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

Hoa Thi NINH\(^1\), Tao Thien NGUYEN\(^{2,}\)*, Nikolai ORLOV\(^3\), Truong Quang NGUYEN\(^4\) & Thomas ZIEGLER\(^5\)

\(^1,2\)Vietnam National Museum of Nature, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Road, Hanoi, Vietnam.
\(^3\)Department of Herpetology, Zoological Institute, Russian Academy of Sciences, 199034, St. Petersburg, Russia.
\(^4\)Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Road, Hanoi, Vietnam.
\(^2,4\)Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Road, Cau Giay, Hanoi, Vietnam.
\(^1\)Hanoi National University of Education, 136 Xuan Thuy Road, Cau Giay, Hanoi, Vietnam.
\(^3\)AG Zoologischer Garten Köln, Riehler Strasse 173, D-50735 Cologne, Germany.
\(^5\)Institute of Zoology, University of Cologne, Zülpicher Strasse 47b, D-50674 Cologne, Germany.

*Corresponding author: nguyenthientao@gmail.com
\(^1\)Email: ninhhoa.hnue@gmail.com
\(^3\)Email: orlov52@gmail.com
\(^4\)Email: nqt2@yahoo.com
\(^5\)Email: ziegler@koelnerzoo.de

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 I\(^1\)-I\(^1\)II\(^0\)-I\(^3\)\(\frac{1}{2}\)-I\(^4\); 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 Subsaharan 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. dorsoviridis (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 (VNMM) 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   | Buergeria buergeri | IABHU 41011 | Hiroshima, Japan | AB127977    | Sano et al. 2004 |
| 2   | *Zhangixalus burmanus* | Rao6239 | Xizang, China | JX219422    | Li et al. 2012b |
| 3   | Z. chenfui | SCUM 060404L | Sichuan, China | EU215534    | Li et al. 2008 |
| 4   | Z. chenfui | RAO ZT 0806013 | Yunnan, China | JX219431    | Li et al. 2012b |
| 5   | Z. dennyi | Li06 | Hunan, China | JX219433    | Li et al. 2012b |
| 6   | Z. dennyi | SCUM 060401L | Guangdong, China | EU215545    | Li et al. 2008 |
| 7   | Z. dorsoviridis | ROM38015 | Lao Cai, Vietnam | JX219423    | Li et al. 2012b |
| 8   | Z. dorsoviridis | Rao060821199 | Yunnan, China | JX219426    | Li et al. 2012b |
| 9   | Z. duboisi | ROM38771 | Lao Cai, Vietnam | JX219413    | Li et al. 2012b |
| 10  | Z. duboisi | VNMN 4103 | Lao Cai, Vietnam | LC010581    | Nguyen et al. 2017 |
| 11  | Z. dugritei | LJTI 060559 | Sichuan, China | JN688875    | Li et al. 2012a |
| 12  | Z. dugritei | SCUM 051017 L 11 | Sichuan, China | EU215540    | Li et al. 2012a |
| 13  | Z. feae | VNMN 3462 | Sa Pa, Vietnam | LC010588    | Nguyen et al. 2017 |
| 14  | Z. feae | SCUM 050642W | Yunnan, China | EU215544    | Nguyen et al. 2017 |
| 15  | Z. hongchibaensis | CIB 097687 | Chongqing, China | JN688883    | Li et al. 2012a |
| 16  | Z. hongchibaensis | CIB 097696 | Chongqing, China | JN688882    | Li et al. 2012a |
| 17  | Z. hungfuensis | SCUM 060425L | Sichuan, China | EU215538    | Li et al. 2012a |
| 18  | Z. hungfuensis | SCUMLi 01 | Sichuan, China | JN688878    | Li et al. 2012a |
| 19  | Z. minimus | KIZ 061214YP | Guangxi, China | EU215539    | Li et al. 2008 |
| 20  | Z. minimus | KUHE:70049 | China | LC386569    | Matsui et al. 2019 |
| 21  | Z. nigropunctatus | Rao3494 | Yunnan, China | JX219429    | Li et al. 2012b |
| 22  | Z. nigropunctatus | GZ070658 | Guizhou, China | JX219430    | Li et al. 2012b |
| 23  | Z. omeimontis | Li02 | Sichuan, China | JX219420    | Li et al. 2012b |
| 24  | Z. smaragdinus | KIZ 06241Rao | Tibet, China | JX219411    | Li et al. 2012a |
| 25  | Z. pinglongensis | NHMG201002011 | Guangxi, China | KU170684    | Mo et al. 2016 |
| 26  | Z. pinglongensis | NHMG201002003 | Guangxi, China | KU170683    | Mo et al. 2016 |
| 27  | Z. puerensis | SCUM 060649L | Yunnan, China | EU215542    | Li et al. 2012a |
| 28  | Z. puerensis | ROM 37996 | Lao Cai, Vietnam | JN688891    | Li et al. 2012a |
| 29  | Z. schlegelii | KUHE 44531 | Okayama, Japan | LC369670    | Matsui et al. 2019 |
| 30  | Z. schlegelii | Genbank | Hiroshima, Japan | AB202078    | Sano et al. 2005 |
| 31  | Z. pachyproctus | VNMN 1534 | Bac Giang, Vietnam | LC010592    | Nguyen et al. 2017 |
| 32  | Z. pachyproctus | VNMN 4113 | Nghe An, Vietnam | LC010593    | Nguyen et al. 2017 |
| 33  | Z. taroensis (burmanus) | SCUM 060614L | Yunnan, China | EU215537    | Li et al. 2008 |
| 34  | Z. wui | CIB 097685 | Hubei, China | JN688881    | Li et al. 2012a |
| 35  | Z. wui | CIB 097690 | Hubei, China | JN688880    | Li et al. 2012a |
| 36  | Z. yaoshanensis | NHMG150408 | Guangxi, China | MG322122    | Chen et al. 2018 |
| 37  | Z. yaoshanensis | NHMG150404 | Guangxi, China | MG322121    | Chen et al. 2018 |
| 38  | Z. zhoukaiyae | AHU-RhaDb-120428 | Anhui, China | KU601502    | Pan et al. 2017 |
| 39  | Z. zhoukaiyae | RhaDb-150420-02 | Anhui, China | KU601501    | Pan et al. 2017 |
| 40  | *Rhacophorus annamensis* | VNMN 07225 | Ninh Thuan, Vietnam | LC548739    | 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

