



# Systematics of the Lao torrent frog, *Amolops cremnobatus* Inger & Kottelat, 1998 (Anura: Ranidae), with descriptions of four new species

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## Abstract

The Lao torrent frog *Amolops cremnobatus* Inger & Kottelat, 1998 was recently hypothesized, based on mitochondrial DNA, to consist of more than a single species across its range in Laos and flanking regions of Vietnam and Thailand. We tested this hypothesis using mitochondrial DNA, nuclear DNA, and quantitative and qualitative morphological data from adults and larvae. We found corroborating lines of evidence for five distinct evolutionary lineages that we hypothesize to be species. *Amolops cremnobatus* sensu stricto is restricted to the southeastern portion of its previous range, and remaining populations are described as four new species. Some of the new species are easier to diagnose with morphology as larvae than as adults. Further sampling in northern Thailand may reveal an additional species of this torrent frog complex.

## Keywords

Amphibians, frogs, morphology, phylogeny, Southeast Asia, tadpoles

## Introduction

The ranid frog genus *Amolops* Cope, 1865 currently contains 80 recognized species (Frost 2023) that occur across hilly regions of South and Southeast Asia and that have a high affinity for stream environments. Species in this genus are almost exclusively found in clear, fast-flowing streams and exhibit adaptations for living in these noisy, turbulent habitats, including having tadpoles with ventral suckers that allow them to cling to rocks (McDiarmid and Altig 1999; Pham et al. 2015), and in at least one species, the ability to call underwater (Zheng 2019).

The Lao torrent frog *Amolops cremnobatus* Inger & Kottelat, 1998 was originally described from north-central Laos near the Vietnam border (Inger and Kottelat 1998) and has since been reported to occur across much of northern Laos and flanking regions of Vietnam and Thailand (Orlov et al. 2002; Stuart 2005; Nguyen et al. 2009; Pham et al. 2015; Pham et al. 2016; Wu et al. 2020; Pham et al. 2022). The species is a geographically disjunct member of the *A. larutensis* group (Wu et al. 2020; Jiang et al. 2021), equivalent to the subgenus *Amo* (Dubois

1992), with *A. larutensis* Boulenger, 1899, *A. australis* Chan, Abraham, Grismer & Grismer, 2018, and *A. gerutu* Chan, Abraham, Grismer & Grismer, 2018, all of which occur south of the Isthmus of Kra in extreme southern Thailand and Peninsular Malaysia (Chan-ard 2003; Chan et al. 2018; Niyomwan et al. 2019). *Amolops larutensis* was recently partitioned into *A. larutensis*, *A. australis*, and *A. gerutu* based on corroborated lines of evidence in mitochondrial DNA, genomic DNA, and morphology (Chan et al. 2017, 2018). Multiple molecular phylogenetic analyses have demonstrated the sister relationship between *A. cremnobatus* in Indochina and the geographically disparate *A. larutensis* (as one or three species) in the Malay Peninsula (Matsui et al. 2006; Cai et al. 2007; Wiens et al. 2009; Kurabayashi et al. 2010; Pyron and Wiens 2011; Goutte et al. 2016; Chan et al. 2017; Wu et al. 2020; Zeng et al. 2020; Mahony et al. 2022). Recent analysis of mitochondrial DNA of *A. cremnobatus* from three localities, one each in Laos, Vietnam, and Thailand, revealed surprisingly high levels of genetic divergences (Wu et al. 2020), and a species delimitation method using Multi-rate Poisson Tree Processor (mPTP, Kapli et al. 2017) interpreted those mitochondrial sequences to represent two distinct species (Wu et al. 2020).

