Distance between anterior corners of eyes
DPE	=	Distance between posterior corners of eyes
ED	=	Eye diameter
EN	=	Distance from anterior corner of eye to nostril
fd3	=	width of disc of finger III
Fel	=	Thigh length, from vent to knee
Fig 1–4	=	Length of fingers I–IV, from base of finger to tip of finger
FLL	=	Upper arm length, from axilla to elbow
FoL	=	Foot length, from tibiotarsal joint to tip of fourth toe
HAL	=	Forearm length, from elbow to tip of third finger

HL	=	Head length, from back of mandible to tip of snout
HW	=	Maximum head width across angle of jaws
IMT	=	Inner metatarsal tubercle length
IN	=	Internarial distance
IPT	=	Inner palmar tubercle length
IOD	=	Interorbital distance, minimal distance between orbits
MBE	=	Distance from back of mandible to back of eye
MFE	=	Distance from back of mandible to front of eye
MN	=	Distance from jaw angle to nostril
NS	=	Distance from nostril to tip of snout
SNL	=	Snout length, from anterior corner of eye to tip of snout
SVL	=	Snout-vent length
TbL	=	Tibia length, from knee to tibiotarsal joint
TbW	=	Maximal tibia width
td4	=	Width of disc of toe 4
Toe 1–5	=	Length of toes I–V from inner metatarsal tubercle to tip of toe
TYD	=	Maximal tympanum diameter
TYE	=	Distance from anterior margin of tympanum to posterior corner of eye
UEW	=	Maximum width of upper eyelid.

Terminology for describing webbing formula followed Glaw and Vences (2007). Sex was determined by the presence of nuptial pads and gonadal inspection.

We compared morphological characters of the new species with congeners from Vietnam and China based on specimen examination (see Appendix) and data obtained from the literature (e.g., Stejneger 1907; Bourret 1937; Liu 1950; Liang & Wang 1978; Maeda & Matsui 1990; Lue *et al.* 1995; Ohler *et al.* 2000; Orlov *et al.* 2001; Harvey *et al.* 2002; Wilkinson 2003; Bain & Nguyen 2004; Goris & Maeda 2004; Wilkinson & Rao 2004; Rao *et al.* 2006; Bordoloi *et al.* 2007; Chou *et al.* 2007; Ohler 2009; Fei *et al.* 2010; Li *et al.* 2012a; Ziegler *et al.* 2014; Jiang *et al.* 2016; Mo *et al.* 2016; Liu *et al.* 2017; Pan *et al.* 2017; Chen *et al.* 2018; Yu *et al.* 2019).

Results

Phylogenetic analyses

Aligned, combined sequences yielded a total of 1,085 nucleotide sites. Of 1,085 nucleotide sites, 304 were variable and 266 were parsimony informative within the in-group. Nucleotide frequencies were A = 37.0%, T = 24.4%, C = 20.9%, and G = 17.7% (data for ingroup only). The ML and BI analyses produced similar topologies with $-\ln L = 7372.186$ and 7549.990 respectively. Phylogenetic analyses employing ML and BI methods yielded identical topologies, and only the BI tree is presented in Figure 1.

Monophyly of *Zhangixalus* with respect to the outgroup species was fully supported (each 100% support in ML bootstrap value and Bayesian posterior probability) and samples were split into two major clades, *Rhacophorus* and *Zhangixalus*. The clade *Zhangixalus* contained 21 taxa and was comprised of four subclades A1, A2, A3 and A4. The subclade A1 contained *Z. burmanus*, *Z. dorsovireidis*, *Z. duboisi*, *Z. omeimontis*, *Z. zhoukaiyae* and the new species from Ha Giang Province with strong support values (MLBS = 83%, BPP = 1.00) (Fig. 1).

The mean interspecific uncorrected genetic p-distances for the 16S rRNA gene fragment examined between the unnamed *Zhangixalus* species from Ha Giang and other known congeners ranged from 2.51% (compared with *Z. duboisi*) to 11.57% (compared with *Z. smaragdinus*). In the genus *Zhangixalus*, the

