

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

## DEAR EDITOR,

We report on a new species, *Zhangixalus melanoleucus* sp. nov., from Phou Samsoum Mountain (PSM) in Xiengkhouang Province, northeastern Laos, based on an integrative taxonomic approach, including morphological, molecular, and bioacoustic lines of evidence. Morphologically, the new species can be distinguished from its congeners by a combination of the following diagnostic characters: medium body size (SVL 34.4–36.3 mm in males, 53.7 mm in a single female); dorsum smooth and green; chest and belly lacking spots; flanks, axillae, ventral surfaces of forearms, inguinal, anterior and posterior surfaces of thighs white, covered with irregular black pattern; finger webbing formula I 2½–3 II 2–3 III 2¼–2 IV; toe webbing formula I 2–2½ II 1–2 III 1–2 IV 2–1 V; toe webbing cream with small black blotches; outer margin of forearms and feet with weak dermal ridges; supraclacal fold and pointed projection at tibiotarsal articulation absent; and iris reddish-orange. The new species is divergent from all other members of *Zhangixalus* based on 16S rRNA gene sequences ( $P=3.4\%–8.8\%$ ) and is reconstructed as the sister species of *Z. nigropunctatus*. The advertisement call of the new species consists of clicking sounds and includes a series of notes, each 0.28 s in duration and consisting of 2–3 pulses with a dominant frequency of ca. 3.14 kHz. To date, *Zhangixalus melanoleucus* sp. nov. is known only from the montane evergreen forests of PSM at elevations of 2 000–2 200 m a.s.l. We preliminarily suggest the new species should be considered as Data Deficient (DD) following the IUCN's Red List categories.

The genus *Zhangixalus* Li, Jiang, Ren & Jiang, 2019 is widely distributed in East and Southeast Asia, ranging from northeastern India, Nepal, Bhutan, Myanmar, southern and southwestern China, Japan, throughout Indochina southwards to Malaysia and Indonesia (Jiang et al., 2019; Poyarkov et al., 2021). Recently regarded as a part of the genus *Rhacophorus* Kuhl & Van Hasselt, 1822, members of *Zhangixalus* can be morphologically diagnosed by: medium to large body size (SVL 30–120 mm); snout rounded; projections on snout, upper eyelids, and tarsal joint absent; dermal folds along forearm or tarsus absent or weak; terminal phalanges of digits Y-shaped; dorsal skin surfaces smooth or scattered with small tubercles;

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fingers webbed; dorsal coloration generally green; and iris lacking X-shaped pattern (Jiang et al., 2019). Currently, there are 40 recognized species of *Zhangixalus*, one-third of which have been described in the last two decades (Frost, 2023). However, the actual diversity of *Zhangixalus* remains underestimated due to the common occurrence of elevated endemism and narrow-ranged species within the genus (Dufresnes et al., 2022). The herpetofauna of Laos remains one of the least studied and poorly known in continental Asia. Presently, only three *Zhangixalus* species are recorded from Laos, i.e., *Z. dennysi* (Blanford), *Z. feae* (Boulenger), and *Z. pachyproctus* Yu, Hui, Hou, Wu, Rao, & Rang (Nguyen et al., 2020; Poyarkov et al., 2021).