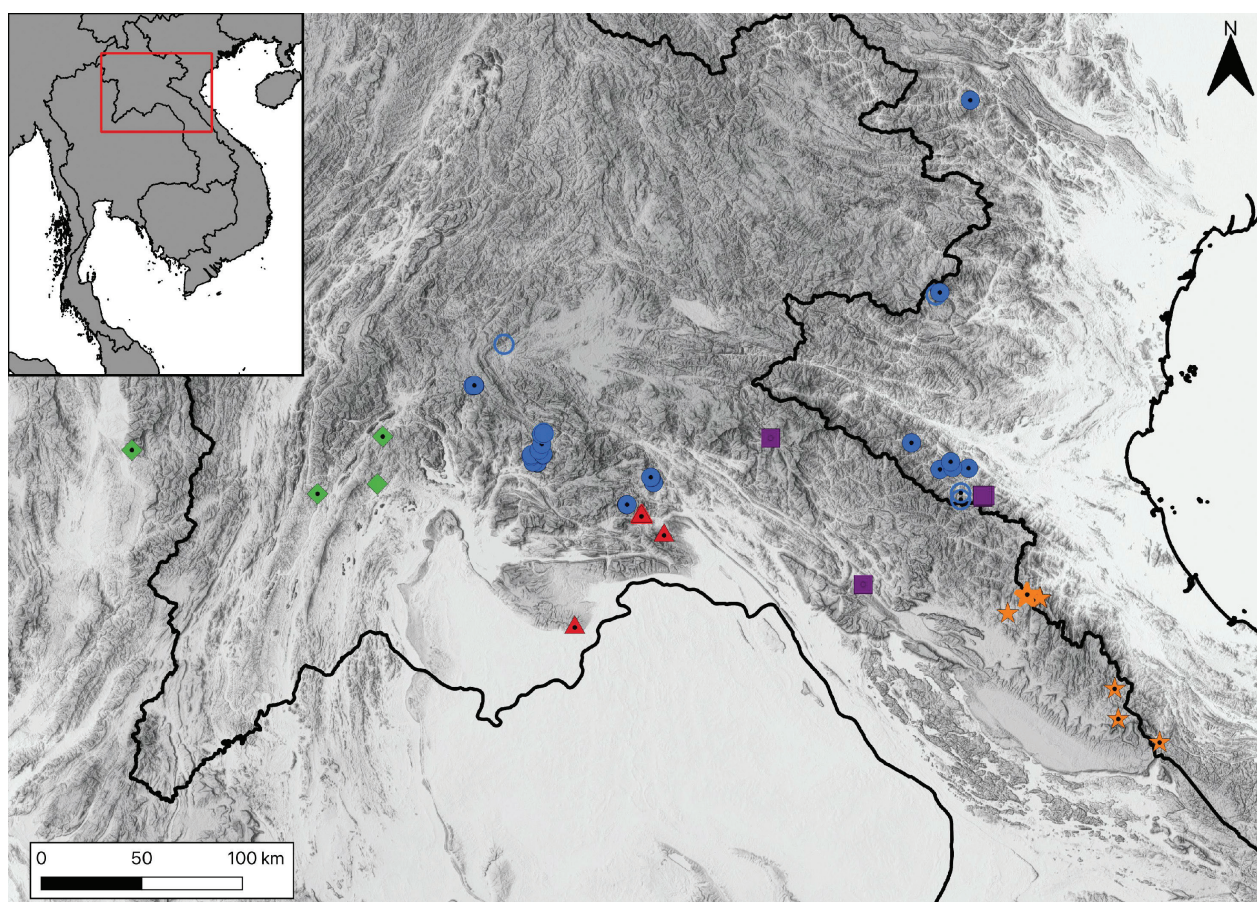
Through expanded sampling from new fieldwork and existing museum collections, we aimed to test the hy-

pothesis of Wu et al. (2020) that the current concept of *A. cremnobatus* consists of more than a single species across its geographic range.

