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# Supplementary Materials

## Mountain jade: A new high-elevation microendemic species of the genus *Zhangixalus* (Amphibia: Anura: Rhacophoridae) from Laos

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## SUPPLEMENTARY MATERIALS AND METHODS

**Specimen collection and preservation.** Field surveys were conducted in July 2020 in Phou Samsoum Mountain (PSM) in Xiengkhouang Province, northeast Laos (Figure 1A). Geographic coordinates and elevation were obtained using a Garmin GPSMAP 64CSx (USA) and recorded in WGS84 datum. Specimens were euthanized with 20% benzocaine and femoral muscles were collected for genetic analysis and stored in 90% ethanol prior to specimen preservation. Specimens were subsequently preserved in 70% ethanol and deposited in the herpetological collections of the Biotechnology and Ecology Institute Ministry of Science and Technology of Laos (BEI, Veintiane, Laos), the School of Agriculture and Natural Resources, University of Phayao (AUP, Phayao, Thailand) and the Zoological Museum of Lomonosov Moscow State University (ZMMU, Moscow, Russia).

**Laboratory methods**. For molecular phylogenetic analyses, we extracted total genomic DNA from ethanol-preserved femoral muscle tissue using standard phenol-chloroform-proteinase K extraction procedures with consequent isopropanol precipitation; to a final concentration of ~1 mg/mL (protocols followed Hillis et al., 1996 and Sambrook & Russell, 2001). We visualized the isolated total genomic DNA in agarose electrophoresis in the presence of ethidium bromide. We measured the concentration of total DNA in 1 μL using a NanoDrop 2000 (Thermo Scientific, USA), and consequently adjusted the concentration to ca. 100 ng DNA/μL.

We amplified mtDNA fragments covering partial sequences of the 16S rRNA mtDNA gene to obtain a 1918 bp length continuous fragment of mtDNA. We also amplified 655 bp of the 5'-end of the first subunit of cytochrome c oxidase mtDNA gene (*COI*). The 16S rRNA gene is widely applied in biodiversity surveys in amphibians (Vences et al., 2005a, 2005b; Vieites et al., 2009), and has been used in most recent phylogenetic studies on Rhacophoridae (Jiang et al., 2019; Li et al., 2008, 2012; Nguyen et al., 2020; Ninh et al., 2020; Pan et al., 2017; Poyarkov et al., 2018). The *COI* gene is widely known as a barcoding marker for amphibians as well as other vertebrates (Murphy et al., 2013). We performed DNA amplification in 20 μL reactions using ca. 50 ng genomic DNA, 10 nmol of each primer, 15 nMol of each dNTP, 50 nMol additional $MgCl_2$, Taq PCR buffer (10 mmol/L Tris-HCl, pH 8.3, 50 mmol/L KCl, 1.1 mmol/L $MgCl_2$, and 0.01% gelatin), and 1 unit of Taq DNA polymerase. Primers used in PCR and sequencing of were obtained from previous studies (for 16S rRNA gene: Hedges, 1994; Li et al., 2008; Poyarkov et al., 2018; for *COI* gene: Che et al., 2012). The PCR conditions included an initial denaturation step of 5 min at 94 °C and 43 cycles of denaturation for 1 min at 94 °C, primer annealing for 1 min with the TouchDown program from 65 °C to 55 °C reducing 1 °C every cycle, extension for 1 min at 72 °C, and final extension step for 5 min at 72 °C.

The PCR products were loaded onto 1.5% agarose gels in the presence of ethidium bromide and visualized via agarose electrophoresis. When distinct bands were produced, we purified the PCR products using 2 μL of a 1:4 dilution of ExoSapIt (Amersham, USA) per 5 μL of PCR product prior to cycle sequencing. The 10 μL sequencing reaction included 2 μL of template, 2.5 μL of sequencing buffer, 0.8 μL of 10 pmol primer, 0.4 μL of BigDye Terminator v3.1 Sequencing Standard (Applie Biosystems, USA), and 4.2 μL of water. The cycle sequencing used 35 cycles of 10 s at 96 °C, 10 s at 50 °C, and 4 min at 60 °C. We purified the cycle sequencing products by ethanol precipitation. We carried out sequence data collection and visualization on an ABI 3730xl Automated Sequencer (Applied Biosystems, USA). The obtained sequences were deposited in GenBank under accession numbers OQ288104-OQ288107, OQ297601, OQ305233-OQ305236 (see Supplementary Table S1).

**Phylogenetic analyses**. To reconstruct the matrilineal genealogy, we used 16S rRNA and *COI* sequences of the *Zhangixalus* sp. from Xiengkhouang Province of Laos, as well as the homologous sequences of 38 out of 40 currently recognized *Zhangixalus* species obtained from the earlier phylogenetic studies of the genus (e.g., Mathipi et al., 2021; Nguyen et al., 2020; Ninh et al., 2020; Pan et al., 2017; Yu et al., 2019; see Supplementary Table S1). GenBank accession numbers, museum vouchers, and localities of origin for sequences used in this study are summarized in Supplementary Table S1. We also added the homologous sequences of *Leptomantis gauni* and *Rhacophorus kio*, representing the sister taxa of *Zhangixalus*; the sequence of *Polypedates leucomystax* was used as an outgroup (Jiang et al., 2019). In total, we obtained 16S rRNA and *COI* sequence data from 45 specimens, including four specimens of *Zhangixalus* sp. from Xiengkhouang, 38 sequences of all other species of *Zhangixalus*, three outgroup sequences of other Rhacophoridae representatives (*Leptomantis*, *Rhacophorus*, and *Polypedates*) (see Supplementary Table S1).

We initially aligned nucleotide sequences using ClustalX 1.81 (Thompson et al., 1997) with default parameters, and then optimized them manually in BioEdit 7.0.5.2 (Hall, 1999). We used MODELTEST v.3.06 (Posada & Crandall, 1998) to estimate the optimal evolutionary models to be used for dataset analysis. The best-fitting model for the 16S rRNA gene fragment was the GTR+I+G model of DNA evolution, as suggested by the Akaike Information Criterion (AIC). The best-fitting models selected for the COI dataset were SYM+I for the first, F81+I for the second, and HKY+G for the third codon positions, as suggested by the Akaike Information Criterion (AIC). We determined mean uncorrected genetic distances (*p*-distances) between sequences with MEGA 6.0 (Tamura et al., 2013).

We inferred matrilineal genealogy using Bayesian inference (BI) and maximum-likelihood (ML) approaches. We conducted BI in MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003); Metropolis-coupled Markov chain Monte Carlo (MCMCMC) analyses were run with one cold chain and three heated chains for one million generations, with sampling every 100 generations. We performed five independent MCMCMC runs and the initial 2 500 trees were discarded as burn-in. We assessed confidence in tree topology by the frequency of nodal resolution (posterior probability; BI PP) (Huelsenbeck et al., 2001). We *a priori* considered BI PP 0.95 or greater as significant support (Leaché & Reeder, 2002).

We conducted the ML analysis in the IQ-TREE webserver (http://iqtree.cibiv.univie.ac.at/). We employed 1,000 bootstrap pseudoreplicates via the ultrafast bootstrap (UFBS; Hoang et al., 2018) approximation algorithm, and nodes having ML UFBS values of 95 and above were a-priori considered highly supported, while the nodes with values of 90–94 were considered well-supported, and the nodes with values of 70–89 were considered as tendencies. Lower values were considered to indicate no support.

**Morphological description**. Measurements were taken to the nearest 0.1 mm with a Mitutoyo digital caliper. The descriptions of the morphological characteristics of adults and larvae followed Poyarkov et al. (2018) and Poyarkov et al. (2015), respectively. Sex was determined by direct observation of calling and by examination of the nuptial pads and vocal sac in males. Comparative data on morphological and bioacoustic characteristics of other *Zhangixalus* species were obtained from previous publications.

The morphometrics of adults and character terminology followed Poyarkov et al. (2018): SVL (snout-vent length); A-G (axilla to groin, distance from posterior base of forelimb at its emergence from body to anterior base of hind limb at its emergence from body); HW (head width at greatest cranial width); HL (head length from rear of lower jaw to tip of the snout); HD (head depth, greatest transverse depth of head, taken beyond interorbital region); UEW (upper eyelid width, greatest width of upper eyelids); IOD (interorbital distance); ED (horizontal diameter of eye); TD (horizontal diameter of tympanum); ESL (tip of snout-eye distance); IND (internarial distance between nostrils); END (eye to nostril distance from anterior corner of eye to nostril); TED (tympanum-eye distance from anterior edge of tympanum to posterior corner of eye); NS (distance from nostril to tip of snout); FLL (length of forelimb from tip of disk of finger III to axilla); HML (humerus length from axilla to elbow); LAL (forearm length, from elbow to base of outer palmar tubercle); ML (hand length from tip of third digit to base of outer palmar tubercle); 1FLi (first finger length, from base of inner palmar tubercle to tip finger); 1FLo (first finger length in inner); 2FLi (second finger length in inner); 3FLi (third finger length in inner); 4FLi (fourth finger length in inner); FTD (maximal diameter of disk of finger III); NPL (nuptial pad length, measured for males only); MCTe (length of external metacarpal tubercle); HLL (length of hindlimb from tip of disk of toe IV to groin); FL (femur length); TL (tibia length); TTL (tibiotarsus length from the posterior edge of tibia to the anterior edge of inner metatarsal tubercle); FOT (foot length from tip of fourth toe to the anterior edge of the inner metatarsal tubercle); 1TLi (first toe length, from the base of inner carpal tubercle to the tip); 1TLo (first toe length in outer); 2TLi (second toe length inner); 3TLi (third toe length in inner); 4TLi (fourth toe length in inner); 5TLi (fifth toe length in inner); HTD (diameter of fourth toe tip, greatest diameter of disk on fourth toe); MTTi (length of internal metatarsal tubercle); IMW (inner metatarsal tubercle width). Additional measurements of the holotype of *Zhangixalus nigropunctatus* (Liu, Hu & Yang) [CIB 590405] followed Li et al. (2011) and included the following characters: FLL-2 (forelimb length excluding the length of the humerus, measured from elbow to tip of third finger); and FTL (length of tarsus and foot from the posterior edge of tibia to the end of the fourth toe). Subarticular tubercle and webbing formulas follow Savage (1975). All measurements were taken on the right side of the examined specimen. Sex was determined by gonadal inspection following dissection.