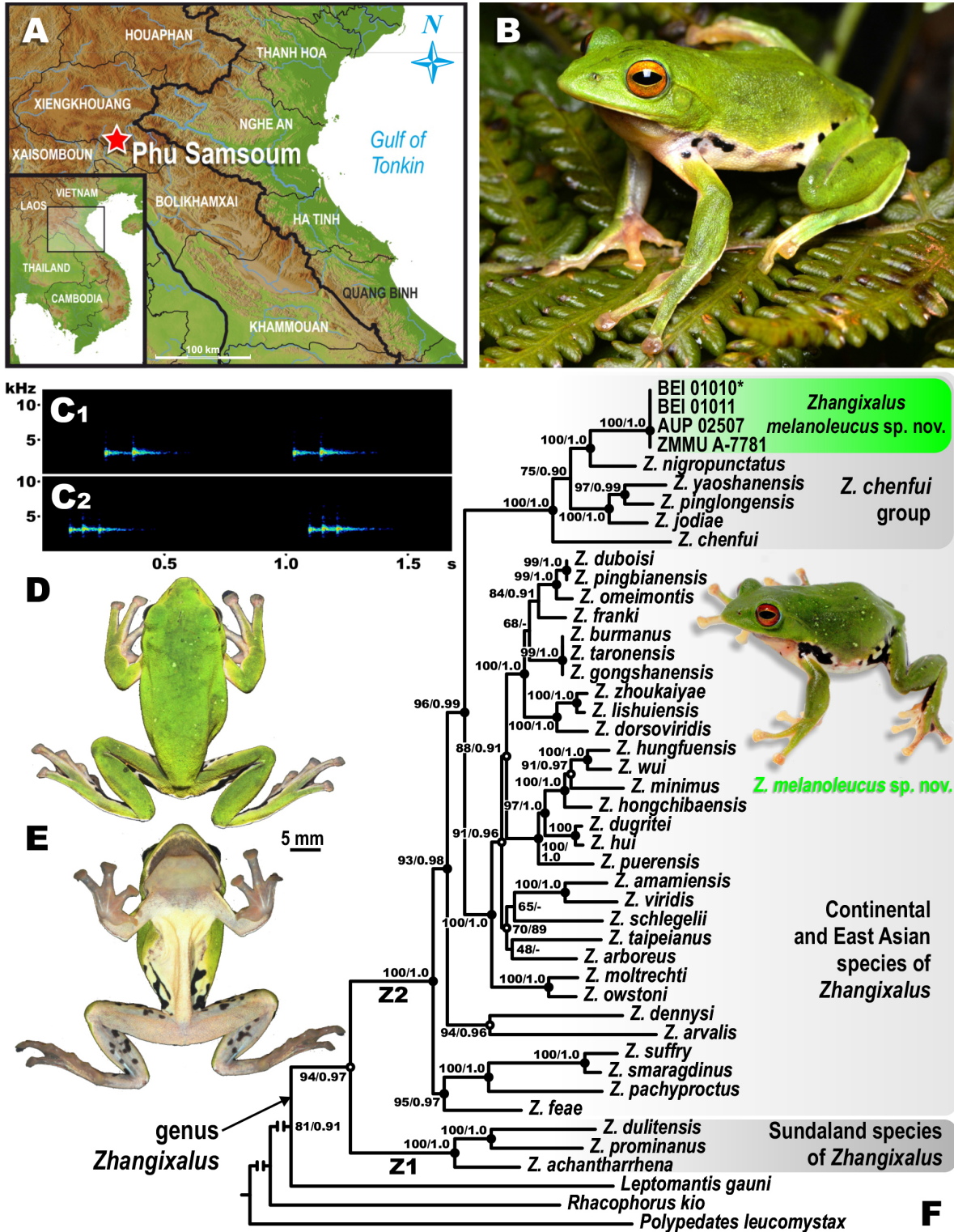
As the third highest summit in Laos, PSM (2 632 m a.s.l.) forms part of the Central Highlands, along with Phou Bia (2 819 m a.s.l.), Phou Xai Lai Leng (2 714 m a.s.l.), and Phou Sao (2 597 m a.s.l.). PSM and the surrounding montane areas of Xiengkhouang Province are recognized as an amphibian diversity hotspot in Indochina, exhibiting a high level of local endemism (see Poyarkov et al., 2021).

During our recent fieldwork to PSM in Xiengkhouang Province, Laos, we encountered several unusual rhacophorid specimens, which were assigned to the genus *Zhangixalus* due to their superficial morphological similarity to *Z. dorsoviridis* (Bourret), *Z. nigropunctatus* (Liu, Hu, & Yang), *Z. pinglongensis* (Mo, Chen, Liao, & Zhou), and *Z. jodiae* Nguyen, Ninh, Orlov, Nguyen, & Ziegler. Subsequent molecular phylogenetic analyses revealed that this population represents a distinct evolutionary lineage, nested within a single clade with *Z. nigropunctatus*. Based on a combination of morphological and bioacoustic characters, as well as molecular divergence analysis, this population is described herein as a new species.