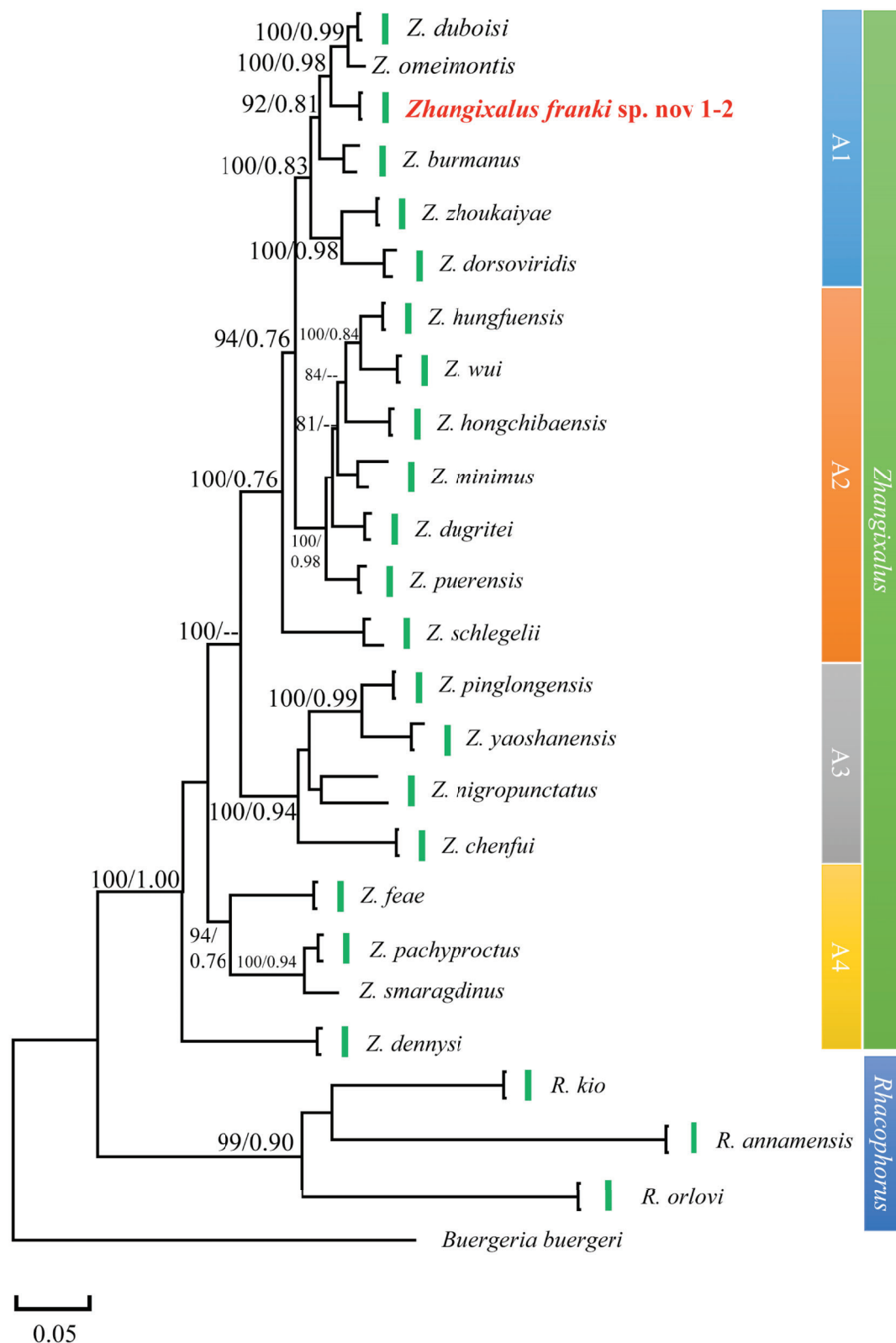


Fig. 1. BI tree from a 1085 bp sequence of mitochondrial 16S rRNA gene of *Zhangixalus* Li, Jiang, Ren & Jiang, 2019 and outgroup species. Numbers above and below branches are Bayesian posterior probabilities (BPP) and ML bootstrap values (only values above 60% are shown), respectively. For GenBank accession numbers, refer to Table 1.

lowest mean genetic distance was between *Z. duboisi* and *Z. omeimontis* at approximately 1.17%. Two other taxa, *Z. minimus* and *Z. puerensis*, showed a genetic distance of approximately 2.40% (Table 2). Furthermore, it is also clearly separated morphologically from all nominal species of *Zhangixalus*. Thus, we describe the population of *Zhangixalus*. Therefore consider the unnamed species of *Zhangixalus* from Ha Giang Province as a distinct species and describe it in the following.

Class Amphibia Gray, 1825
Order Anura Fischer von Waldheim, 1813
Family Rhacophoridae Hoffman, 1932
Genus *Zhangixalus* Li, Jiang, Ren & Jiang, 2019

***Zhangixalus franki* sp. nov.**

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Fig. 2

Diagnosis

The new species can be assigned to the genus *Zhangixalus* based on the following morphological characters: snout pointed; presence of intercalary cartilage between terminal and penultimate phalanges of digits, distal end of terminal phalanx in Y-shape; tips of digits expanded into large disks, bearing