Morphological description of larval stages included the following Poyarkov et al. (2015): TL (total length); BL (body length); TaL (tail length); BW (maximal body width); BH (maximal body height); TH (maximal tail height); SVL (snout-vent length); SSp (snout-spiracle length); UF (maximal upper tail fin height); LF (maximal lower fin height); IN (internarial distance); IP (interpupilar distance); RN (rostro-narial distance); NP (naro-pupilar distance); ED (eye diameter); ODW (oral disk width). LTRF (labial tooth row formula) was recorded following Wassersug et al. (1981). Tadpoles were staged after Gosner (1960); morphometrics followed Grosjean (2001).

Comparative data on the morphology and taxonomy of *Zhangixalus* were obtained from previous publications on the genus (Chen et al., 2018; Chou et al., 2007; Fei et al., 2010; Jiang et al., 2016, 2019; Li et al., 2012; Liu et al., 2017, 2020; Mo et al., 2016; Nguyen et al., 2020; Ninh et al., 2020; Ohler & Deuti, 2018; Ohler et al., 2000; Orlov et al., 2001; Pan et al., 2017; Rao et al., 2006; Yu et al., 2019; Zhang et al., 2011). Comparative data on the morphology of *Zhangixalus nigropunctatus* (Liu, Hu & Yang) tadpoles were obtained from Editorial Committee of Zoology of China, Chinese Academy of Sciences (2009) and Fei et al. (2010).

**Bioacoustic analysis**. Advertisement calls of the newly discovered *Zhangixalus* population were recorded in situ at the breeding site (coordinates N 19.131 °, E 103.784 °; elevation 2066 m asl.) on 15 July 2020 at 2030 h and at 16.5 °C using a portable digital audio recorder Zoom h5 (ZOOM Corporation, Tokyo, Japan) in stereo mode with 48 kHz sampling frequency and 16-bit precision. The temperature was measured at the calling site immediately after the audio recording with a digital thermometer KTJ TA218A Digital LCD Thermometer-Hydrometer. Calls were analyzed using Avisoft SASLab Pro software v.5.2.05 (Avisoft Bioacoustics, Germany); the analyses generally followed Poyarkov et al. (2018).

In total, six advertisement calls from two individuals (holotype AUP02505 and paratype ZMMU A-7781) were recorded. The total duration of the recordings was 541.86 s. Calls were analyzed using Avisoft SASLab Pro software v. 5.2.05; spectrograms for analysis were created using Hamming window, FFT-length 1024 points, frame 100%, and overlap 93.75%. Figure spectrograms were created using Hamming window, FFT-length 512 points, frame 50%, and overlap 93.75%. We measured the duration of each note (s) number of pulses per note, pulse duration (measured separately for the first, the second and the third pulses, s), internote interval (s), note repetition rate (notes per second), and the dominant frequency (= frequency of maximum amplitude, Hz). Notes (or pulses) per second were calculated by counting the number of notes (or pulses) within each call, minus one, and dividing that number by the call duration (or duration of the note). All numeral parameters are given as mean ± SE, the minimum and maximum values are given in parentheses (min–max).

Comparative advertisement call characteristics for *Zhangixalus* species were taken from references, with advertisement calls known only for five of the 40 known species of *Zhangixalus* (Fang et al., 2019; Matsui & Wu, 1994; Nguyen et al., 2020; Wang et al., 2012).

## SUPPLEMENTARY RESULTS

**Measurements of the holotype and additional morphological information on *Zhangixalus nigropunctatus* (Liu, Hu & Yang).** The following measurements of the holotype of *Zhangixalus nigropunctatus* (Liu, Hu & Yang) [CIB 590405] were taken by one of us (NAP), additional measurements of this specimen were obtained from the paper by Li et al. (2011). For abbreviations of the additional measurements see Supplementary materials and methods. Measurements of CIB 590405 (all in mm): SVL: 36.5; A-G: 20.0; HW: 12.5; HL: 12.2; HD: 5.1; UEW: 3.0; IOD: 4.0; ED: 4.5; TD: 1.5; ESL: 5.1; IND: 3.4; END: 2.6; TED: 1.5; NS: 2.5; FLL: 26.0; HML: 7.0; LAL: 8.0; ML: 10.2; FLL-2: 18.2; FTD: 1.8; HLL: 48.0; FL: 12.0; TL: 14.0; TTL: 8.0; FOT: 14.8; FTL: 22.8; HTD: 1.8.

Photograph of a male *Zhangixalus nigropunctatus* (Liu, Hu & Yang) from Yushe National Forest Park (N 26.46 °, E 104.81 °; elevation 2070 m a.s.l.), Guizhou Province, China, kindly provided by Jian Wang (SYS, China) is presented in Supplementary Figure S5.

**Larval morphology description.** Tadpoles in the developmental stage 35 of Gosner (1960) were assigned to the new species based on 16S partial sequences obtained for one specimen ZMMU A-7783. Measurements of tadpoles of the new species are presented in Supplementary Table S4;

*General appearance of the tadpoles in preservative*: The tadpoles are medium-sized (TL = 25.1–39.8 mm), lentic: benthic (Altig & McDiarmid, 1999), and are classified as generalized exotrophic tadpoles of Orton's (Orton, 1953) type IV lacking obvious specializations. Dorsal coloration is uniform light-brown from the snout to the tip of the tail including fins (Supplementary Figure S4A). Dark-brown marbled pattern is present on dorsal tail fin and on the dorsal surfaces of body. Dorsal and dorsolateral pigmentation of the body is same dense as the tail pigmentation. The tail musculature coloration varies from light brown to ochre (see Supplementary Figure S4A). The ventral and ventrolateral body sides are white to yellow and more or less pigmented. Belly is translucent and the intestine is visible through the body.

The following description in is based on a single tadpole ZMMU A-7783-1 with SVL 39.8 mm. In dorsal view, body elliptical with a slightly pointed snout (Supplementary Figure S4A) with its widest portion being at midbody (body width 0.56 times of body length). Eyes of moderate size (eye diameter 0.11 times of body length), with dorsolateral orientation, directed more laterally than anteriorly, slightly bulging, not visible in ventral view. Nares small, rounded, not rimmed, positioned dorsolaterally in slightly anterolateral direction. Naris notably closer to snout than to pupil (rostro-narial distance 0.36 times of naropupilar distance). Internarial distance about 0.44 times of interpupilar distance. Nasolacrimal duct from the naris to the anterior corner of the eye not discernable.

In lateral view, body slightly depressed (body height 0.86 times of body width), snout slightly rounded. Spiracle sinistral, positioned at midbody with ventrolateral orientation (distance from snout tip to opening of spiracle 0.59 times of body length), conical, with posterodorsal orientation and entirely attached to the body. Spiracle opening oval; vent tube partially reduced. Myotomes of the tail musculature well-developed; with parallel orientation in the anterior part of the tail, then gradually tapering, reaching the tip of the tail. Tail fin moderate, tapering at the end. Highest point of the upper fin at the middle of the tail length (maximum height of upper tail fin 0.30 times of maximum tail height). Lower fin slightly smaller than dorsal fin (maximum height of lower tail fin 0.78 times of maximum tail height). Lateral line organs well developed on body and along the the caudal musculature.

Oral disk anteroventrally positioned comprising about 0.34 times of body width, ovoid in shape in relaxed state (see Supplementary Figure S4B), laterally emarginated. Oral disk framed by finger shaped papillae of moderate size except for a large medial gap of the upper labium is slightly narrower than the first keratodont row. Submarginal papillae on the upper labium not discernable; posterior border of the lower labium emarginated with an additional row of submarginal papillae of the same length as the lowest keratodont row. Keratodont row A1 continuous, A2 – A3 divided, A4 – A6 entirely separated by the upper jaw sheath. Keratodont row P1 divided; keratodont rows P2 – P3 of the lower labium undivided. Keratodont row formula (KRF): 1:5+5/1+1:2. Jaw sheaths black, notably serrated (see Supplementary Figure S4B); with upper jaw sheath narrow, stretched into a wide arch; lower jaw sheath V-shaped.

**Morphological comparisons.** The green dorsum, the white belly, flanks, axilla, ventral surface of forearms, inguinal, anterior and posterior surfaces of thighs covered with irregular black pattern; and the reddish-orange iris distinguishes the new species from 25 nominal *Zhangixalus* species distributed in Indochina, China, India and Myanmar (comparisons detailed in Supplementary Tables S5–S7).

Morphological comparisons of the new species with its sister species *Z. nigropunctatus* appear to be the most pertinent (see Supplementary Figure S5 for life photo of *Z. nigropunctatus*; also see Supplementary Results for measurements of the holotype of this species [CIB 590405]). The new species can be readily distinguished from *Z. nigropunctatus* by coloration in life, in particular by the presence of large irregular black blotches on axilla, flanks, anterior and posterior surfaces of thighs forming continuous pattern (vs. small separated indistinct black spots), by having small back spots on the ventral surfaces of thighs and tarsus (vs. yellowish lacking back spots), and by having bright reddish-orange iris (vs. yellowish-gold). In morphometrics males of the new species can be easily differentiated from *Z. nigropunctatus* by comparatively larger head (HL/SVL 36.7% [N=4] vs. 34.5% [N=20, data from Editorial Committee of Zoology of China, Chinese Academy of Sciences, 2009] in *Z. nigropunctatus*, 33.4% in the holotype of *Z. nigropunctatus*, see Supplementary Results); by having a larger tympanum (TD/SVL 5.9% [N=4] vs. 4.9% [N=20, data from Editorial Committee of Zoology of China, Chinese Academy of Sciences, 2009] in *Z. nigropunctatus*, 4.1% in the holotype of *Z. nigropunctatus*); by having comparatively larger eyes

(ED/SVL 16.7% [N=4] vs. 14.0% in the holotype of *Z. nigropunctatus*); by having larger internarial distance (IND/SVL 12.2% [N=4] vs. 9.3% in the holotype of *Z. nigropunctatus*); and by having comparatively longer hindlimbs (HLL/SVL 141.7% [N=4] vs. 131.5% in the holotype of *Z. nigropunctatus*). Furthermore, the new species is clearly different from *Z. nigropunctatus* in keratodont row formula (KRF) of tadpole mouth discs (1:5+5/1+1:2 vs. 1:3+3/1+1:1 in *Z. nigropunctatus*, data from Editorial Committee of Zoology of China, Chinese Academy of Sciences, 2009). Moreover, the closest known population of *Z. nigropunctatus* in Guizhou Province of China is separated from the range of *Zhangixalus melanoleucus* **sp. nov.** by over 800 km distance, which provides further support for our hypothesis that the differentiation between these taxa reaches the species level. Comparisons of *Zhangixalus melanoleucus* **sp. nov.** with other congeners are detailed in Supplementary Data and summarized in Supplementary Table S5.