Field surveys were conducted in July 2020 in PSM, Xiengkhouang Province, northeast Laos (Figure 1A). Details on specimen collection and preservation are presented in the Supplementary Materials. Specimens were deposited in the herpetological collections of the Biotechnology and Ecology Institute, Ministry of Science and Technology of Laos (BEI, Veintiane, Laos), School of Agriculture and Natural Resources, University of Phayao (AUP, Phayao, Thailand),

Received: 16 January 2023; Accepted: 09 March 2023; Online: 14 March 2023

Foundation items: This work was supported by Thailand Research Fund 2019 (MRG6280203), the Unit of Excellence 2023 on Biodiversity and Natural Resources Management, University of Phayao (FF66-UoE003, specimen collection) to C.S., and partially by the Russian Science Foundation (22-14-00037, molecular phylogenetic analyses) to N.A.P.



**Figure 1** Phylogenetic relationships, distribution, acoustics, and external morphology of *Zhangixalus melanoleucus* sp. nov.

A: Distribution of *Zhangixalus melanoleucus* sp. nov. in Phou Samsoum Mountain, Xiengkhouang Province, northeast Laos. B: Holotype of *Zhangixalus melanoleucus* sp. nov. (AUP 02505), adult male, *in situ* in dorsolateral view. C: Sonogram of male advertisement call of *Zhangixalus melanoleucus* sp. nov., with two pulses (C1) and three pulses (C2) per note, recorded at 16.5 °C (at type locality). D: Dorsal life coloration of *Zhangixalus melanoleucus* sp. nov. holotype (AUP 02505). E: Ventral life coloration of *Zhangixalus melanoleucus* sp. nov. holotype (AUP 02505). F: Maximum-likelihood topology of *Zhangixalus* based on 4 432 bp of concatenated 16S rRNA and *COI* gene sequences. Ancestral nodes for all species show strong UFB and BI PP support (100 and 1.00, respectively). Values at nodes correspond to UFB/BI PP, respectively; black circles represent nodes with BI PP and UFB support of >0.95 and 95%, respectively; white circles represent nodes with BI PP support of >0.90 only or UFB support of >90% only; nodes lacking circles are not supported. For specimen and sequence data see Supplementary Table S1. Photos by P. Pawangkhanant and N.A. Poyarkov.

and Zoological Museum of Lomonosov Moscow State University (ZMMU, Moscow, Russia). Descriptions of morphological characteristics of adults and larvae followed Poyarkov et al. (2015, 2018). Comparative data on morphological and bioacoustic characteristics of other *Zhangixalus* species were obtained from previous publications (see Supplementary Materials for details). Advertisement calls of the new species were recorded at the breeding site. Analyses of acoustic data generally followed Poyarkov et al. (2018) and are detailed in the Supplementary Materials.

To reconstruct the matrilineal genealogy of *Zhangixalus*, we obtained partial 16S rRNA and *COI* mtDNA sequences from the PSM population and compared them with the 16S rRNA and *COI* sequences of all available *Zhangixalus* species reported in earlier phylogenetic studies (e.g., Dufresnes et al., 2022). Information on GenBank accession numbers, museum vouchers, and origin locality of sequences used in this study is summarized in Supplementary Table S1. DNA extraction, amplification, and sequencing protocols followed Poyarkov et al. (2018) and are detailed in the Supplementary Materials.

We inferred matrilineal genealogy using maximum-likelihood (ML) and Bayesian inference (BI) approaches (see Supplementary Materials for details). Pairwise uncorrected *P*-distances among 16S rRNA sequences of *Zhangixalus* species were used to estimate genetic divergence among species (see Supplementary Table S2). In total, the concatenated alignment (total length 4 432 bp) included 45 16S rRNA and 32 *COI* gene sequences from 45 Rhacophoridae specimens, representing 38 nominal species of *Zhangixalus* and three outgroup taxa (see Supplementary Table S1).