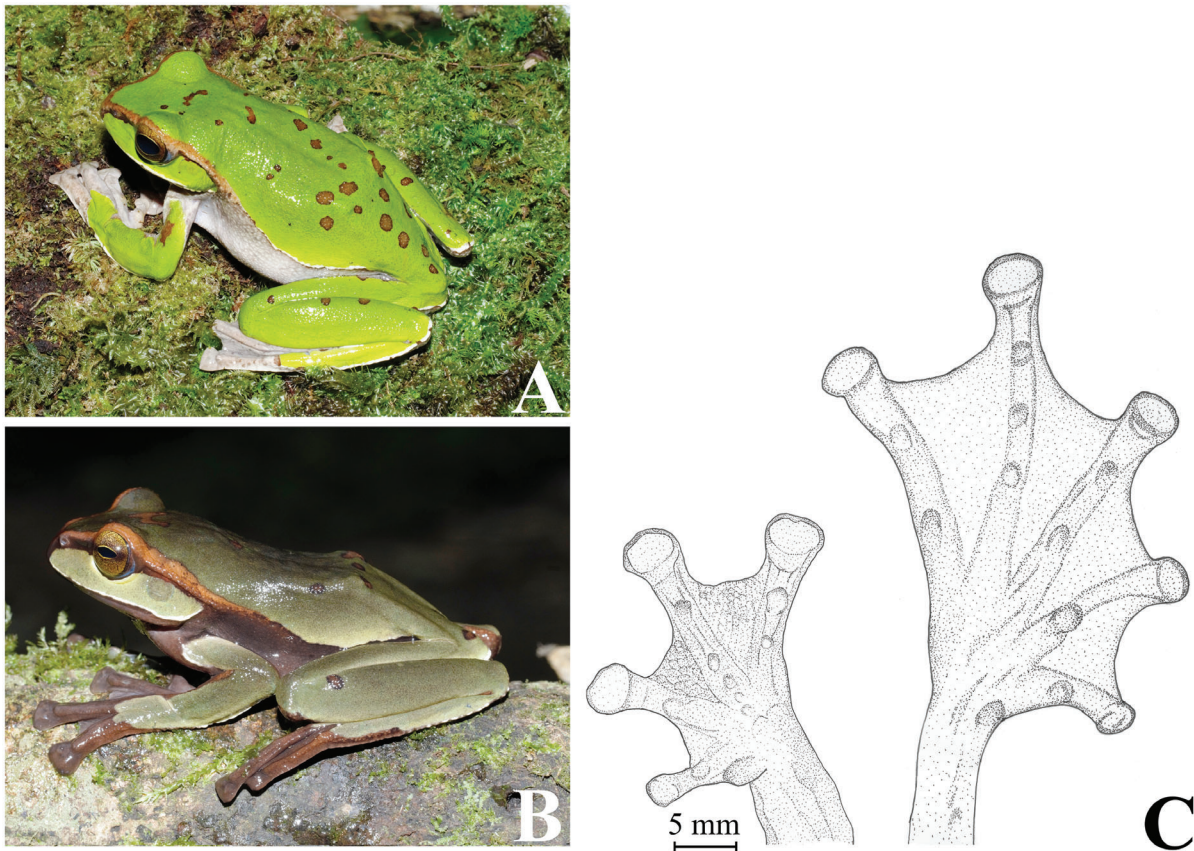


Fig. 2. A–B. Dorsal and lateral views. C. Drawing of ventral view of left hand and right foot of the adult male holotype (VNMN 011682) of *Zhangixalus franki* sp. nov. from Quan Ba District, Ha Giang Province, northern Vietnam.