*Zhangixalus melanoleucus* **sp. nov.** can be distinguished from other members of *Z. chenfui* species group by the following combination of morphological characters. The new species differs from *Z. chenfui* (Liu) by having whitish belly without spots (vs. cream with small pale yellow spots), by the presence of irregular black pattern on white flanks, anterior and posterior surfaces of thighs (vs. absence); from *Z. pinglongensis* by having flanks and anterior and posterior surfaces of thighs white with irregular black pattern (vs. black with small white spots), by having ventral surfaces of feet and webbing cream (vs. tangerine), and by reddish-orange iris (vs. silver); from *Z. yaoshanensis* (Liu & Hu) by having flanks, anterior and posterior surfaces of thighs white with irregular black pattern (vs. orange-red without spots), and by having reddish orange iris (vs. grayish-gold); from *Z. jodiae* by having flanks and anterior and posterior surfaces of thighs white with irregular black pattern (vs. axilla cream with large black blotches, groin and front-rear parts of the thigh, ventral surface of tibia black with orange blotches), and by reddish-orange iris (vs. silver).

From other *Zhangixalus* species which have immaculate green dorsum, *Zhangixalus melanoleucus* **sp. nov.** can be further distinguished as follows: from *Z. dorsoviridis* by the presence of nuptial pads (vs. absence), by flanks, anterior and posterior surfaces of thighs white with irregular large black pattern (vs. white to orange with variable small black spots); from *Z. feae* by smaller body size (34.4–36.3 mm in males, 53.7 mm in female vs. 86–111 mm in males, 68–116 mm in females), by having white or grey throat (vs. pale green), by the presence of irregular black pattern on white flanks, anterior and posterior surfaces of thighs (vs. absence), by reddish-orange iris (vs. greenish-gold), by finger webbing reduced (vs. complete); from *Z. leucofasciatus* (Liu & Hu) by having smaller body size in males (34.4–36.3 mm vs. 47.5–49.4 mm), by the presence of an irregular black pattern on flanks, anterior and posterior surfaces of thighs (vs. absence), by reddish-orange iris (vs. yellowish-brown); from *Z. lishuiensis* (Liu, Wang & Jiang) by having the entire belly whitish (vs. anteriorly white, posteriorly yellow), by the presence of an irregular black pattern on flanks, anterior and posterior surfaces of thighs (vs. absence), by reddish-orange iris (vs. yellowish-gold); from *Z. minimus* (Rao, Wilkinson & Liu) by having immaculate white ventral surfaces (vs. spots on belly), by the presence of an irregular black pattern on flanks, anterior and posterior surfaces of thighs (vs. absence), by reddish-orange iris (vs. yellowish-gold); from *Z. pachyproctus* by having smaller body size (34.4–36.3 mm in males, 53.7 mm in female vs. 73.4–78.2 mm in males, 102.4 mm in female), by the presence of an irregular black pattern on flanks, anterior and posterior surfaces of thighs (vs. absence), by reddish-orange iris (vs. yellowish-gold), and by reduced finger webbing (vs. complete); from *Z. smaragdinus* (Blyth) by having smaller body size (34.4–36.3 mm in males, 53.7 mm in female vs. 57–84 mm in males, 85–112 mm in females), by the presence of an irregular black pattern on flanks, anterior and posterior surfaces of thighs (vs. absence), by reddish-orange iris (vs. yellowish-gold), and by reduced finger webbing (vs. complete); from *Z. zhoukaiyae* (Pan, Zhang & Zhang) by having ventral surface whitish (vs. yellowish), by the presence of an irregular black pattern on flanks, anterior and posterior surfaces of thighs (vs. small brown spots), and by reddish-orange iris (vs. yellowish-gold).

*Zhangixalus melanoleucus* **sp. nov.** further differs from all other congeners by having immaculate green dorsum (vs. green to dark green with small pale yellow to brown dots in *Z. burmanus* (Andersson); green with small brown spots in *Z. dennysi* (Blanford); greenish with red-brown spots in *Z. duboisi* (Ohler, Marquis, Swan & Grosjean); green with round golden spots in *Z. dugritei* (Boulenger); green with dark brown spots in *Z. franki* Ninh, Nguyen, Orlov, Nguyen & Ziegler; green with yellowish-brown spots edged with dark brown in *Z. hongchibaensis* (Li, Liu, Chen, Wu, Murphy, Zhao, Wang & Zhang); green with brown spots in *Z. hui* (Liu); green with small white spots in *Z. hungfuensis* (Liu & Hu); green with brown pattern in *Z. omeimontis* (Stejneger); greenish-yellow with small white or brown spots in *Z. prominanus* (Smith); green with brownish-red spots in *Z. puerensis* (He); numerous light-brown spots with dark yellowish brown edges in *Z. wui* (Li, Liu, Chen, Wu, Murphy, Zhao, Wang & Zhang); and green with few fine white spots in *Z. yinggelingensis* (Chou, Lau & Chan).

**Discussion on synonymy of particular *Zhangixalus* species.** Our data confirms the synonymy of *Rhacophorus taronensis* Smith and *R. gongshanensis* Yang & Su with *Z. burmanus* (Andersson) as proposed earlier by Ohler (2009); and of *Polypedates pingbianensis* Kou, Hu & Gao with *Z. duboisi* (Ohler, Marquis, Swan & Grosjean) as proposed by Orlov et al. (2002).