Monophyly of the genus *Zhangixalus* obtained only moderate nodal support (94/0.97, corresponding to ML ultra-fast bootstrap (UFB)/BI posterior probability (PP) values, respectively (same below); see Figure 1F), consistent with earlier studies (Jiang et al., 2019). Two well-supported major clades were revealed within *Zhangixalus* (as in Dufresnes et al., 2022): one joining three species inhabiting the Malayan Peninsula, Sumatra, and Borneo (clade Z1, 100/1.0; see Figure 1F), the other comprising all remaining species from East Asia and continental Southeast Asia (clade Z2, 100/1.0; see Figure 1F). Within the latter clade, analyses uncovered several subclades: i.e., *Z. feae*+*Z. pachyproctus*+*Z. smaragdinus*+*Z. suffry* (95/0.97; Figure 1F); *Z. dennysi*+*Z. arvalis* (94/0.96; Figure 1F); majority of species from Japan, China, and northern Indochina (*Z. chenfui* group, 100/1.0; Figure 1F); and six species from southern mainland China (*Z. chenfui*, *Z. nigropunctatus*, *Z. pinglongensis*, and *Z. yaoshanensis*), northernmost Vietnam (*Z. jodiae*), and the newly discovered population of *Zhangixalus* sp. from PSM. The latter was strongly suggested to be a sister lineage of *Z. nigropunctatus* from Guizhou Province in China (100/1.0; Figure 1F).

Uncorrected genetic distances between the 16S rRNA gene sequences of *Zhangixalus* sp. from PSM and other congeners varied from *P*=3.4% (with *Z. nigropunctatus*) to *P*=8.8% (with *Z. suffry*). Values were generally higher than the formal *P*=3.0% threshold for the 16S rRNA gene, which is widely used as an indicator of species-level divergence in anurans (Vieites et al., 2009). At the same time, genetic distances between several recognized species of the genus *Zhangixalus* were much lower, e.g., no divergence in the 16S rRNA gene was revealed between *Z. burmanus*, *Z. taronensis*, and *Z.*

*gongshanensis* (*P*=0.0%), *Z. duboisi* and *Z. pingbianensis* (*P*=0.0%), and only minimal divergence was revealed between *Z. dugritei* and *Z. hui* (*P*=0.2%), consistent with Dufresnes et al. (2022). These results suggest that the taxonomy of the genus *Zhangixalus* is far from complete and further integrative studies are needed to clarify the status of the abovementioned taxa. The congruent molecular, morphological, and acoustic differences of *Zhangixalus* sp. from PSM compared to other congeners suggest evolutionary distinctiveness. As such, we consider the Phou Samsoum population as a distinct species, which we formally describe below.

## Taxonomic account

### *Zhangixalus melanoleucus* sp. nov.

(Figure 1; Supplementary Figures S1–S4; Supplementary Tables S1–S5)

**Holotype:** Adult male BEI 01010 (field ID NAP-09192), collected 16 July 2020 by P. Brakels, T.V. Nguyen, P. Pawangkhanant, S. Idiitullina, and N.A. Poyarkov from the montane evergreen forest on Phou Samsoum Mountain, Xiengkhouang Province, northeast Laos (N19.13101°, E103.78408°; at an elevation of 2 066 m a.s.l.).

**Paratypes:** Three adult males BEI 01011 (field ID NAP-09193), AUP 02507 (field ID NAP-09195), and ZMMU A-7781 (field ID NAP-09194), and one adult female ZMMU A-7782 (field ID NAP-09196), with the same collection information as the holotype.

**Referred materials:** Three tadpoles ZMMU A-7783 (field ID NAP-09244), with the same collection information as the holotype.