Table 2. Mean uncorrected (*p*) distance (%) among 1085 bp fragments of 16S rARN of the genus *Zhangixalus* Li, Jiang, Ren & Jiang, 2019.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1. <i>Zhangixalus franki</i> sp. nov.																								
2. <i>Z. burmanus</i>	3.29																							
3. <i>Z. chenfui</i>	9.06	8.56																						
4. <i>Z. dennysi</i>	9.25	9.12	11.29																					
5. <i>Z. dorsovireidis</i>	4.87	5.32	9.44	9.47																				
6. <i>Z. duboisi</i>	2.51	3.63	9.19	8.89	4.18																			
7. <i>Z. dugritei</i>	4.98	4.65	8.81	9.11	6.03	4.42																		
8. <i>Z. feae</i>	8.80	9.16	8.96	9.59	9.62	8.82	8.82																	
9. <i>Z. hongchibaensis</i>	6.29	5.70	10.05	10.67	6.76	5.48	3.52	9.58																
10. <i>Z. hungfuensis</i>	5.69	5.93	9.80	9.80	6.17	5.24	3.29	9.47	3.51															
11. <i>Z. minimus</i>	5.34	5.01	9.69	8.68	6.16	5.01	2.39	9.20	3.63	2.85														
12. <i>Z. nigropunctatus</i>	8.68	8.68	6.96	10.71	8.83	9.07	8.45	9.19	9.80	9.68	9.19													
13. <i>Z. omeimontis</i>	2.95	3.63	9.45	9.41	4.41	1.17	4.43	8.70	5.83	5.12	5.13	8.73												
14. <i>Z. pachyproctus</i>	10.38	9.86	11.19	10.19	9.99	9.55	10.26	7.85	10.89	10.77	10.27	11.64	9.52											
15. <i>Z. pinglongensis</i>	7.96	7.62	7.22	9.97	8.46	7.74	8.18	9.32	9.20	8.59	7.98	6.72	7.88	10.63										
16. <i>Z. puerensis</i>	5.68	5.34	9.29	9.05	6.62	5.34	3.51	9.06	4.31	3.63	2.40	9.42	5.23	10.38	8.47									
17. <i>Z. schlegelii</i>	6.51	6.86	9.92	10.78	7.71	6.06	6.30	8.80	6.91	6.92	6.66	9.32	6.31	9.52	9.44	6.76								
18. <i>Z. smaragdinus</i>	11.57	11.42	11.39	11.13	11.67	11.09	11.73	8.85	12.48	12.16	12.01	10.85	10.84	8.28	11.10	11.49	10.82							
19. <i>Z. wui</i>	5.36	5.94	9.45	9.79	6.77	5.02	3.41	9.96	4.20	2.50	3.52	9.69	5.02	10.91	8.95	4.42	6.93	12.80						
20. <i>Z. yaoshanensis</i>	8.48	9.23	8.22	11.58	9.48	8.62	9.42	10.47	10.82	10.08	9.95	7.24	9.13	11.84	4.32	10.07	10.09	11.96	10.08					
21. <i>Z. zhoutaiyae</i>	4.97	4.96	8.82	9.37	3.28	4.18	5.67	9.02	6.99	5.93	6.27	9.07	4.07	9.01	8.82	6.14	7.22	10.32	6.74	9.72				
22. <i>Rhacophorus annamensis</i>	19.14	17.91	19.38	19.06	17.95	18.28	19.09	18.59	18.56	18.37	18.98	19.78	18.64	19.99	18.95	19.71	18.81	21.27	19.06	19.76	18.05			
23. <i>R. kio</i>	17.33	15.92	17.62	15.13	16.80	16.52	16.21	15.73	16.50	16.28	16.50	18.04	16.42	16.10	16.93	16.65	16.86	16.85	16.79	18.30	15.84	14.77		
24. <i>R. orlovi</i>	17.69	16.91	19.88	18.20	16.21	17.79	16.23	16.90	18.01	17.10	16.91	19.28	17.55	17.81	18.01	17.70	18.03	18.80	17.65	19.59	17.12	17.06	15.61	
25. <i>Buergeria buergeri</i>	19.78	19.72	19.16	21.23	21.34	20.85	20.66	19.67	21.12	21.12	20.56	19.88	21.13	22.03	19.68	19.90	20.84	19.98	21.79	20.82	20.14	25.60	22.49	24.02

circum-marginal grooves; fingers webbed; presence of supra-cloacal dermal ridge; and pupil horizontal (Jiang *et al.* 2019). In addition, the new species is unambiguously nested in the genus *Zhangixalus* by molecular phylogenetic evidence. It is closely related to *Z. duboisi* and *Z. omeimontis* and nested in the same clade with *Zhangixalus dugritei* (David, 1872), the type species of this genus.

The new species is distinguished from its congeners by a combination of the following morphological characters: 1) size large (SVL 77.9–85.8 mm for the males); 2) head slightly wider than long; 3) snout pointed; 4) dorsal skin smooth; 5) finger webbing formula I1-II0-III1/2-0IV; 6) dorsal surface of head and body green with dark brown spots; 7) lower jaw region greyish, throat, chest and belly white; 8) white stripe along edge of jaw, insertion of limbs, and along lateral ridges of fore limbs, hind limbs and flank, separating upper green part from lower grey part.

Etymology

We name this new species in honor of Dr Frank Mutschmann, late amphibian veterinarian and conservationist from Berlin, Germany, in recognition of his support of our amphibian research and conservation projects in Vietnam. As common names we suggest Frank's Tree Frog (English),Ếch cây frank (Vietnamese) and Franks Ruderfrosch (German).

Material examined

Holotype

VIETNAM • adult ♂; northern Vietnam, Ha Giang Province, Quan Ba District, in the forest of Tung Vai Commune; 23002'45" N, 104050'59" E; elevation 1360 m a.s.l.; 11 Jun. 2019; collected by T.T. Nguyen; VNMN 011682.

Paratypes

VIETNAM • 4 adult ♂♂; same data as for holotype; 13 Jun. 2019; collected by T.T. Nguyen; VNMN 011683, VNMN 011684, IEBR A.2019.7-A.2019.8 (VNMN 011685, VNMN 011686) and one sub-adult VNMN 011687.

Description of holotype

SIZE. Large, body robust (SVL 82.8 mm).

HEAD. Slightly compressed, wider than long (HW 26.5 mm, HL 25.7 mm), convex above; snout pointed, slightly protruding beyond lower jaw in lateral view, and longer than horizontal diameter of eye (SNL 11.6 mm, ED 8.2 mm).

CANTHUS ROSTRALIS. Rounded, loreal region oblique, concave; interorbital distance greater than internarial distance and upper eyelid width (IOD 9.2 mm, IN 8.1 mm, UEW 6.2 mm); distance between anterior corners of eyes approximately 69% of distance between posterior corners of eyes.