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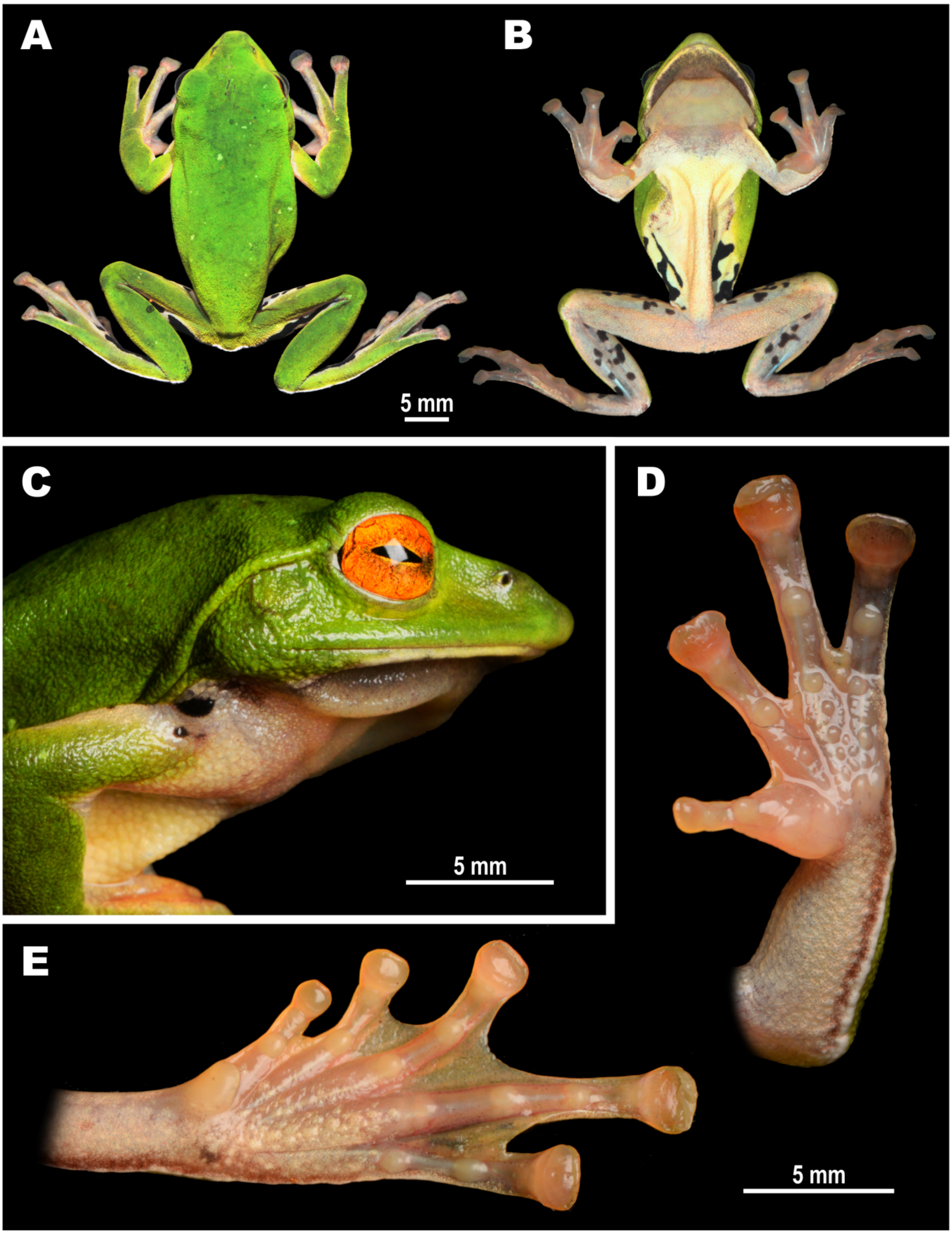
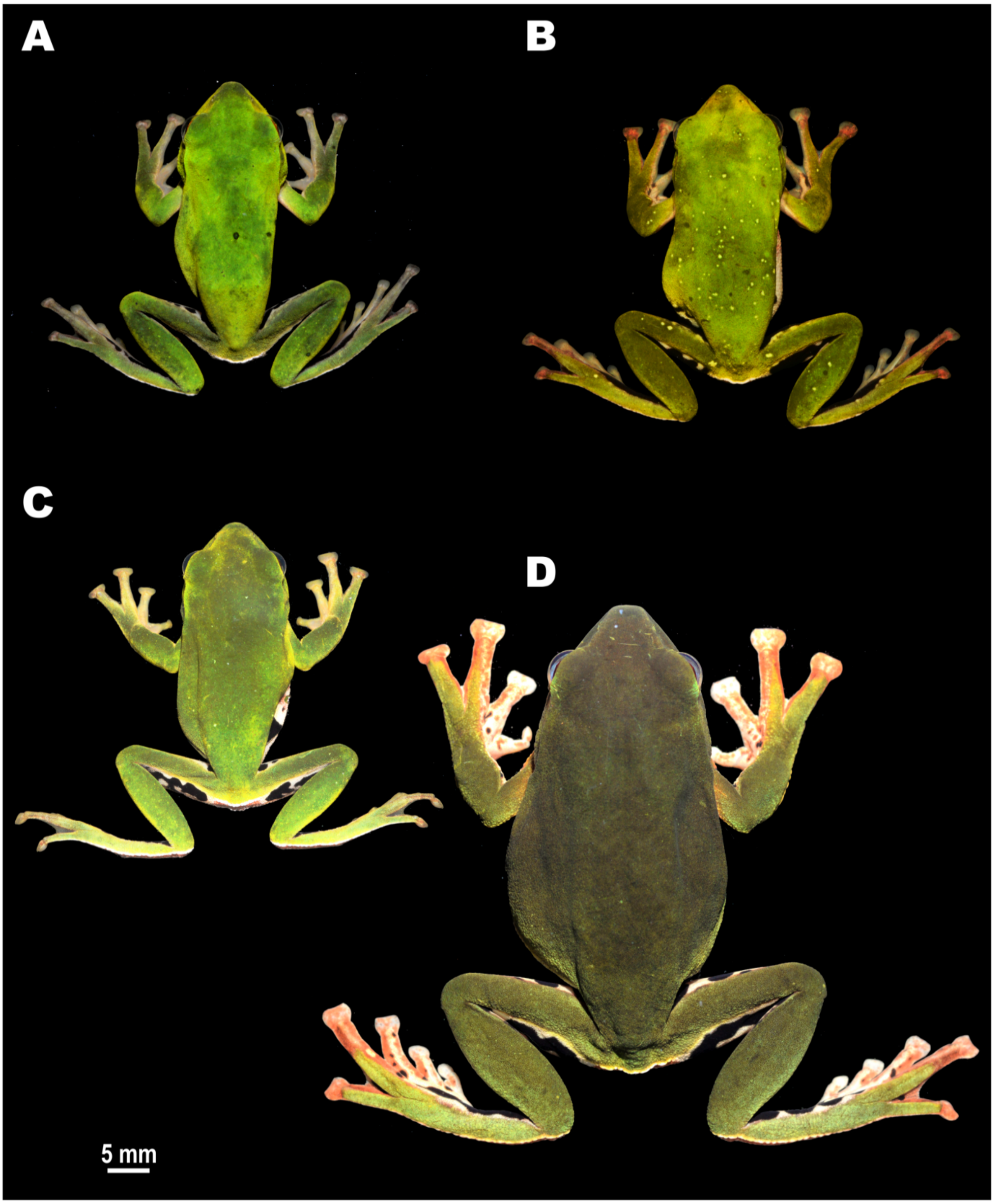
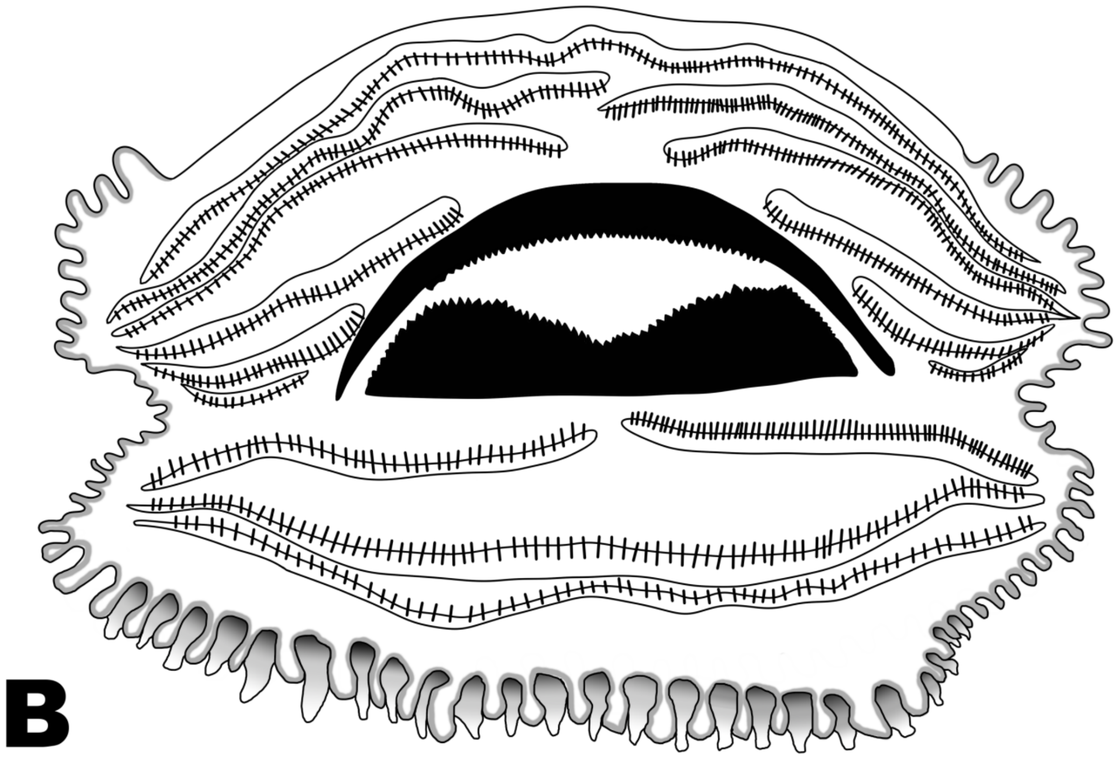



**Supplementary Figure S1** Life coloration of the holotype of *Zhangixalus melanoleucus* **sp. nov.** (AUP02505), adult male, in (A) Dorsal view; (B) ventral view; (C) head lateral view; (D) volar view of left hand; (E) plantar view of left foot. Scale bar equals 5 mm. Photographs by N. A. Poyarkov.




**Supplementary Figure S2** Variation in life dorsal coloration within the type series of *Zhangixalus melanoleucus* **sp. nov.** (A) AUP 02506, adult male; (B) ZMMU A-7781, adult male; (C) AUP 02507, adult male; (D) ZMMU A-7782, adult female. Scale bar equals 5 mm. Photographs by N. A. Poyarkov.

**Supplementary Figure S3** Amplexus of *Zhangixalus melanoleucus* **sp. nov.** (ZMMU A-7781 male and ZMMU A-7782 female). Photograph by P. Pawangkhanant.


**Supplementary Figure S4** Tadpole of *Zhangixalus melanoleucus* **sp. nov.** (ZMMU A-7783-1) (Gosner stage 35). (A) In dorsolateral view in situ, photograph by P. Pawangkhanant; (B) oral disk morphology, drawing by S. Idiiatullina.

**Supplementary Figure S5** Male *Zhangixalus nigropunctatus* (Liu, Hu & Yang) from Yushe National Forest Park (N 26.46 °, E 104.81 °, elevation 2070 m a.s.l.), Guizhou Province, China, in life; photograph by Jian Wang.

**Supplementary Table S1.** Localities, voucher information, and GenBank accession numbers for all specimens used in molecular analyses in this study. For references see Supplementary materials and methods section. (n.a. - not available)

| Species | Specimen ID | 12S-16S | COI | Locality | Reference |
|---|---|---|---|---|---|
| *Z. melanoleucus* **sp. nov.** | BEI 01010 | OQ305233 | OQ288104 | Phou Samsoum Mt., Xiengkhoang, Laos | *this study* |
| *Z. melanoleucus* **sp. nov.** | BEI 01011 | OQ305235 | OQ288106 | Phou Samsoum Mt., Xiengkhoang, Laos | *this study* |
| *Z. melanoleucus* **sp. nov.** | AUP 02507 | OQ305236 | OQ288107 | Phou Samsoum Mt., Xiengkhoang, Laos | *this study* |
| *Z. melanoleucus* **sp. nov.** | ZMMU A-7781 | OQ305234 | OQ288105 | Phou Samsoum Mt., Xiengkhoang, Laos | *this study* |
| *Z. achantharrhena* | ENS 7597 | MF066239 | n.a. | Indonesia | O'Connell et al. (2018) |
| *Z. amamiensis* | KUHE 22524 | LC386575 | LC386524 | Amamioshima, Japan | Matsui et al. (2019) |
| *Z. arboreus* | KUHE 47945 | LC386562 | LC386500 | Iida-shi, Nagano, Japan | Matsui et al. (2019) |
| *Z. arvalis* | 17560 | OQ297601 | MH034328 | Douliu, Yunlin, Taiwan, China | Jang-Liaw unpublished data |
| *Z. burmanus* | SCUM 060614L | EU215537 | KP996738 | Mt Gaoligong, Yunnan, China | Li et al. (2008) |
| *Z. chenfui* | Li05 | JX219432 | KP996815 | Emeishan, Sichuan, China | Li et al. (2012) |
| *Z. dennysi* | RDEN 20150618 | KT191129 | n.a. | Ningguo, Meilin, Anhui, China | Huang et al. (2016) |
| *Z. dorsoviridis* | ROM 38015 | JX219423 | n.a. | Sa Pa, Lao Cai, Vietnam | Li et al. (2012) |
| *Z. duboisi* | KIZ 060821289 | EF564567 | EF564567 | Jinping, Yunnan, China | Yu et al. (2008) |
| *Z. dugritei* | KUHE 27701 | LC010584 | n.a. | Emeishan, Sichuan, China | Nguyen et al. (2014) |
| *Z. dulitensis* | BORNEENSIS 09087 | AB847123 | KP996755 | Sabah, Borneo, Malaysia | Matsui et al. (2014) |
| *Z. feae* | SCUM 050642W | EU215544 | KP996749 | Daweishan, Pingbian, Yunnan, China | Li et al. (2008) |
| *Z. franki* | VNMN 011686 | LC548745 | n.a. | Tung Vai, Quan Ba, Ha Giang, Vietnam | Ninh et al. (2020) |
| *Z. gongshanensis* | KIZ 1049 | EF564569 | EF564569 | Gonghan, Yunnan, China | Yu et al. (2008) |
| *Z. hongchibaensis* | CIB 097696 | JN688882 | n.a. | Hongchiba, Wuxi, Chongqing, China | Li et al. (2012) |
| *Z. hui* | SCUM 0504111L | JN688878 | KP996701 | Yanwotang, Zhaojue, Sichuan, China | Li et al. (2012) |
| *Z. hungfuensis* | SCUM 060425L | EU215538 | LC386532 | Wenchuan, Sichuan, China | Li et al. (2008) |