**Diagnosis:** The new species is assigned to *Zhangixalus* based on the following morphological characters: (1) medium body size (SVL 34.4–36.3 mm in males, 53.7 mm in female); (2) supraclacal fold and dermal projections on tibiotarsal joint and eyelids absent; (3) dermal folds on limbs absent; and (4) dorsal coloration green (Jiang et al., 2019). The new species can be distinguished from all congeners by a combination of the following morphological characters: dorsum smooth and uniform green with several dark and light-green spots; chest and belly immaculate white; flanks, axilla, inguinal region, ventral surface of forearms, anterior and posterior surfaces of thighs white, covered with irregular black pattern; finger webbing formula I 2½–3 II 2–3 III 2¼–2 IV; toe webbing formula I 2–2½ II 1–2 III 1–2 IV 2–1 V; toe webbing cream with small black blotches; outer margin of forearms and feet with weak dermal ridges; supraclacal fold and pointed projection at tibiotarsal articulation absent; iris reddish-orange; tadpole mouth disc keratodont row formula (KRF) 1:5+5/1+1:2.

**Description of holotype:** Medium-sized frog specimen in a good state of preservation; body moderately robust (Figure 1D–E), SVL 35.0 mm. Head much longer than wide (HW/HL 0.98), quite deep (HD/HL 0.50), convex above; dorsally smooth with skin not co-ossified to skull, calcified warts lacking; snout long (ESL/HL 0.45) and tapering, snout tip rounded in dorsal view (Supplementary Figure S1A), rounded in profile (Supplementary Figure S1C), snout notably projecting beyond margin of lower jaw (Supplementary Figure S1C); nostril ovoid, slightly protuberant (Supplementary Figure S1C), oriented dorsolaterally, located closer to eye than to tip of snout (END/ESL 0.39; END/NS 0.67); canthus rostralis distinct, rounded; loreal region slightly concave; eyes large (ED/HL 0.34), eye diameter less than snout length (ED/ESL 0.75), notably protuberant in dorsal view (Supplementary

Figure S1A) and profile (Supplementary Figure S1C), pupil horizontal, ovoid (Supplementary Figure S1C); tympanum barely distinct, rounded, with vertical tympanum diameter equal to horizontal; supratympanic fold distinct, glandular, elevated above skin of temporal region (Supplementary Figure S1C), tympanum comprising half eye diameter (TD/ED 0.49), located close to eye (TED/ED 0.22); vomerine teeth present, in two oblique series, closer to choanae than to each other, separated by distance about as long as each series; choanae ovoid; tongue attached anteriorly, deeply notched posteriorly; single external vocal sac.

Forelimbs relatively robust; relative finger lengths:  $I < II < IV < III$ ; tips of all fingers with well-developed disks with distinct circummarginal grooves (Supplementary Figure S1D), disks rounded, slightly expanded transversally, disk on finger III slightly larger than tympanum (FTD/TD 1.07); dermal fringing along fingers weak (Supplementary Figure S1D); fingers moderately webbed, webbing formula:  $I \frac{2}{2}-3 \quad II \frac{2}{2}-3 \quad III \frac{2}{2}-2 \quad IV$ ; subarticular tubercles rather large, rounded, notably protruding, distinct on all fingers, finger subarticular tubercle formula: 1, 1, 2, 2; two metacarpal (palmar) tubercles present, inner metacarpal tubercle large, ovoid; outer metacarpal tubercle smaller in size, flattened and heart-shaped; four supernumerary metacarpal tubercles at bases of fingers II–IV (Supplementary Figure S1D); nuptial pad present, ovoid, elongated, covering prepollex area (MCTi/1FLi 0.42).

Hindlimbs robust, moderately short, tibiotarsal articulation not reaching beyond tip of snout; tibia slightly over half snout-vent length (TL/SVL 0.42); dermal ridge along outer surface of tibia weak; toes moderately webbed, webbing formula:  $I \frac{2}{2}-2 \frac{1}{2} \quad II \frac{1}{2}-2 \quad III \frac{1}{2}-2 \quad IV \frac{2}{2}-1 \quad V$ ; weak dermal fringes reaching disks of all toes (Supplementary Figure S1E). Tips of toes bearing disks with distinct circummarginal and transverse grooves; disks rounded, slightly smaller than those of fingers (HTD/FTD 0.91); relative toe lengths:  $I < II < V < III < IV$ ; round, distinct, and protuberant subarticular tubercles present on all toes, toe subarticular tubercle formula: 1, 1, 2, 3, 2; inner metatarsal tubercle well-developed, bean-shaped, and notably protuberant; outer metatarsal tubercle or supernumerary tubercles absent (Supplementary Figure S1E).