NOSTRILS. Round, without lateral flap of skin, closer to tip of snout than to eye.

PUPIL. Oval, horizontal.

TYMPANUM. Distinct, round, about half of eye diameter, and two times greater than distance between tympanum and eye.

PINEAL OCELLUS. Absent; spinules on upper eyelid absent; vomerine teeth well developed, in two oblique ridges.

CHOANAE. Round.

TONGUE. Deeply notched posteriorly; supratympanic fold distinct, extending from behind eye to beyond level of axilla.

FORELIMBS. Upper arm short, about one third of forearm length (FLL 14.7 mm, HAL 41.7 mm), dermal fringe present along outer edge of forearm; not well developed; relative finger lengths $I < II < V < III$; tips of fingers with enlarged discs with distinct circum-marginal grooves, disc of finger III approximately 1.5 times width of finger III ($fd3/fw3$ 1.5), greater than tympanum diameter ($fd3/TYD$ 1.27); webbing formula $I1-I1II0-I1II\frac{1}{2}-0IV$; subarticular tubercles distinct, blunt, round, formula 1, 1, 2, 2.

HINDLIMBS. Heels overlapping when held at right angles to body; tibia length about five times greater than tibia width (TbL 39.3 mm, TbW 8.5 mm), longer than thigh length (FeL 34.1 mm), shorter than foot length (FoL 53.4 mm); relative toe lengths $I < II < III < V < IV$; tips of toes with enlarged discs with distinct circum-marginal grooves, discs slightly smaller than those of fingers; webbing formula $I0-0II0-\frac{1}{2}III0-IIV0-0V$; subarticular tubercles distinct, blunt, round, formula 1, 1, 2, 3, 2; inner metatarsal tubercle small (IMT 3.2 mm); dermal ridge present along outer edge of tibia and tarsus; dermal projection present at tibiotarsal articulation.

SKIN TEXTURE. Dorsal surface of head and body smooth, canthal and supratympanic folds on each side developed, throat and chest smooth, belly rough, ventral surface of fore and hind limbs smooth, a short dermal appendage above vent.

COLORATION IN LIFE. Iris bronze, pupil black; dorsal surface of head and body green with some small dark brown spots, various in size and shape; tympanum region green or dark green; a yellowish brown stripe running from tip of snout, along canthus rostralis, bordering upper eyelid, along supratympanic fold to posterior of axilla; dorsal surface of fore and hind limbs green, fingers and toes grey; axilla, lower part of flank and belly grey; white stripe present along edge of upper jaw, limb insertions, and dermal fringes of fore and hind limbs; flank with white stripe, separating upper green part from lower grey part; ventral surface of arm and thigh grey; anterior and posterior parts of thigh and ventral surface of tibia grey; lower jaw and throat region grey; supracloacal area grey; ventral side of webbing greyish brown, nuptial pad grey. Color can change from green to dark green depending on activity and environmental conditions.

COLORATION IN PRESERVATIVE. As in life, but with green dorsal surface fading to blue and cream white ventral surface fading to greyish brown, brown line extending from tip of snout to axilla.

MALE SECONDARY SEXUAL CHARACTERS. Male specimens with smooth oval nuptial pad on prepollex and inner edge of finger I, and a subgular vocal sac.

Variation of paratypes

The ground color of dorsum is dark green or green, ventral surface is grey or greyish brown. The number of dark brown spots on dorsum ranges from 9 to 45, variable in size and shape. For measurements of type series see Table 3.

Comparisons

In the following, we compared the new species with other members of *Zhangixalus* distributed in Vietnam and neighboring countries.

Zhangixalus franki sp. nov can be distinguished from the following 29 species in the genus *Zhangixalus* by having a larger size in males (SVL 77.9–85.8) versus *Z. achantharrhena* at 36.52–40.56 mm (Harvey

Table 3. Measurements (in mm) of *Zhangixalus franki* sp. nov.