## Materials and Methods

### Specimen sampling

Specimens collected in the field by the authors were humanely euthanized by immersion in tricaine methanesulfonate (MS-222) and fixed in 10% buffered formalin after preserving liver (adults) or part of the tail (representative larvae) in 20% DMSO-salt saturated storage buffer, RNAlater (Invitrogen), or 95% ethanol. Adult specimens were later transferred to 70% ethanol for permanent storage at the Field Museum of Natural History (FMNH), North Carolina Museum of Natural Sciences (NCSM), and National University of Laos, Faculty of Natural Sciences, Department of Biology (NUOL). Specimens were examined (Table S1) and associated tissues were sequenced (Table S2 and Table S3) from the holdings of these institutions and the American Museum of Natural History (AMNH), Australian Museum, Sydney (AMS),



**Figure 1.** Map of specimen sampling locations of *Amolops cremnobatus* (orange stars), *A. tanfuiianae* sp. nov. (blue circles), *A. sengae* sp. nov. (green diamonds), *A. kottelati* sp. nov. (red triangles), and *A. attiguus* sp. nov. (purple squares). Filled symbols are adults and open symbols are tadpoles. Symbols with black dots are sequenced individuals, and symbols without black dots are unsequenced individuals. The sample in Thailand is provisionally referred to *A. sengae* sp. nov. (see text).



California Academy of Sciences (CAS), La Sierra University Herpetological Collection (LSUHC), and Royal Ontario Museum (ROM).

## Sequencing

Total genomic DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen) from muscle or liver tissue of 94 individuals of *A. cremnobatus* and seven outgroups consisting of *Staurois latopalmaris* and six species of *Amolops* (Table S2). Two mitochondrial (mt) and two nuclear (nu) genes were sequenced from all samples. A 597–602 bp fragment of mt DNA that encodes part of the 16S ribosomal RNA (16S) gene was amplified by the polymerase chain reaction (PCR) at annealing temperature ( $T_m$ ) 55°C and sequenced from all samples using the primers L-16SRanaIII and H-16SRanaIII (Stuart et al. 2006). A larger, overlapping fragment of 953–960 bp of 16S was obtained from some samples by amplifying at  $T_m$  55°C with the primers L-16SRanaIII and 16Sbr-3' (Palumbi 1996) and sequencing with the amplifying primers, H-16SRanaIII, and 16Sar-3' (Palumbi 1996). A 1,060 bp fragment of mt DNA that encodes part of the tRNA-Met gene, the complete NADH dehydrogenase subunit 2 gene, and part of the tRNA-Trp gene (ND2) was amplified at  $T_m$  49°C with the primers Met-LND2 and Trp-HND2 (Stuart et al. 2006) and sequenced using the amplifying primers and the internal primers L-ND2crem (5'-TCAACTACGCAAAATTATTGC-3'), H-ND2crem (5'-GTGAATATGGATGTTGTTATTA-3'), or H-ND2crem2 (5'-TTTGGTARTTTGAGTTTGAGC-3'). A ca. 1,000 bp fragment of nuclear (nu) DNA that encodes part of the sodium/calcium exchanger 1 (NCX1) gene was amplified at  $T_m$  49.5°C and sequenced using the primers NCX\_1F and NCX\_3R (Shimada et al. 2011). A 601 bp fragment of nu DNA that encodes part of exon 1 of the tyrosinase gene was amplified at  $T_m$  52°C and sequenced using the primers Tyr1B and Tyr1G (Bossuyt and Milinkovitch 2000).

An additional 11 nu genes (for a total of 13 nu genes) were amplified and sequenced from a subset of 15 individuals (Table S3) consisting of three representatives of each of the five major mt DNA lineages recovered (below). A 697–700 bp fragment that encodes part of the brain-derived neurotrophic factor (BDNF) gene was amplified at  $T_m$  53°C and sequenced using the primers BDNF\_DRV\_F1 and BDNF\_DRV\_R1 (Vieites et al. 2007). A 589 bp fragment that encodes part of the neurotrophin-3 (NTF3) gene was amplified at  $T_m$  58°C using the primers NTF\_F3 and NTF\_R3 (Santos and Cannatella 2011). A 1,233 bp fragment that encodes part of the recombination activating protein 1 (RAG-1) gene was amplified and sequenced following Stuart (2008). A 316 bp fragment that encodes part of exon 1 of the rhodopsin gene was amplified at  $T_m$  58°C and sequenced using the primers Rhod1A and Rhod1C (Bossuyt and Milinkovitch 2000). A 535 bp fragment that encodes part of the solute carrier family 8 member 3 (SLC8A3) gene was amplified at  $T_m$  49.5°C using the primers SCF\_1F and SCF\_1R (Shimada et al. 2011).