| | | | | | |
|---|---|---|---|---|---|
| *Z. jodiae* | VNMN 07122 | LC545595 | n.a. | Tung Vai, Quan Ba, Ha Giang, Vietnam | Nguyen et al. (2020) |
| *Z. lishuiensis* | YPX 47791 | KY653718 | n.a. | Lishui, Zhejiang, China | Liu et al. (2017) |
| *Z. minimus* | KUHE 70049 | LC386569 | LC386532 | China | Matsui et al. (2019) |
| *Z. moltrechti* | KUHE 31070 | LC386570 | LC386533 | Taipei, Taiwan, China | Matsui et al. (2019) |
| *Z. nigropunctatus* | GZ 070658 | JX219430 | JN700897 | Weining, Guizhou, China | Li et al. (2012) |
| *Z. omeimontis* | CIB 20060104 | LC010595 | LC386536 | Sichuan, China | Nguyen et al. (2014) |
| *Z. owstoni* | KUHE 12764 | LC386572 | LC386537 | Ishigakijima, Japan | Matsui et al. (2019) |
| *Z. pachyproctus* | KUHE 35130 | LC386568 | LC386531 | Pilok, Thailand | Matsui et al. (2019) |
| *Z. pingbianensis* | YN 080484 | JX219418 | KP996808 | Pingbian, Yunnan, China | Li et al. (2012) |
| *Z. pinglongensis* | NHMG 201002011 | KU170684 | n.a. | Pinglongshan, Shangsi, Guangxi, China | Mo et al. (2016) |
| *Z. prominanus* | Rao 081201 | JX219434 | LC386529 | Malaysia | Li et al. (2012) |
| *Z. puerensis* | SCUM 060649L | EU215542 | KP996810 | Puer, Yunnan, China | Li et al. (2008) |
| *Z. schlegelii* | KUHE 45531 | LC369670 | LC386405 | Okayama, Japan | Matsui et al. (2019) |
| *Z. smaragdinus* | KUHE 34511 | LC386567 | LC386530 | Kachin, Myanmar | Matsui et al. (2019) |
| *Z. suffry* | MZMU1390 | MT808304 | n.a. | Mizoram, India | Lalremsanga et al. unpublished data |
| *Z. taipeianus* | KUHE 34347 | LC386574 | LC386539 | Taipei, Taiwan, China | Matsui et al. (2019) |
| *Z. taronensis* | SCUM 060614L | EU215537 | n.a. | Gaoligong Mt., Yunnan, China | Li et al. (2008) |
| *Z. viridis* | KUHE 35354 | LC386576 | LC386525 | Okinawajima, Okinawa, Japan | Matsui et al. (2019) |
| *Z. wui* | CIB 097685 | JN688881 | KP996819 | Hanchi, Lichuan, Hubei, China | Li et al. (2012) |
| *Z. yaoshanensis* | NHMG 150408 | MG322122 | n.a. | Jinxiu, Guangxi, China | Chen et al. (2018) |
| *Z. zhoukaiyae* | AHU-RhaDb-150418-02 | KU601494 | n.a. | Qianping, Jinzhai, Anhui, China | Pan et al. (2017) |
| **Outgroup** | | | | | |
| *Leptomantis gauni* | FMNH 273928 | JX219456 | n.a. | Bintulu, Sarawak, Malaysia | Li et al. (2012) |
| *Rhacophorus kio* | SCUM 37941C | EU215532 | KR087903 | Xishuangbanna, Yunnan, China | Li et al. (2008) |
| *Polypedates leucomystax* | KUHE 33881 | AB728168 | n.a. | Chatthin, Sagaing, Myanmar | Kuraishi et al. (2013) |

**Supplementary Table S2.** Uncorrected *p*-distance (percentage) 16S rRNA sequences of *Zhangixalus* species included in phylogenetic analyses (below diagonal), average intraspecific genetic *p*-distances (on diagonal), and standard error estimates (above diagonal).

| | **Taxon** | **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** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | *Z. melanoleucus* **sp. nov.** | 0.0 | | | | | | | | | | | | | | | | | | | | | | | | |
| **2** | *Z. dugritei* | 4.5 | – | | | | | | | | | | | | | | | | | | | | | | | |
| **3** | *Z. hui* | 4.3 | 0.2 | – | | | | | | | | | | | | | | | | | | | | | | |
| **4** | *Z. minimus* | 4.9 | 1.4 | 1.6 | – | | | | | | | | | | | | | | | | | | | | | |
| **5** | *Z. hongchibaensis* | 5.4 | 1.4 | 1.6 | 1.8 | – | | | | | | | | | | | | | | | | | | | | |
| **6** | *Z. hungfuensis* | 5.8 | 2.3 | 2.0 | 2.5 | 1.8 | – | | | | | | | | | | | | | | | | | | | |
| **7** | *Z. wui* | 5.6 | 2.0 | 1.8 | 2.7 | 2.5 | 1.8 | – | | | | | | | | | | | | | | | | | | |
| **8** | *Z. puerensis* | 5.8 | 2.3 | 2.0 | 2.7 | 2.3 | 2.3 | 2.5 | – | | | | | | | | | | | | | | | | | |
| **9** | *Z. amamiensis* | 5.4 | 2.9 | 2.7 | 3.6 | 2.7 | 2.7 | 2.9 | 2.3 | – | | | | | | | | | | | | | | | | |
| **10** | *Z. moltrechti* | 4.7 | 2.9 | 2.7 | 3.6 | 3.2 | 3.4 | 3.6 | 3.2 | 3.2 | – | | | | | | | | | | | | | | | |
| **11** | *Z. owstoni* | 4.7 | 3.8 | 3.6 | 4.5 | 4.0 | 4.0 | 4.3 | 4.0 | 3.2 | 1.8 | – | | | | | | | | | | | | | | |
| **12** | *Z. taipeianus* | 5.2 | 2.9 | 2.7 | 4.0 | 3.2 | 4.0 | 3.8 | 3.2 | 3.4 | 2.9 | 4.3 | – | | | | | | | | | | | | | |
| **13** | *Z. duboisi* | 5.2 | 3.4 | 3.6 | 4.0 | 3.2 | 3.6 | 3.8 | 3.6 | 2.5 | 3.4 | 4.3 | 2.7 | – | | | | | | | | | | | | |
| **14** | *Z. pingbianensis* | 5.5 | 3.5 | 3.7 | 4.2 | 3.5 | 4.0 | 3.7 | 3.7 | 2.7 | 3.5 | 4.5 | 2.7 | 0.0 | – | | | | | | | | | | | |
| **15** | *Z. omeimontis* | 5.4 | 3.6 | 3.4 | 4.3 | 3.4 | 3.4 | 3.6 | 3.4 | 2.5 | 3.2 | 4.0 | 2.9 | 0.7 | 0.8 | – | | | | | | | | | | |
| **16** | *Z. gongshanensis* | 4.5 | 3.8 | 3.5 | 4.5 | 4.3 | 4.8 | 4.5 | 4.5 | 3.5 | 3.5 | 4.0 | 3.3 | 2.3 | 2.3 | 2.5 | – | | | | | | | | | |
| **17** | *Z. burmanus* | 4.3 | 3.4 | 3.2 | 4.0 | 4.0 | 4.5 | 4.3 | 4.0 | 3.4 | 3.2 | 3.6 | 2.9 | 2.3 | 2.2 | 2.5 | 0.0 | – | | | | | | | | |
| **18** | *Z. taronensis* | 4.3 | 3.4 | 3.2 | 4.0 | 4.0 | 4.5 | 4.3 | 4.0 | 3.4 | 3.2 | 3.6 | 2.9 | 2.3 | 2.2 | 2.5 | 0.0 | 0.0 | – | | | | | | | |
| **19** | *Z. franki* | 4.0 | 3.2 | 2.9 | 3.8 | 3.8 | 3.8 | 3.6 | 3.4 | 2.7 | 3.4 | 3.4 | 2.9 | 1.6 | 1.5 | 1.8 | 1.3 | 1.1 | 1.1 | – | | | | | | |
| **20** | *Z. schlegelii* | 6.5 | 4.7 | 4.5 | 5.4 | 4.5 | 4.7 | 5.2 | 4.5 | 4.3 | 4.7 | 5.6 | 4.0 | 4.9 | 5.2 | 4.9 | 4.5 | 4.3 | 4.3 | 4.3 | – | | | | | |
| **21** | *Z. viridis* | 5.4 | 3.2 | 2.9 | 3.6 | 3.2 | 3.6 | 3.8 | 2.7 | 2.7 | 2.9 | 4.3 | 2.7 | 3.6 | 3.7 | 3.4 | 4.0 | 3.6 | 3.6 | 3.8 | 4.3 | – | | | | |
| **22** | *Z. arboreus* | 4.7 | 3.4 | 3.2 | 3.6 | 3.6 | 3.8 | 4.0 | 3.6 | 3.6 | 2.5 | 3.8 | 2.7 | 3.2 | 3.2 | 2.9 | 3.0 | 2.7 | 2.7 | 3.4 | 4.3 | 2.3 | – | | | |

(Continued on the next page)