Field no	VNMN 011682	VNMN 011683	VNMN 011684	VNMN 011685	VNMN 011686	Mean + SD (n = 5 males)	VNMN 011687
Type status	Holotype	Paratype	Paratype	Paratype	Paratype		Paratype
Sex	M (adult)	M (adult)	M (adult)	M (adult)	M (adult)		M (subadult)
SVL	82.8	85.8	82.3	79.7	77.9	81.7±3.01	61.9
HW	26.5	27.9	27.5	26.2	25.1	26.6±1.12	23.1
HL	25.7	27.2	26.7	26.0	24.6	26.0±1.01	22.0
MN	21.7	23.1	22.4	21.7	21.1	22.0±0.77	18.6
MFE	16.6	17.4	17.0	16.3	16.5	16.8±0.45	14.1
MBE	9.8	10.0	9.3	9.5	9.6	9.6±0.27	8.2
SNL	11.6	13.1	12.2	11.9	11.8	12.1±0.58	9.7
ED	8.2	8.6	9.5	8.6	8.1	8.6±0.54	6.9
UEW	6.2	7.3	7.1	7.1	6.5	6.8±0.47	6.1
IN	8.1	7.6	7.2	7.7	7.7	7.6±0.30	7.5
IOD	9.2	10.5	8.9	10.0	9.3	9.6±0.67	8.2
DAE	16.3	17.7	16.7	17.0	15.7	16.7±0.76	13.2
DPE	23.6	24.3	24.3	22.8	22.9	23.6±0.74	20.6
NS	5.4	5.3	5.1	5.1	5.0	5.2±0.17	4.8
EN	6.9	7.5	6.8	6.7	6.6	6.9±0.34	5.5
TYD	4.5	4.8	5.4	5.1	5.0	4.9±0.33	3.9
TYE	2.2	4.5	2.1	2.1	1.9	2.6±1.09	2.0
FLL	14.7	14.0	15.6	12.9	13.8	14.2±1.00	11.6
HAL	41.7	44.1	40.4	40.0	40.9	41.4±1.62	34.0
IPT	5.2	5.2	5.4	5.6	5.3	5.3±0.15	3.6
Fig 1	8.3	9.0	9.4	7.7	8.2	8.5±0.66	6.3
Fig 2	13.0	13.3	14.0	12.2	13.5	13.2±0.66	10.8
Fig 3	18.6	19.6	18.6	18.2	17.3	18.4±0.84	14.4
Fig 4	14.8	15.4	15.6	14.0	14.9	14.9±0.64	12.4
fd3	5.7	6.2	5.6	5.3	6.2	5.8±0.39	5.0
FeL	34.1	35.7	36.7	35.6	36.4	35.7±0.99	29.3
TbL	39.3	39.7	37.4	38.4	38.3	38.6±0.90	33.6
TbW	8.5	9.3	9.1	8.7	8.9	8.9±0.34	5.9
FoL	53.4	54.9	50.8	50.7	51.0	52.2±1.90	43.6
Toe 1	14.1	14.5	13.1	13.8	11.9	13.5±1.01	11.9
Toe 2	20.7	21.2	19.8	20.4	18.1	20.0±1.18	16.5
Toe 3	29.0	29.6	27.3	27.5	24.9	27.7±1.81	22.9
Toe 4	36.3	36.3	35.5	34.4	34.8	35.4±0.86	28.3
Toe 5	31.2	31.4	30.5	30.3	29.7	30.6±0.68	25.3
td4	4.6	5.0	4.4	4.6	3.2	4.4±0.68	4.2
IMT	3.2	3.6	3.5	3.3	2.1	3.1±0.61	2.4

et al. 2002); *Z. arboreus* at 42–60 mm (Wilkinson 2003); *Z. arvalis* at 39–46.4 mm (Lue *et al.* 1995); *Z. aurantiventris* at 48–54 mm (Lue *et al.* 1994); *Z. chenfu* at 33–41 mm (Fei *et al.* 2010); *Z. dorsovireidis* (Bourret, 1937) at 31.31–2.43 mm (Orlov *et al.* 2001); *Z. dugritei* at 31.65–47 mm (Liu 1950; Orlov *et al.* 2001; Fei *et al.* 2010); *Z. dulitensis* at 38.39–39.66 mm (Harvey *et al.* 2002); *Z. hongchibaensis* at 46.5–49.7 mm (Li *et al.* 2012a); *Z. hungfuensis* at 31–37 mm (Fei *et al.* 2010); *Z. jarujini* at 33.7–40 mm (Fei *et al.* 2010); *Z. leucofasciatus* at 35–48 mm (Fei *et al.* 2010); *Z. lishuiensis* at 34.2–35.8 mm (Liu *et al.* 2017); *Z. minimus* at 21.3–33.0 mm (Rao *et al.* 2006); *Z. moltrechti* at 33–46 mm (Fei *et al.* 2010); *Z. nigropunctatus* at 32–37 mm (Fei *et al.* 2010); *Z. owstoni* at 42–51 mm (Maeda & Matsui 1990); *Z. pinglongensis* at 32.0–38.5 mm (Mo *et al.* 2016); *Z. prasinatus* at 49–56 mm (Fei *et al.* 2010); *Z. prominatus* at 50.46–51.26 mm (Harvey *et al.* 2002); *Z. puerensis* at 35.5–41 mm (Bain & Nguyen 2004); *Z. schlegelii* at 49 mm (Stejneger 1907); *Z. suffry* at 38.5–52.9 mm (Bordoloi *et al.* 2007); *Z. taipeianus* at 30.7–36.7 mm (Liang & Wang 1978); *Z. viridis* at 45–56 mm (Goris & Maeda 2004); *Z. wui* at 35.2–38.2 mm (Li *et al.* 2012a); *Z. yaoshanensis* at 31.6–36.4 mm (Chen *et al.* 2018); *Z. yinggelingsensis* at 43–43.4 mm (Chou *et al.* 2007); *Z. zhukaiyae* at 27.9–36.88 mm (Pan *et al.* 2017).