**Skin texture and skin glands:** Dorsal skin smooth; ventral surface of chest, venter, and thighs coarsely granular; dorsal and ventral surfaces of limbs smooth; cloaca and posterior surface of thighs granular; supracloacal fold and pointed projection at tibiotarsal articulation absent (Supplementary Figure S1).

**Coloration in life:** Dorsal surface uniform grass-green; flanks, axilla, ventral surfaces of forearms, inguinal, anterior and posterior surfaces of thighs, ventral surface of shanks, dorsal surfaces of feet, and fingers I, II, III white-cream covered with irregular black pattern; larger black spot above insertion of arm. Venter and chest immaculate cream; throat gray with dark-gray margins, groin region and ventral surfaces of thighs cream; dark-brown stripe from elbow to outer metacarpal tubercle. Webbing grayish-pink with small black blotches; iris reddish-orange, scleral ring grayish-blue; pupil horizontal, black (Figure 1; Supplementary Figure S1).

**Coloration in preservative:** After two years in ethanol, green coloration turned bluish-gray; lower part of flanks and ventral surface of body and limbs gray white, contrasting black pattern on flanks well discernable.

**Variation and sexual dimorphism:** Male individuals of the type series were very similar in morphology, body proportions,

and body coloration (Supplementary Figure S2); SVL varied from 34.4–36.3 mm in males and 53.7 mm in single female; measurements of type series are shown in Supplementary Table S3. Male paratype BEI 01011 had several dark-brown spots on mid-dorsum; male paratype ZMMU A-7781 had numerous light-green to yellow spots on dorsum (Supplementary Figure S2A, B). Female paratype ZMMU A-7782 had duller dark bottle-green dorsal coloration (Supplementary Figure S2D). Coloration of *Zhangixalus melanoleucus* sp. nov. showed slight variation in response to day period and microhabitat conditions. In life, dorsum was somewhat lighter nocturnally than during the day, with dorsal surfaces appearing light to dark green (Supplementary Figure S3). Males can be distinguished from the female based on nuptial pads present; single external subgular vocal sac present; and throats grayish.

**Larval morphology:** Tadpoles at Gosner developmental stage 35 were assigned to the new species based on 16S partial sequences obtained for one specimen ZMMU A-7783. Measurements of the new species tadpoles are presented in Supplementary Table S4. Tadpole coloration in life and mouth apparatus are shown in Supplementary Figure S4. Detailed description of larval morphology is given in the Supplementary Materials.

**Advertisement call:** Call description is based on six advertisement calls from two individuals (holotype BEI 01010 and paratype ZMMU A-7781). The advertisement call of the new species represented a series of clicking sounds (notes). Each note was 0.24–0.30 s in duration (mean  $0.28 \pm 0.02$  s,  $n=50$ ) and consisted of 2–3 pulses (mean  $2.25 \pm 0.38$ ,  $n=50$ ). The first pulse was always shorter in duration (0.05–0.11 s, mean  $0.09 \pm 0.03$  s;  $n=90$ ) than the second and third pulses (0.06–0.19 s, mean  $0.15 \pm 0.04$  s;  $n=70$ ). The inter-note interval was 0.48–0.85 s (mean  $0.61 \pm 0.09$  s;  $n=40$ ) and the dominant frequency was uniformly 3.14 kHz (3 040–3 280 Hz, mean  $3 140 \pm 47.06$  Hz).