**Supplementary Table S2.** (Continued)

| | Taxon | 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 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **23** | *Z. dorsoviridis* | 5.8 | 4.9 | 5.2 | 5.6 | 4.7 | 4.3 | 5.4 | 5.2 | 4.0 | 4.9 | 5.4 | 4.3 | 2.9 | 3.2 | 3.6 | 4.5 | 4.3 | 4.3 | 4.0 | 5.4 | 4.3 | 3.4 | – | | |
| **24** | *Z. zhoukaiyae* | 6.3 | 5.3 | 5.1 | 6.0 | 5.8 | 4.9 | 5.3 | 4.9 | 3.9 | 5.1 | 5.1 | 3.9 | 3.9 | 4.1 | 4.2 | 4.9 | 4.4 | 4.4 | 4.2 | 5.6 | 4.2 | 3.5 | 2.6 | – | |
| **25** | *Z. lishuiensis* | 5.8 | 5.2 | 5.2 | 5.6 | 5.4 | 4.7 | 5.4 | 4.9 | 3.8 | 4.9 | 4.9 | 4.3 | 3.6 | 3.7 | 4.0 | 4.8 | 4.3 | 4.3 | 4.0 | 5.4 | 4.0 | 3.4 | 2.0 | 1.2 | – |
| **26** | *Z. pachyproctus* | 6.3 | 6.3 | 6.1 | 6.1 | 6.5 | 6.5 | 6.7 | 6.3 | 5.8 | 5.4 | 6.3 | 5.6 | 6.1 | 6.5 | 5.8 | 6.3 | 5.6 | 5.6 | 6.3 | 7.2 | 4.5 | 4.5 | 5.8 | 5.1 | 4.9 |
| **27** | *Z. dennysi* | 6.5 | 6.1 | 5.8 | 6.3 | 6.3 | 6.5 | 5.8 | 5.8 | 5.6 | 6.1 | 6.3 | 6.3 | 6.1 | 6.5 | 6.3 | 6.5 | 5.8 | 5.8 | 5.4 | 7.0 | 4.7 | 6.3 | 7.0 | 7.2 | 7.0 |
| **28** | *Z. suffry* | 8.8 | 9.7 | 9.4 | 9.7 | 9.2 | 9.0 | 9.7 | 8.5 | 8.3 | 8.3 | 8.3 | 8.5 | 8.3 | 8.7 | 8.3 | 8.8 | 8.3 | 8.3 | 8.1 | 8.3 | 8.3 | 7.9 | 9.2 | 7.0 | 8.3 |
| **29** | *Z. smaragdinus* | 8.5 | 9.0 | 8.8 | 9.2 | 8.5 | 8.8 | 9.4 | 7.9 | 7.6 | 8.1 | 8.1 | 8.3 | 8.1 | 8.7 | 8.1 | 9.3 | 8.5 | 8.5 | 8.3 | 8.5 | 8.1 | 7.4 | 8.5 | 6.3 | 7.6 |
| **30** | *Z. feae* | 4.0 | 4.7 | 4.5 | 4.9 | 4.5 | 4.9 | 5.2 | 3.8 | 4.0 | 3.6 | 4.9 | 3.2 | 4.0 | 4.2 | 4.3 | 4.0 | 3.6 | 3.6 | 3.8 | 4.9 | 3.4 | 3.2 | 4.5 | 3.9 | 4.0 |
| **31** | *Z. chenfui* | 5.6 | 6.5 | 6.3 | 7.2 | 6.7 | 6.5 | 6.1 | 5.6 | 5.2 | 5.4 | 5.2 | 5.6 | 6.1 | 6.5 | 6.1 | 5.8 | 5.4 | 5.4 | 5.2 | 6.1 | 5.6 | 5.8 | 6.5 | 6.0 | 6.1 |
| **32** | *Z. nigropunctatus* | 3.4 | 5.9 | 5.6 | 6.1 | 6.1 | 6.5 | 6.8 | 6.5 | 5.4 | 4.7 | 5.0 | 5.2 | 4.3 | 4.8 | 4.5 | 3.5 | 3.4 | 3.4 | 3.4 | 7.0 | 6.5 | 5.2 | 5.9 | 6.7 | 6.3 |
| **33** | *Z. yaoshanensis* | 4.9 | 6.5 | 6.3 | 6.3 | 6.7 | 6.7 | 7.4 | 6.3 | 5.4 | 6.1 | 6.3 | 6.1 | 5.6 | 6.2 | 5.8 | 5.3 | 5.2 | 5.2 | 4.7 | 7.4 | 6.7 | 6.5 | 6.7 | 7.0 | 6.7 |
| **34** | *Z. pinglongensis* | 4.5 | 5.4 | 5.2 | 5.4 | 5.8 | 5.8 | 6.5 | 5.4 | 5.4 | 4.9 | 5.8 | 4.3 | 4.3 | 4.7 | 4.5 | 4.8 | 4.5 | 4.5 | 4.0 | 7.0 | 5.8 | 5.2 | 5.8 | 6.3 | 6.1 |
| **35** | *Z. achantharrhena* | 7.4 | 7.9 | 7.6 | 8.5 | 8.3 | 8.5 | 7.9 | 7.6 | 7.2 | 7.4 | 6.5 | 7.4 | 7.2 | 7.7 | 7.4 | 6.8 | 6.3 | 6.3 | 6.1 | 9.0 | 7.9 | 8.1 | 8.5 | 8.8 | 8.8 |
| **36** | *Z. dulitensis* | 6.7 | 6.5 | 6.3 | 6.7 | 7.0 | 6.7 | 6.5 | 6.3 | 5.8 | 6.1 | 6.1 | 5.6 | 6.3 | 6.7 | 6.5 | 6.5 | 6.1 | 6.1 | 6.1 | 7.6 | 6.5 | 6.1 | 6.7 | 6.5 | 6.5 |
| **37** | *Z. prominanus* | 6.3 | 7.2 | 7.0 | 7.4 | 7.9 | 7.4 | 7.2 | 6.5 | 6.1 | 6.7 | 6.7 | 5.2 | 5.6 | 6.0 | 5.8 | 5.8 | 5.4 | 5.4 | 4.9 | 8.1 | 6.3 | 6.3 | 7.2 | 6.5 | 6.7 |
| **38** | *Z. arvalis* | 8.3 | 8.3 | 8.5 | 8.3 | 7.0 | 8.3 | 8.5 | 8.1 | 7.6 | 8.8 | 9.2 | 8.1 | 7.6 | 7.7 | 8.1 | 8.5 | 8.5 | 8.5 | 8.3 | 10.1 | 7.9 | 9.4 | 9.0 | 9.5 | 9.4 |
| **39** | *Leptomantis gauni* | 9.5 | 9.5 | 9.2 | 9.7 | 8.8 | 9.0 | 9.2 | 9.0 | 7.9 | 9.2 | 8.8 | 8.3 | 9.5 | 10.0 | 9.5 | 9.3 | 8.8 | 8.8 | 8.8 | 7.9 | 8.3 | 8.8 | 9.5 | 9.8 | 9.7 |
| **40** | *Rhacophorus kio* | 11.0 | 10.8 | 10.6 | 11.2 | 11.2 | 10.6 | 11.0 | 11.2 | 10.8 | 9.4 | 8.8 | 10.8 | 11.5 | 12.2 | 11.5 | 11.3 | 10.6 | 10.6 | 11.0 | 11.7 | 11.0 | 10.8 | 11.9 | 11.8 | 12.1 |
| **41** | *Polypedates leucomystax* | 14.7 | 16.3 | 16.3 | 15.8 | 16.7 | 16.7 | 16.3 | 16.0 | 16.3 | 14.9 | 14.7 | 16.0 | 15.4 | 16.0 | 15.8 | 16.3 | 15.6 | 15.6 | 15.4 | 16.0 | 16.0 | 16.3 | 15.4 | 16.1 | 14.7 |

(Continued on the next page)

**Supplementary Table S2.** (Continued)

| | Taxon | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **26** | *Z. pachyproctus* | – | | | | | | | | | | | | | | | |
| **27** | *Z. dennysi* | 7.0 | – | | | | | | | | | | | | | | |
| **28** | *Z. suffry* | 8.1 | 9.7 | – | | | | | | | | | | | | | |
| **29** | *Z. smaragdinus* | 7.6 | 9.7 | 1.6 | – | | | | | | | | | | | | |
| **30** | *Z. feae* | 4.3 | 5.6 | 6.7 | 6.5 | – | | | | | | | | | | | |
| **31** | *Z. chenfui* | 7.2 | 7.6 | 8.3 | 8.3 | 4.7 | – | | | | | | | | | | |
| **32** | *Z. nigropunctatus* | 7.0 | 7.2 | 9.2 | 8.8 | 5.4 | 6.3 | – | | | | | | | | | |
| **33** | *Z. yaoshanensis* | 7.6 | 8.3 | 9.0 | 9.0 | 5.6 | 5.8 | 4.1 | – | | | | | | | | |
| **34** | *Z. pinglongensis* | 6.7 | 7.9 | 8.5 | 8.3 | 4.7 | 6.3 | 4.1 | 3.4 | – | | | | | | | |
| **35** | *Z. achantharrhena* | 8.8 | 7.6 | 9.4 | 9.2 | 7.0 | 7.2 | 7.9 | 8.3 | 7.9 | – | | | | | | |
| **36** | *Z. dulitensis* | 7.0 | 8.3 | 9.4 | 8.8 | 5.6 | 7.0 | 6.8 | 7.6 | 7.0 | 3.4 | – | | | | | |
| **37** | *Z. prominanus* | 7.4 | 7.9 | 9.0 | 9.2 | 5.8 | 6.3 | 6.3 | 6.7 | 5.6 | 4.5 | 2.9 | – | | | | |
| **38** | *Z. arvalis* | 8.1 | 7.4 | 10.1 | 10.3 | 7.9 | 9.4 | 8.6 | 9.7 | 9.4 | 9.4 | 9.9 | 9.7 | – | | | |
| **39** | *Leptomantis gauni* | 9.5 | 10.4 | 10.4 | 10.4 | 7.9 | 8.8 | 10.4 | 11.0 | 10.6 | 9.5 | 8.6 | 9.7 | 9.9 | – | | |
| **40** | *Rhacophorus kio* | 11.9 | 11.0 | 13.9 | 13.7 | 11.7 | 10.8 | 11.5 | 12.8 | 12.1 | 10.3 | 10.6 | 11.7 | 13.0 | 11.0 | – | |
| **41** | *Polypedates leucomystax* | 14.5 | 15.8 | 16.9 | 17.2 | 16.0 | 16.5 | 14.7 | 15.8 | 14.7 | 14.7 | 15.4 | 15.4 | 15.4 | 16.0 | 15.1 | – |