Zhangixalus franki sp. nov. differs from the following seven species by having dorsum smooth; dorsal surface green with some small dark spots; chest and belly grey; supratympanic fold well developed, brown or gold; white stripe along lateral ridges of fore and hind limbs and flank, separating upper green part from lower grey part; and webbing between fingers incomplete versus dorsum of *Z. duboisi* green and brown, skin granular with horny spinules, venter fleshy with brown spots (Ohler *et al.* 2000); snout of *Z. hui* yellowish brown (Li *et al.* 2012a); dorsum and dorsal aspect of limbs of *Z. omeimontis* green with large brown markings (Liu 1950), supratympanic fold of *Z. pachyproctus*, *Z. smaragdinus* and *Z. dennysi* green and weakly developed (Yu *et al.* 2019; Fei *et al.* 2010); lateral ridges of fore and hind limbs and flank of *Z. dennysi* without white stripe; supratympanic fold yellow and webbing between fingers complete in *Z. feae* (Ziegler *et al.* 2014).

The new species mostly resembles *Z. burmanus*, but with clearly distinguishing characters: males of *Zhangixalus franki* sp. nov. with SVL at 77.9–85.5 mm larger than those of *Z. burmanus* with SVL at 47–70.5 (Ohler 2009; Jiang *et al.* 2016; Wilkinson & Rao 2004); webbing between fingers in males of *Zhangixalus franki* sp. nov. is more developed than that in males of *Z. burmanus* (I1–II0–III¹/₂–0IV vs I1–1¹/₄II1–2¹/₂III1–IIIV in *Z. burmanus* Ohler 2009); color on upper and, lower parts of flank separated from each other by white line, and dark spots on anterior and posterior sides of thigh are absent in *Zhangixalus franki* sp. nov. whereas, dark brown spots enclosing cream spots on flanks and thigh present in *Z. burmanus* (Wilkinson & Rao 2004).

Distribution

Zhangixalus franki sp. nov. is currently known only from the type locality in Ha Giang Province, northern Vietnam (Fig. 3). The species was recorded at elevations between 1320 and 1360 m a.s.l.

Natural history

Specimens of the new species were collected from 19:00 to 24:00 on trees, close to small ponds in undisturbed evergreen forest (Fig. 4). Other tree frogs that were found at the same site were *Polypedates* sp. (of the *P. leucomystax* species complex), *Z. duboisi* and *Kurixalus* sp. Females, larval stages and eggs of the new species are unknown.

Conservation status

The new species is expected to be found in evergreen forests of Guangxi Province, southern China. However, the actual distributional range should be confirmed in further studies. Given the available information, we suggest this species be considered as Data Deficient following IUCN's Red List categories (IUCN 2020).

Discussion

Ziegler *et al.* (2014) reported *Z. feae* for the first time from Ha Giang Province, northern Vietnam based a photograph which was taken at the type locality of *Z. franki*. Although the voucher specimen is not available, the color pattern of the individual in Ziegler *et al.* (2014) agreed well with the new species (dorsal surface green with some dark spots; a golden stripe running from tip of snout, along canthus rostralis, bordering