### Table 1. Continuation

| No. | Scientific name               | Voucher    | Locality              | Genbank No. | Source       |
|-----|-------------------------------|------------|-----------------------|-------------|--------------|
| 41  | *R. annamensis*               | HAO 01     | Ninh Thuan, Vietnam   | LC548740    | This study   |
| 42  | *R. kio*                      | VN.2018.057| Kon Tum, Vietnam      | LC548742    | This study   |
| 43  | *R. kio*                      | VN.2018.082| Kon Tum,Vietnam       | LC548741    | This study   |
| 44  | *R. orlovi*                   | TQ.2018.20 | Tuyen Quang, Vietnam  | LC548743    | This study   |
| 45  | *R. orlovi*                   | TQ.2018.56 | Tuyen Quang, Vietnam  | LC548744    | This study   |
| 46  | *Zhangixalus franki* sp. nov  | VNMN 011686| Ha Giang, Vietnam     | LC548745    | This study   |
| 47  | *Zhangixalus franki* sp. nov  | VNMN 011687| Ha Giang, Vietnam     | LC548746    | This study   |
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| Symbol | Description |
|--------|-------------|
| 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 -lnL = 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. dorsoviridis*, *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.  
urn:lsid:zoobank.org:act:346FA039-3D9A-4F41-A4F2-57F5E7ABA793  
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

![Image of frog](image_url)