**Supplementary Table S3.** Measurements of the type series (in mm) of *Zhangixalus melanoleucus* **sp. nov.**

| **Specimen ID** | **BEI 01010** | **BEI 01011** | **ZMMU A-7781** | **AUP 02507** | **Min–Max (4 males)** | **Mean±SD (4 males)** | **ZMMU A-7782** |
|---|---|---|---|---|---|---|---|
| **Type** | **Holotype** | **Paratype** | **Paratype** | **Paratype** | | | **Paratype** |
| **Sex** | **Male** | **Male** | **Male** | **Male** | | | **Female** |
| SVL | 35.0 | 34.4 | 36.3 | 34.4 | 34.4–36.3 | 35.03±0.88 | 53.7 |
| A-G | 17.8 | 17.6 | 17.9 | 18.3 | 17.6–18.3 | 17.89±0.29 | 26.8 |
| HW | 13.3 | 12.2 | 13.4 | 12.0 | 12.0–13.4 | 12.72±0.71 | 19.5 |
| HL | 13.6 | 12.6 | 13.3 | 12.0 | 12.0–13.6 | 12.87±0.69 | 18.7 |
| HD | 6.8 | 7.1 | 7.8 | 6.0 | 6.0–7.8 | 6.94±0.75 | 10.8 |
| UEW | 3.2 | 3.0 | 3.1 | 3.1 | 3.0–3.2 | 3.12±0.12 | 4.0 |
| IOD | 4.7 | 4.1 | 4.8 | 4.2 | 4.1–4.8 | 4.43±0.37 | 6.3 |
| ED | 4.6 | 4.2 | 4.7 | 4.1 | 4.1–4.7 | 4.39±0.29 | 6.6 |
| TD | 2.3 | 1.9 | 2.2 | 1.9 | 1.9–2.3 | 2.05±0.21 | 3.0 |
| ESL | 6.1 | 5.8 | 6.1 | 5.4 | 5.4–6.1 | 5.85±0.36 | 7.7 |
| IND | 4.3 | 4.2 | 4.4 | 4.2 | 4.2–4.4 | 4.28±0.09 | 6.5 |
| END | 2.4 | 2.0 | 2.6 | 2.4 | 2.0–2.6 | 2.35±0.26 | 3.9 |
| TED | 1.0 | 0.9 | 1.0 | 1.0 | 0.9–1.0 | 0.98±0.08 | 1.5 |
| NS | 3.6 | 3.9 | 3.8 | 3.7 | 3.6–3.9 | 3.74±0.13 | 4.0 |
| FLL | 27.3 | 25.4 | 25.4 | 24.8 | 24.8–27.3 | 25.72±1.08 | 37.7 |
| HML | 7.5 | 7.1 | 6.8 | 7.1 | 6.8–7.5 | 7.09±0.29 | 9.2 |
| LAL | 8.3 | 7.5 | 8.0 | 7.4 | 7.4–8.3 | 7.80±0.43 | 11.4 |
| ML | 11.5 | 10.8 | 10.7 | 10.3 | 10.3–11.5 | 10.83±0.50 | 17.0 |
| 1FLi | 5.2 | 5.1 | 4.9 | 5.0 | 4.9–5.2 | 5.04±0.13 | 7.6 |
| 1FLo | 4.1 | 3.6 | 3.8 | 3.5 | 3.5–4.1 | 3.73±0.26 | 5.1 |
| 2FLi | 5.4 | 5.6 | 5.2 | 5.3 | 5.2–5.6 | 5.36±0.18 | 8.7 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 3FLi | 7.8 | 8.3 | 7.9 | 7.8 | 7.8–8.3 | 8.00±0.22 | 11.8 |
| 4FLi | 6.2 | 6.6 | 7.0 | 5.7 | 5.7–7.0 | 6.39±0.57 | 10.0 |
| FTD | 2.4 | 2.0 | 2.5 | 2.3 | 2.0–2.5 | 2.28±0.21 | 3.9 |
| NPL | 2.2 | 2.4 | 3.0 | 2.0 | 2.0–3.0 | 2.38±0.44 | 0.0 |
| MCTe | 0.8 | 0.9 | 1.0 | 0.7 | 0.7–1.0 | 0.84±0.14 | 2.0 |
| HLL | 50.8 | 49.1 | 51.2 | 47.4 | 47.4–51.2 | 49.63±1.74 | 73.6 |
| FL | 12.3 | 12.4 | 13.0 | 12.1 | 12.1–13.0 | 12.43±0.39 | 18.8 |
| TL | 14.7 | 14.0 | 14.3 | 13.8 | 13.8–14.7 | 14.18±0.36 | 21.6 |
| TTL | 8.0 | 7.8 | 8.0 | 7.2 | 7.2–8.0 | 7.75±0.35 | 10.8 |
| FOT | 15.9 | 14.9 | 16.0 | 14.3 | 14.3–16.0 | 15.28±0.83 | 22.3 |
| 1TLi | 5.1 | 4.5 | 5.9 | 4.5 | 4.5–5.9 | 5.01±0.64 | 8.6 |
| 1TLo | 2.7 | 2.5 | 3.2 | 2.2 | 2.2–3.2 | 2.63±0.42 | 3.6 |
| 2TLi | 5.2 | 4.7 | 5.5 | 5.2 | 4.7–5.5 | 5.14±0.34 | 6.8 |
| 3TLi | 7.2 | 6.7 | 6.9 | 6.6 | 6.6–7.2 | 6.84±0.28 | 9.9 |
| 4TLi | 8.5 | 8.2 | 8.8 | 8.9 | 8.2–8.9 | 8.62±0.32 | 13.1 |
| 5TLi | 6.4 | 5.7 | 6.0 | 5.8 | 5.7–6.4 | 5.97±0.31 | 9.5 |
| HTD | 2.2 | 1.8 | 2.2 | 1.8 | 1.8–2.2 | 2.01±0.24 | 3.0 |
| MTTi | 1.9 | 1.8 | 1.9 | 1.7 | 1.7–1.9 | 1.81±0.09 | 3.1 |
| IMW | 1.2 | 1.2 | 1.2 | 1.3 | 1.2–1.3 | 1.21±0.05 | 2.3 |

**Supplementary Table S4.** Measurements of the series of *Zhangixalus melanoleucus* **sp. nov.** tadpoles (ZMMU A-7783; all in mm). For character abbreviations see Supplementary materials and methods.

| **Character** | **ZMMU A-7783-1** | **ZMMU A-7783-2** | **ZMMU A-7783-3** |
|---|---|---|---|
| TL | 39.8 | 37.0 | 25.1 |
| BL | 17.0 | 16.7 | 12.9 |
| TaL | 22.8 | 20.4 | 12.1 |
| BW | 9.5 | 10.2 | 8.1 |
| BH | 8.1 | 7.5 | 5.5 |
| TH | 9.3 | 10.9 | 7.2 |
| SVL | 18.7 | 17.4 | 13.9 |
| SSp | 10.0 | 7.0 | 6.6 |
| UF | 2.7 | 3.4 | 2.0 |
| LF | 2.1 | 2.5 | 1.4 |
| IN | 2.7 | 2.6 | 2.2 |
| IP | 6.1 | 6.0 | 3.7 |
| RN | 0.9 | 1.2 | 1.0 |
| NP | 2.6 | 2.3 | 2.0 |
| ED | 1.9 | 2.0 | 1.1 |
| ODW | 3.2 | 2.6 | 2.2 |
| LTRF | 1:5+5/1+1:2 | 1:5+5/1+1:2 | 1:5+5/1+1:2 |

**Supplementary Table S5**. Basic morphological characters for the species of *Zhangixalus* distributed in Indochina, China, India and Myanmar as compared to *Zhangixalus melanoleucus* **sp. nov.** Symbol characters are: ① SVL in males (mm); ② SVL in females (mm); ③ Color of iris; ④ Finger webbing; ⑤ Colorations on dorsum; ⑥ Colorations on ventral; ⑦ Colorations on flank; ⑧ Colorations on thigh; '?': no data.

| **Species** | ① | ② | ③ | ④ | ⑤ | ⑥ |
|---|---|---|---|---|---|---|
| *melanoleucus* **sp. nov** | 34.4–36.3 | 53.7 | reddish orange | reduced | immaculate green | immaculate white |
| *burmanus* | 47.0–59.5 | 64.2–77.3 | yellow | reduced | green/dark green with small pale yellow/brown/dark dots | cream with small pale yellow |
| *chenfui* | 32.7–40.5 | 46.0–55.0 | reddish-orange | reduced | immaculate green | cream with small pale yellow |
| *dennysi* | 68–92 | 83–109 | yellowish-gold | reduced | green with small brown spots | immaculate cream |
| *dorsoviridis* | 31.3–42.4 | 37.9–42.8 | reddish-white | reduced | immaculate green | cream to orange without spots |
| *duboisi* | 61.5–65.7 | 71.1–74.1 | yellowish-gold | reduced | green with red-brown spots | fleshy with brown spots |
| *dugritei* | 41.5–45.4 | 57.7–64.3 | yellowish-brown | reduced | green with round spots of a golden metallic ash | cream yellow with dark grey |
| *feae* | 86–111 | 68–118 | green-gold | complete | immaculate green | anteriorly white, posteriorly pinkish, pale green throat |
| *franki* | 77.9–85.8 | ? | bronze | reduced | green with dark brown spots | immaculate grey |
| *hongchibaensis* | 46.5–49.7 | 55.3 | yellowish-brown | reduced | yellowish brown spots edged with dark brown | creamy white with vaguely greyish brown blotches |
| *hui* | 40.0–45.4 | 51.0–66.0 | reddish-brown | reduced | green with brow spots | cream/yellow with dark grey |
| *hungfuensis* | 30.8–36.8 | 45.5 | green-gold | reduced | green with small white spots | pinkish with pale yellow |
| *jodiae* | 36.1–39.8 | ? | silver | reduced | immaculate green | immaculate cream |
| *leucofasciatus* | 47.5–49.4 | ? | yellowish-brown | reduced | immaculate green | immaculate white |
| *lishuiensis* | 34.2–35.8 | 45.9 | yellowish-gold | reduced | immaculate pure green | anteriorly white, posteriorly yellow without spots |
| *minimus* | 28.1 | 37 | yellowish-gold | reduced | immaculate green | immaculate cream/grey |

| | | | | | | |
|---|---|---|---|---|---|---|
| *nigropunctatus* | 32.0–37.0 | 44.0–45.0 | yellowish-gold | reduced | immaculate green | immaculate white |
| *omeimontis* | 52–66 | 70–80 | yellowish-gold | reduced | green with brown interweave patterns | cream with very small dark spots |
| *pachyproctus* | 73.4–78.2 | 102.4 | yellowish-gold | complete | immaculate green | immaculate light brown/white |
| *pinglongensis* | 32–38.5 | ? | silvery | reduced | immaculate green | immaculate white |
| *puerensis* | 35.5–41 | 52–55.2 | yellowish-gold | reduced | green with brownish-red spots | white with small spots |
| *smaragdinus* | 57.0–84.0 | 85.0–112.0 | yellowish-gold | complete | immaculate green | immaculate light brown/white |
| *wui* | 35.2–38.2 | 48.6 | grayish-gold | reduced | numerous light-brown spots with dark yellowish brown edges | creamy white with vague greyish brown blotches |
| *yaoshanensis* | 31.6–36.4 | 49.2–51.1 | grayish-gold | reduced | immaculate green | immaculate cream |
| *yinggelingensis* | 43.0–43.4 | ? | grayish-gold | reduced | green with few fine white spots | immaculate yellowish |
| *zhoukaiyae* | 27.9–37.1 | 41.1–44.7 | yellowish-gold | reduced | immaculate green | immaculate pure paler yellowish |