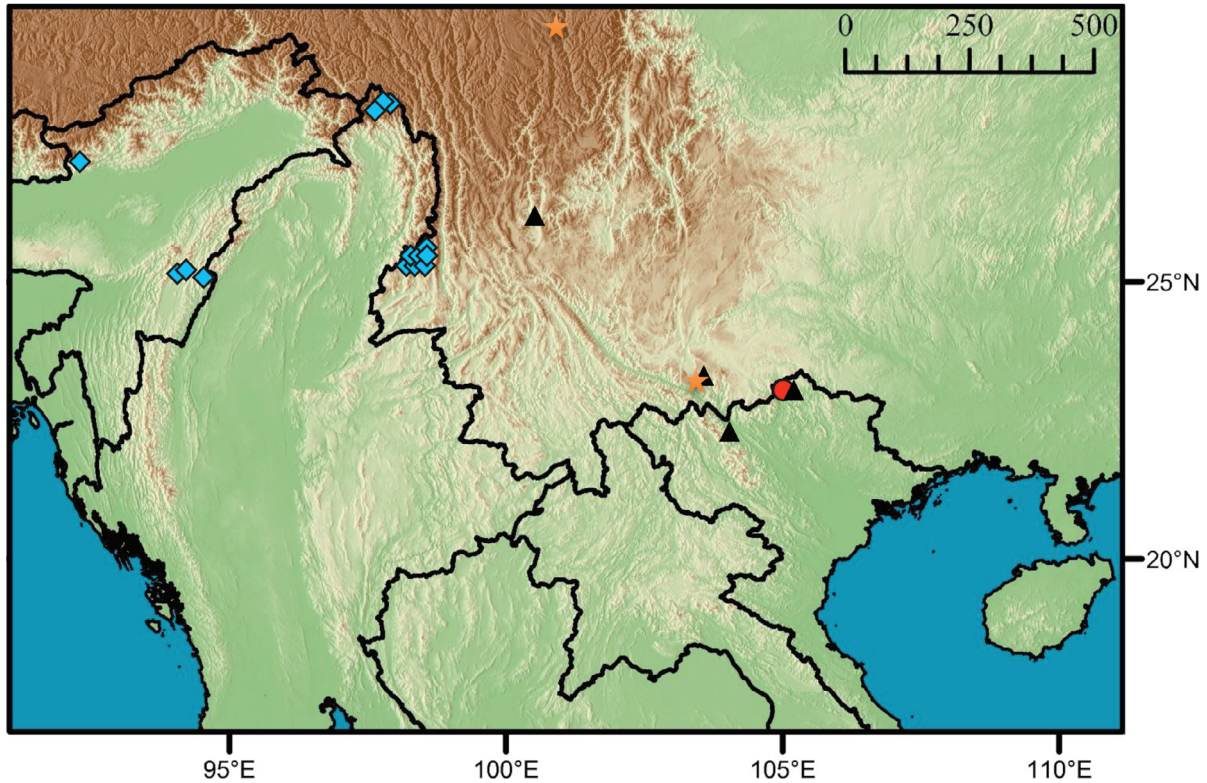


Fig. 3. Map showing the type locality of *Zhangixalus franki* sp. nov. (●) in Quan Ba District, Ha Giang Province, northern Vietnam and distribution of *Z. burmanus* (Andersson, 1939) (◆), *Z. duboisi* (Ohler, Marquis, Swan & Grosjean, 2000) (▲) and *Z. omeimontis* (Stejneger, 1924) (★ orange star).



Fig. 4. Habitat of *Zhangixalus franki* sp. nov. at the type locality in Quan Ba District, Ha Giang Province, Vietnam.

upper eyelid, and along supratympanic fold to shoulder; white stripes present along upper jaw, dermal fringes of fore and hind limbs, as well as along flank; webbing grey). Therefore the previous record of *Z. feae* in Ha Giang Province by Ziegler *et al.* (2014) should be re-identified as *Z. franki*. In our phylogenetic analyses, *Z. franki* was clustered in the same clade with *Z. burmanus*, *Z. dorsovirens*, *Z. duboisi*, *Z. omeimontis*, and *Z. zhengkaiyae*, whereas *Z. feae* was embedded in the same clade with *Z. smaragdinus* and *Z. dennysi*. The discovery of *Z. franki* from Ha Giang Province brings the total number of known species in the genus *Zhangixalus* to 37 and the species number reported from Vietnam to nine (Frost 2020).

Tropical montane forests in the border region between Vietnam and China are known to harbor a high level of species richness and local endemism (Sterling *et al.* 2006). One of the main reasons assumed to be responsible for this richness is greater environmental heterogeneity observed in the montane regions as opposed to the lowland regions, allowing for a larger number of habitats to be occupied by species (Keller *et al.* 2009). Therefore it is not unexpected that new species from this region are still being discovered and further studies in this region are required to discover the actual species richness of tree frogs in Vietnam.

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Appendix 1. Specimens examined for morphological comparisons.

Zhangixalus dennysi

VIETNAM • 4 ♂, 2 ♀; Vinh Phuc Province, Me Linh District; ML.2019.1, ML.2019.2, VNMN 06188, IEBR 132, IEBR 133, IEBR 134 • 1 ♀; Tuyen Quang Province, Cham Chu Nature Reserve; TQ.2019.100.

Zhangixalus dorsoviridis

VIETNAM • 3 ♂; Lao Cai Province, Y Ty District; VNMN 04314, VNMN 04331, VNMN 04343 • 1 ♀; Lai Chau Province, Sin Ho District; VNMN 05858.

Zhangixalus duboisi

VIETNAM • 1 ♀, 1 ♂; Lai Chau Province: Phong Tho District; VNMN 010238, VNMN 010239 • 2 ♂; Ha Giang Province, Quan Ba District; VNMN 07069, VNMN 07070.

Zhangixalus dugritei

CHINA • 2 ♂; Sichuan; CIB 20050194, KUHE 27701.

Zhangixalus puerensis

VIETNAM • 2 ♀, 1 ♂; Lai Chau Province, Phong Tho District; VNMN 010288, VNMN 010289, VNMN 010293.

Zhangixalus pachyproctus

VIETNAM • 2 ♂; Lang Son Province, Trang Dinh District; TD-LS.2019.174, TD-LS.2019.117 • 1 ♂, 1 ♀; Thanh Hoa Province, Xuan Lien National Park; XL.2013.2, XL.2013.4 • 1 ♂; Tuyen Quang, Cham Chu Nature Reserve; TQ.2019.85 • 1 ♂; Lao, Hua Phan District; VNMN 06405.

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