**Fig. 2.** A–B. Dorsal and lateral views. C. Drawing of ventral view of left hand and right foot of the adult male holotype (VNHN 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 | 2.9  |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| 2 | 3.29 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| 3 | 9.06 | 8.56 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| 4 | 9.25 | 9.12 | 11.29|      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| 5 | 4.87 | 5.32 | 9.44 | 9.47 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| 6 | 2.51 | 3.63 | 9.19 | 8.89 | 4.18 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| 7 | 4.98 | 4.65 | 8.81 | 9.11 | 6.03 | 4.42 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| 8 | 8.80 | 9.16 | 8.96 | 9.59 | 9.62 | 8.82 | 8.82 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| 9 | 6.29 | 5.70 | 10.05| 10.67| 6.76 | 5.48 | 3.52 | 9.58 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| 10| 5.69 | 5.93 | 9.80 | 9.80 | 6.17 | 5.24 | 3.29 | 9.47 | 3.51 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| 11| 5.34 | 5.01 | 9.69 | 8.68 | 6.16 | 5.01 | 2.39 | 9.20 | 3.63 | 2.85 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| 12| 8.68 | 8.68 | 6.96 | 10.71| 8.83 | 9.07 | 8.45 | 9.19 | 9.80 | 9.68 | 9.19 |      |      |      |      |      |      |      |      |      |      |      |      |      |
| 13| 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| 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| 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| 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| 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| 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| 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| 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| 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| 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| 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| 16.79| 18.30| 15.84| 14.77|      |      |      |
| 24| 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| 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-I1I0-IIII½-0IV; 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; 2300′245″ 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<IV<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-II0-III½-IV0; 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<IV; tips of toes with enlarged discs with distinct circum-marginal grooves, discs slightly smaller than those of fingers; webbing formula I0-0-II0-½-III0-IV0; 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 ofibia 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) |
|----------|-------------|-------------|-------------|-------------|-------------|------------------------|
| Type status | Holotype M (adult) | Paratype M (adult) | Paratype M (adult) | Paratype M (adult) | Paratype M (adult) | Paratype M (adult) |
| Sex | SVL | 82.8 | 85.8 | 82.3 | 79.7 | 77.9 | 81.7 ± 3.01 |
| | HW | 26.5 | 27.9 | 27.5 | 26.2 | 25.1 | 26.6 ± 1.12 |
| | HL | 25.7 | 27.2 | 26.7 | 26.0 | 24.6 | 26.0 ± 1.01 |
| | MN | 21.7 | 23.1 | 22.4 | 21.7 | 21.1 | 22.0 ± 0.77 |
| | MFE | 16.6 | 17.4 | 17.0 | 16.3 | 16.5 | 16.8 ± 0.45 |
| | MBE | 9.8 | 10.0 | 9.3 | 9.5 | 9.6 | 9.6 ± 0.27 |
| | SNL | 11.6 | 13.1 | 12.2 | 11.9 | 11.8 | 12.1 ± 0.58 |
| | ED | 8.2 | 8.6 | 9.5 | 8.6 | 8.1 | 8.6 ± 0.54 |
| | UEW | 6.2 | 7.3 | 7.1 | 7.1 | 6.5 | 6.8 ± 0.47 |
| | IN | 8.1 | 7.6 | 7.2 | 7.7 | 7.7 | 7.6 ± 0.30 |
| | IOD | 9.2 | 10.5 | 8.9 | 10.0 | 9.3 | 9.6 ± 0.67 |
| | DAE | 16.3 | 17.7 | 17.0 | 15.7 | 16.7 ± 0.76 |
| | DPE | 23.6 | 24.3 | 24.3 | 22.8 | 22.9 | 23.6 ± 0.74 |
| | NS | 5.4 | 5.3 | 5.1 | 5.1 | 5.0 | 5.2 ± 0.17 |
| | EN | 6.9 | 7.5 | 6.8 | 6.7 | 6.6 | 6.9 ± 0.34 |
| | TYD | 4.5 | 4.8 | 5.4 | 5.1 | 5.0 | 4.9 ± 0.33 |
| | TYE | 2.2 | 4.5 | 2.1 | 2.1 | 1.9 | 2.6 ± 1.09 |
| | FLL | 14.7 | 14.0 | 15.6 | 12.9 | 13.8 | 14.2 ± 1.00 |
| | HAL | 41.7 | 44.1 | 40.4 | 40.0 | 40.9 | 41.4 ± 1.62 |
| | IPT | 5.2 | 5.2 | 5.4 | 5.6 | 5.3 | 5.3 ± 0.15 |
| | Fig 1 | 8.3 | 9.0 | 9.4 | 7.7 | 8.2 | 8.5 ± 0.66 |
| | Fig 2 | 13.0 | 13.3 | 14.0 | 12.2 | 13.5 | 13.2 ± 0.66 |
| | Fig 3 | 18.6 | 19.6 | 18.6 | 18.2 | 17.3 | 18.4 ± 0.84 |
| | Fig 4 | 14.8 | 15.4 | 15.6 | 14.0 | 14.9 | 14.9 ± 0.64 |
| | fd3 | 5.7 | 6.2 | 5.6 | 5.3 | 6.2 | 5.8 ± 0.39 |
| | FeL | 34.1 | 35.7 | 36.7 | 35.6 | 36.4 | 35.7 ± 0.99 |
| | TbL | 39.3 | 39.7 | 37.4 | 38.4 | 38.3 | 38.6 ± 0.90 |
| | TbW | 8.5 | 9.3 | 9.1 | 8.7 | 8.9 | 8.9 ± 0.34 |
| | FoL | 53.4 | 54.9 | 50.8 | 50.7 | 51.0 | 52.2 ± 1.90 |
| | Toe 1 | 14.1 | 14.5 | 13.1 | 13.8 | 11.9 | 13.5 ± 1.01 |
| | Toe 2 | 20.7 | 21.2 | 19.8 | 20.4 | 18.1 | 20.0 ± 1.18 |
| | Toe 3 | 29.0 | 29.6 | 27.3 | 27.5 | 24.9 | 27.7 ± 1.81 |
| | Toe 4 | 36.3 | 36.3 | 35.5 | 34.4 | 34.8 | 35.4 ± 0.86 |
| | Toe 5 | 31.2 | 31.4 | 30.5 | 30.3 | 29.7 | 30.6 ± 0.68 |
| | td4 | 4.6 | 5.0 | 4.4 | 4.6 | 3.2 | 4.4 ± 0.68 |
| | IMT | 3.2 | 3.6 | 3.5 | 3.3 | 2.1 | 3.1 ± 0.61 |

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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. chenfui at 33–41 mm (Fei et al. 2010); Z. dorsovirdis (Bourret, 1937) at 31.31–2.43 mm (Orlov et al. 2001); Z. dugreitai 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. yinggelingensis at 43–43.4 mm (Chou et al. 2007); Z. zhokaiyae 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 verses 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* (II–II10–III1–III3–IV vs II–I½II1–II½II1–IV 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 *Kurilxalus* 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

![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).]

![Habitat of Zhangixalus franki sp. nov. at the type locality in Quan Ba District, Ha Giang Province, Vietnam.]

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. dorsovirdis*, *Z. duboisi*, *Z. omeimontis*, and *Z. zhoukaiyae*, 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.