(Continued on the next page)

**Supplementary Table S5**. (Continued)

| Species | ⑦ | ⑧ | Sources |
|---|---|---|---|
| *melanoleucus* **sp. nov** | white with irregular black pattern | white with irregular black pattern | 18 |
| *burmanus* | with small brown/yellow/dark blotches | cream with scattered mottling | 8 |
| *chenfui* | grey without blotches | grey without blotches | 5 |
| *dennysi* | cream/gray with small white spots | cream without blotches | 5, 18 |
| *dorsoviridis* | white with variable black spots | cream with small black spots | 2, 18 |
| *duboisi* | blackish with white spots | white with dark brown marbling | 1, 7 |
| *dugritei* | marbled with cream yellow | marbled with cream yellow | 5, 7 |
| *feae* | uniform green without blotches | uniform green without blotches | 5, 18 |
| *franki* | with a white stripe, separating upper green part from lower cream part | immaculate grey | 16,17 |
| *hongchibaensis* | light green with numerous large spots of light yellowish | lightly red, marbled with grey | 7 |
| *hui* | marbled with cream yellow | marbled with cream yellow | 7 |
| *hungfuensis* | cream without blotches | grey without blotches | 5 |
| *jodiae* | cream with irregular black and orange blotches | black blotches interposed by orange | 15 |
| *leucofasciatus* | cream with wide white band in middle | gray without blotches | 5 |
| *lishuiensis* | cream without blotches | gray without blotches | 10 |
| *minimus* | grey with narrow white band in middle | cream with scattered mottling | 3, 5 |
| *nigropunctatus* | green above, white bellow with small black spots in posteriorly | cream/yellowish with black blotches | 5 |
| *omeimontis* | dark brow mottling | dark brow without blotches | 5 |
| *pachyproctus* | cream/grey scattered with clouded light brown spots | cream/grey scattered with cloudy light brown | 14, 18 |

| | | | |
|---|---|---|---|
| | | spots | |
| *pinglongensis* | black blotches with white spots | black blotches with white spots and faint orange tint | 9 |
| *puerensis* | black with irregular white pattern | black with irregular white pattern | 7, 18 |
| *smaragdinus* | cream/grey scattered with clouded light brown spots | cream/grey scattered with cloudy light brown spots | 13, 14 |
| *wui* | light green with numerous light-brown spots | light green with numerous light-brown spots | 7 |
| *yaoshanensis* | cream with small spots | orange-red without spots | 12 |
| *yinggelingensis* | immaculate cream | orange-red without spots | 4, 5 |
| *zhoukaiyae* | cream bellow with small brow spots | yellowish with grayish blotching | 11 |

**Sources**: 1= Ohler et al. (2000); 2= Orlov et al. (2001); 3= Rao et al. (2006); 4= Chou et al. (2007); 5= Fei et al. (2010); 6= Zhang et al. (2011); 7= Li et al. (2012); 8= Jiang et al. (2019); 9= Mo et al. (2016); 10= Liu et al. (2017); 11= Pan et al. (2017); 12= Chen et al. (2018); 13= Ohler & Deuti (2018); 14= Yu et al. (2019); 15= Nguyen et al. (2020); 16= Ninh et al. (2020); 17= Liu et al. (2020); 18= our data

**Supplementary Table S6**. Morphological comparisons of *Zhangixalus lishuiensis* (Liu, Wang & Jiang) with *Zhangixalus zhoukaiyae* (Pan, Zhang & Zhang).

| **Species** | ***Zhangixalus lishuiensis*** | | | ***Zhangixalus zhoukaiyae*** | | | |
|---|---|---|---|---|---|---|---|
| **Sex** | Males (n=3) | | Female (n=1) | Males (n=6) | | Females (n=3) | |
| | Min-Max | Mean ±SD | | Min-Max | Mean ±SD | Min-Max | Mean ±SD |
| **SVL** | 34.20-35.80 | 35.80±0.92 | 45.9 | 27.9-37.12 | 33.96±3.40 | 42.12-44.67 | 43.49±1.28 |
| **HL** | 14.90-15.80 | 15.27±0.47 | 19.2 | 9.49-12.66 | 11.47±1.16 | 14.19-14.65 | 14.46±0.24 |
| **HW** | 13.90-14.70 | 14.17±0.46 | 17.6 | 11.56-14.44 | 13.38±1.06 | 14.80-17.94 | 16.34±1.57 |
| **SL** | 5.70-6.10 | 5.90±0.20 | 7.5 | 3.80-5.67 | 5.00±0.66 | 5.60-5.83 | 5.74±0.12 |
| **ED** | 4.10-4.50 | 4.37±0.23 | 5.6 | 3.26-4.77 | 4.20±0.53 | 4.58-5.25 | 5.01±0.37 |
| **TD** | 2.30-3.20 | 2.60±0.52 | 2.6 | 2.16-2.54 | 2.34±0.17 | 2.73-3.12 | 2.91±0.20 |
| **TL** | 14.70-15.70 | 15.2±0.05 | 18.6 | 12.43-16.66 | 15.01±1.41 | 18.97-20.26 | 19.65±0.65 |
| **HL/SVL** | 0.42-0.44 | 0.43±0.01 | 0.42 | 0.31-0.37 | 0.34±0.02 | 0.32-0.35 | 0.33±0.01 |
| **HW/SVL** | 0.39-0.43 | 0.40±0.02 | 0.38 | 0.35-0.42 | 0.40±0.03 | 0.34-0.40 | 0.38±0.03 |
| **SL/SVL** | 0.16-0.17 | 0.17±0.00 | 0.16 | 0.13-0.17 | 0.15±0.01 | 0.13-0.14 | 0.13±0.01 |
| **ED/SVL** | 0.12-0.13 | 0.12±0.00 | 0.12 | 0.11-0.15 | 0.12±0.01 | 0.10-0.12 | 0.12±0.01 |
| **TD/SVL** | 0.06-0.09 | 0.07±0.01 | 0.06 | 0.06-0.08 | 0.07±0.01 | 0.06-0.07 | 0.07±0.00 |
| **TL/SVL** | 0.41-0.46 | 0.43±0.02 | 0.41 | 0.40-0.48 | 0.44±0.03 | 0.43-0.47 | 0.45±0.02 |
| **Color of iris** | yellowish-gold | | | yellowish-gold | | | |
| **Finger webbing** | reduced | | | reduced | | | |
| **Coloration of dorsum** | immaculate pure green | | | immaculate green | | | |
| **Coloration of belly** | anteriorly white, posteriorly yellow without spots | | | immaculate pure pale-yellow | | | |
| **Colorations of flanks** | cream without blotches | | | cream bellow with small brow spots | | | |
| **Colorations of thighs** | gray without blotches | | | yellowish with grayish blotching | | | |
| **Source** | Liu et al. (2017) | | | Pan et al. (2017) | | | |

**Supplementary Table S7**. Morphological comparisons of *Zhangixalus yaoshanensis* (Liu & Hu) and *Zhangixalus pinglongensis* (Mo, Chen, Liao & Zhou).

| **Species** | ***Zhangixalus yaoshanensis*** | | | ***Zhangixalus pinglongensis*** | |
|---|---|---|---|---|---|
| **Sex** | Males (n=12) | | Females (n=2) | Males (n=13) | |
| | Min-Max | Mean±SD | Min-Max | Min-Max | Mean±SD |
| **SVL** | 31.6-36.4 | 33.9±1.3 | 49.2-51.1 | 32.0-38.5 | 35.9±2.3 |
| **HL** | 10.1-12.2 | 11.2±0 | 14.7-14.9 | 12.3-15.2 | 13.8±1.1 |
| **HW** | 12.4-14.8 | 13.5±0.7 | 18.5-18.7 | 12.7-15.7 | 14.5±1.1 |
| **SL** | 5.5-6.2 | 5.9±0.2 | 8.1-8.2 | 5.5-7.2 | 6.3±0.6 |
| **UEW** | 4.4-5.3 | 4.8±0.2 | 6.1-6.7 | 3.3-4.7 | 3.9±0.4 |
| **IOD** | 4.0-4.8 | 4.5±0.3 | 5.9-6.0 | 4.4-5.5 | 5.0±0.4 |
| **ED** | 4.1-4.9 | 4.5±0.2 | 5.2-6.2 | 4.3-5.5 | 4.8±0.3 |
| **TD** | 2.0-2.5 | 2.3±0.2 | 3.4-3.6 | 2.2-3.1 | 2.6±0.3 |
| **TL** | 13.4-15.8 | 14.9±0.6 | 21.1-21.3 | 15.3-18.1 | 16.3±0.9 |
| **Color of iris** | grayish-gold | | | silvery | |
| **Finger webbing** | reduced | | | reduced | |
| **Coloration of dorsum** | immaculate green | | | immaculate green | |
| **Coloration of belly** | immaculate cream | | | immaculate white | |
| **Colorations of flanks** | cream with small spots | | | black blotches with white spots | |
| **Colorations of thighs** | orange-red without spots | | | black blotches with white spots and faint orange tint | |
| **Source** | Chen et al. (2018) | | | Mo et al. (2016) | |

**Supplementary Table S8**. Basic call parameters of *Zhangixalus melanoleucus* **sp. nov.** as compared to other members of the genus *Zhangixalus*.

| Species | Number of pulses per note | Dominant Frequency (Hz) | Sources |
|---|---|---|---|
| *Z. melanoleucus* **sp. nov.** | 2–3 (2.25 ± 0.38) | 3140 ± 47.06 | *this work* |
| | | | |
| *Z. chenfui* | 2–6 | 2348.8 ± 53.6 | Matsui & Wu (1994) |
| *Z. dennysi* | 3–5 (3.5 ± 0.6) | 1360.6 ± 77.9 | Wang et al. (2012) |
| *Z. dugritei* | 10 or more | 1675.0 ± 41.8 | Matsui & Wu (1994) |
| *Z. jodiae* | 6 | 2000 | Nguyen et al.(2020) |
| *Z. omeimontis* | 2–5 | 977.1 ± 49.8 | Matsui & Wu (1994) |
| *Z. zhoukaiyae* | 10 or more (19.95 ± 4.7) | 1510.3 ± 60.9 | Fang et al. (2019) |