**Natural history notes:** Our knowledge on *Zhangixalus melanoleucus* sp. nov. biology is scarce. Specimens were found at night during the rain between 1900h and 2200h in tropical montane evergreen forest on PSM in Xiengkhouang Province at elevations of 2 000–2 200 m a.s.l. Most specimens were found on tree or grass leaves, ca. 1.5–3.0 m above the ground, in patches of primary undisturbed forest with complete multi-layered canopy and heavy undergrowth, suggesting the new species is a strict forest-dwelling specialist. Breeding was observed from April to July; in July, males called loudly from the grass or while sitting on the edge or within temporary rain puddles. Tadpoles of the new species were recorded in the same puddles. Other amphibian species were found at the same site, including *Duttaphrynus* cf. *melanostictus* (Schneider), *Limnonectes taylori* Matsui, Panha, Khonsue, & Kuraishi, *Nanorana aenea* (Smith), *Leptobrachium masatakasatoi* Matsui, *Boulenophrys palpebralespinosa* (Bourret), *Microhyla butleri* Boulenger, *Nidirana lini* (Chou), *Polypedates impresus* Yang, *Zhangixalus feae*, *Gracixalus yunnanensis* Yu, Li, Wang, Rao, Wu, & Yang, and *Rhacophorus* cf. *rhodopus* Liu & Hu.

**Distribution and biogeography:** The new species is currently known only from the type locality in high-elevation montane evergreen forest of PSM, Xiengkhouang Province, Laos (Figure 1A). Further records from other mountain areas in the Houaphan, Xaisomboun, and Bolikhamxay provinces of Laos and adjacent Nghe An Province in Vietnam are

anticipated.

**Conservation status:** To date, *Zhangixalus melanoleucus* sp. nov. is known only from a narrow area within PSM in Xiengkhouang Province, northeast Laos, which belongs to the newly gazetted 98 873 ha Yod Nam Mo–Phou Samsoum National Protected Area (NPA). Further research is required to clarify the extent of its distribution, population size, trends, and conservation status. We preliminarily suggest the new species be considered as DD following the IUCN's Red List categories (IUCN Standards and Petitions Committee, 2022).

**Etymology:** The specific epithet “*melanoleucus*” is an adjective in the nominative case derived from the Ancient Greek “*μέλανος*” for “black” and “*λευκός*” for “white” and is given in reference to the characteristic white and black spots on the flank of the new species. We recommend “*Phou Samsoum Treefrog*” as the common English name.

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

Morphological comparisons of *Zhangixalus melanoleucus* sp. nov. with its sister species *Z. nigropunctatus* appear to be the most pertinent (see Supplementary Figure S5 for life photo of *Z. nigropunctatus* and 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 presence of large irregular black blotches on axilla, flanks, anterior and posterior surfaces of thighs forming continuous pattern (vs. small separated indistinct black spots), small back spots on ventral surfaces of thighs and tarsus (vs. yellowish, back spots absent), and iris bright reddish-orange (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 holotype of *Z. nigropunctatus*, see Supplementary Results); 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 holotype of *Z. nigropunctatus*); comparatively larger eyes (ED/SVL 16.7% ( $n=4$ ) vs. 14.0% in holotype of *Z. nigropunctatus*); larger internarial distance (IND/SVL 12.2% ( $n=4$ ) vs. 9.3% in holotype of *Z. nigropunctatus*); and comparatively longer hindlimbs (HLL/SVL 141.7% ( $n=4$ ) vs. 131.5% in 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 (China) is separated from the range of *Zhangixalus melanoleucus* sp. nov. by over 800 km, providing further support for our hypothesis that the differentiation between these taxa reaches species level. Comparisons of *Zhangixalus melanoleucus* sp. nov. with other congeners are detailed in the Supplementary Materials and summarized in Supplementary Table S5.

The advertisement call of *Zhangixalus melanoleucus* sp. nov. can be readily distinguished from the calls of six other congeners for which call descriptions are available by higher dominant frequency (3 140±47.06 Hz) and lower number of pulses per note (2.25±0.38); comparative bioacoustic information is presented in Supplementary Table S8.