A 694 bp fragment of the carbohydrate (keratan sulfate Gal-6) sulfotransferase 1 (CHST1) gene, 745 bp fragment of the dolichol kinase (DOLK) gene, 601 bp fragment of the dopey family member 1 (DOPEY1) gene, 794 bp fragment of the frizzled family receptor 4 (FZD4) gene, 690 bp fragment of the glutamate receptor metabotropic 2 (GRM2) gene, and a ca. 900 bp fragment of the suppressor of cytokine signaling 5 (SOCS5) gene were amplified and sequenced following Shen et al. (2013), except that the second round of PCR primers were untailed and used for sequencing.

PCR products were cleaned using ExoSAP-IT and sequenced in both directions by direct double strand cycle sequencing using the BigDye Terminator version 3.1 Cycle Sequencing Kit on a 3130 or 3500xl DNA Analyzer (all by Applied Biosystems). Sequences were edited using Geneious Prime 2022.0.1 (Biomatters Ltd.) and deposited in GenBank under accession numbers OQ980661–OQ981099, OQ994639–OQ994732 (Table S2, Table S3).

## Phylogenetic analyses

Homologous sequences of 16S, ND2, NCX1, and tyrosinase of all available *A. cremnobatus* and the seven outgroup samples were downloaded from GenBank. 16S sequences were available in GenBank from three individuals also sequenced in this study, and these were downloaded and concatenated (Table S2) with the newly generated sequences for use in analyses. Sequences were aligned using the default parameters in the MAFFT 7.45 alignment algorithm (Katoh and Standley 2013) implemented in Geneious Prime 2022.0.1. Alignments were visually checked to ensure that insertion-deletions did not disrupt translation of coding regions. Uncorrected pairwise distances (p) of 16S were calculated using PAUP\* version 4.0a165 (Swofford 2002).

The four-gene dataset consisting of two mt and two nu genes obtained from all samples was partitioned by 16S, tRNA, and codon positions for a total of 11 partitions. The best-fit partitioning scheme and models of sequence evolution were selected using the Akaike Information Criterion (AICc) in PartitionFinder 2 (Lanfear et al. 2017). Nine partitions were selected, with the first and second codon positions of tyrosinase combined into one partition, and 16S and tRNA combined into one partition.

The 13-gene nu DNA dataset containing a subset of 15 individuals of *A. cremnobatus* was partitioned by codon positions for a total of 39 partitions. Fifteen partitions were selected by PartitionFinder 2, with combined partitions consisting of first codon positions of DOPEY1, CHST1, BDNF, and NTF3; first codon positions of FZD4 and tyrosinase; first codon positions of NCX1, SLC8A3, and RAG1; first codon positions of rhodopsin and second codon positions of NCX1, DOPEY1, and RAG1; first codon positions of DOLK and third codon positions of FZD4; second codon positions of BDNF, tyrosinase, SOCS5, NTF3, and CHST1; second codon positions of FZD4, GRM2, rhodopsin, and SLC8A3; third codon