Our phylogenetic study agrees with previous research of *Zhangixalus* in recognizing two distinct well-supported major clades within the genus (Dufresnes et al., 2022; Jiang et al., 2019). Moreover, several currently recognized species of *Zhangixalus* showed almost no or minimal differentiation in mtDNA genes (Supplementary Table S2), and their diagnosis from morphological data alone is also not possible (summarized in Supplementary Tables S5–S7). Hence, our data confirmed the synonymy of *Rhacophorus taronensis* Smith and *R. gongshanensis* Yang & Su with *Z. burmanus* (Andersson), as proposed by Ohler (2009); and of *Polypedates pingbianensis* Kou, Hu, & Gao with *Z. duboisi* (Ohler, Marquis, Swan, & Grosjean), as proposed by Orlov et al. (2002). We also tentatively propose that *Rhacophorus hui* Liu should be considered as a junior subjective synonym of *Z. dugritei* (David), as our study demonstrated that these taxa did not differ in 16S rRNA sequences (0.2% divergence, see Supplementary Table S2) and were morphologically indistinguishable from each other (Supplementary Table S5). Furthermore, several currently recognized *Zhangixalus* species demonstrated only shallow 16S rRNA gene divergence (see Supplementary Table S2), including *Z. lishuiensis* (Liu, Wang, & Jiang) and *Z. zhoukaiyae* (Pan, Zhang, & Zhang) with only 1.6% divergence, as well as a lack of reliable diagnostic characters to differentiate them (summarized in Supplementary Table S6). Further integrative studies are required to clarify the taxonomic status of *Z. yaoshanensis* (Liu & Hu) and *Z. pinglongensis* (Mo, Chen, Liao, & Zhou) due to shallow divergence (3.4% in 16S rRNA) and unclear morphological differentiation between these species (summarized in Supplementary Table S7). Although we refrain herein from formally proposing synonymy of the abovementioned taxa, we call for additional investigations to clarify the taxonomic status of these species.

Our study highlights the important role of the northern Laos mountains as a crucial center of amphibian diversity and endemism in Indochina (Poyarkov et al., 2021). Additional field surveys and integrative taxonomic analyses are required to expand our understanding of this region's exceptional herpetofaunal diversity and effectively develop science-based measures for its conservation.

## NOMENCLATURAL ACTS REGISTRATION

The electronic version of this article in portable document format represents a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new names contained in the electronic version are effectively published under that Code from the electronic edition alone (see Articles 8.5–8.6 of the Code). This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information can be viewed through any standard web browser by appending the LSID to the prefix <http://zoobank.org/>.

Publication LSID: urn:lsid:zoobank.org:pub:601DF810-CCDE-4D0A-A8AF-81837DDE02EA

*Zhangixalus melanoleucus*, LSID: urn:lsid:zoobank.org:act:A987B391-AFFD-4A47-BEB2-F51C0468F977

### SCIENTIFIC FIELD SURVEY PERMISSION INFORMATION

Fieldwork in Laos was permitted by the Biotechnology and Ecology Institute, Ministry of Science and Technology, Lao PDR (permit No. 009 of 23 June 2020). Specimens were collected under approval from the Institute of Animals for Scientific Purposes Development (IAD), which issued fieldwork permission (No. 610104022).

### SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

### COMPETING INTERESTS

The authors declare that they have no competing interests.

### AUTHORS' CONTRIBUTIONS

P.B., T.V.N., C.S., and N.A.P. designed the study. P.B., N.A.P., T.V.N., P.P., S.S.I., and S.L. collected specimens in the field. N.A.P., S.S.I., and S.L. performed molecular experiments. T.V.N., S.S.I., and N.A.P. performed data analyses. P.B., C.S., T.V.N., and N.A.P. wrote the manuscript. C.S., T.V.N., and N.A.P. revised the manuscript. All authors read and approved the final version of the manuscript.

### ACKNOWLEDGEMENTS

NAP thanks Andrei N. Kuznetsov (JVRTRTC, Vietnam), Leonid P. Korzoun (MSU, Russia), Vyacheslav V. Rozhnov (IPEE RAS, Russia), and Hoi Dang Nguyen (JVRTRTC, Vietnam) for organizing and supporting his work in Indochina. TVN thanks Thai Van Nguyen (SVW, Vietnam) and Toan Quoc Phan (DTU, Vietnam) for considerable support. We thank the anonymous reviewers for commenting on an earlier draft of the manuscript. We are grateful to Jian Wang (SYS, China) for providing a photo of *Zhangixalus nigropunctatus* in life, and to Chung-Wei You (Taipei, China) for assistance.

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