**Table 1.** Mean  $\pm$  SD (range) of adult body measurements (mm) of *Amolops* examined in the present study.

	<i>A. tanfuiianae</i> sp. nov. “A” (300–1594 m asl)		<i>A. cremnobatus</i> “B” (200–700 m asl)		<i>A. sengae</i> sp. nov. “C” (200–300 m asl)		<i>A. kottelati</i> sp. nov. “D” (214–987 m asl)		<i>A. attiguus</i> sp. nov. “E” (170–454 m asl)	
	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
N	47	52	15	12	2	5	4	2	2	5
SVL	40.0 $\pm$ 1.8 (35.0–43.1)	32.2 $\pm$ 1.6 (28.0–35.3)	38.6 $\pm$ 1.7 (35.3–40.4)	31.0 $\pm$ 1.3 (29.6–34.2)	n/a (39.2–39.6)	29.8 $\pm$ 1.5 (27.2–31.2)	39.0 $\pm$ 1.8 (38.0–41.7)	n/a (33.3–35.0)	n/a (39.2–39.4)	30.2 $\pm$ 0.9 (28.9–31.3)
HDL	13.5 $\pm$ 0.6 (12.1–14.9)	11.2 $\pm$ 0.6 (9.8–12.0)	13.0 $\pm$ 0.6 (11.8–14.2)	10.4 $\pm$ 0.6 (9.2–11.1)	n/a (12.1–13.0)	10.6 $\pm$ 0.6 (9.5–11.1)	13.3 $\pm$ 0.7 (12.8–14.3)	n/a (10.9–11.0)	n/a (12.6–13.0)	10.3 $\pm$ 0.2 (10.0–10.5)
HDW	13.8 $\pm$ 0.7 (11.8–15.1)	11.3 $\pm$ 0.5 (9.9–12.5)	13.2 $\pm$ 0.6 (11.6–14.1)	11.0 $\pm$ 0.4 (10.4–11.5)	n/a (13.2–13.4)	10.5 $\pm$ 0.6 (9.5–10.9)	14.0 $\pm$ 0.8 (13.5–15.1)	n/a (11.6–11.8)	n/a (13.1–13.8)	10.6 $\pm$ 0.4 (10.2–11.1)
SNT	5.8 $\pm$ 0.4 (4.7–6.8)	4.8 $\pm$ 0.3 (4.1–5.5)	5.4 $\pm$ 0.3 (4.5–6.0)	4.3 $\pm$ 0.3 (3.9–4.9)	n/a (5.2–5.8)	4.9 $\pm$ 0.3 (4.4–5.2)	5.7 $\pm$ 0.7 (5.1–6.7)	n/a (5.0–5.0)	n/a (5.4–5.5)	4.1 $\pm$ 0.1 (4.5–4.7)
EYE	5.4 $\pm$ 0.3 (4.6–6.0)	4.7 $\pm$ 0.3 (4.0–5.4)	4.7 $\pm$ 0.3 (4.4–5.6)	4.4 $\pm$ 0.3 (4.1–5.1)	n/a (5.2–5.6)	4.3 $\pm$ 0.1 (4.2–4.5)	5.4 $\pm$ 0.4 (5.0–6.0)	n/a (4.8–5.1)	n/a (5.3–6.1)	4.7 $\pm$ 0.3 (4.1–4.8)
IOD	3.9 $\pm$ 0.2 (3.4–4.5)	3.4 $\pm$ 0.2 (2.8–4.0)	3.7 $\pm$ 0.5 (2.0–4.1)	3.4 $\pm$ 0.2 (3.1–3.7)	n/a (4.0–4.0)	3.2 $\pm$ 0.3 (2.8–3.5)	3.7 $\pm$ 0.3 (3.3–4.1)	n/a (3.2–3.5)	n/a (3.8–3.9)	3.2 $\pm$ 0.2 (2.9–3.5)
IND	4.5 $\pm$ 0.3 (3.9–5.1)	3.9 $\pm$ 0.2 (3.4–4.5)	4.1 $\pm$ 0.4 (3.3–4.9)	3.7 $\pm$ 0.1 (3.5–3.9)	n/a (4.2–4.7)	3.6 $\pm$ 0.2 (3.4–3.8)	4.6 $\pm$ 0.4 (4.0–4.9)	n/a (3.7–3.9)	n/a (4.3–4.6)	3.5 $\pm$ 0.2 (3.3–3.8)
TMP	1.9 $\pm$ 0.2 (1.4–2.6)	1.7 $\pm$ 0.1 (1.3–1.9)	1.8 $\pm$ 0.2 (1.5–2.0)	1.6 $\pm$ 0.1 (1.4–1.8)	n/a (1.6–1.6)	1.6 $\pm$ 0.1 (1.6–1.7)	1.9 $\pm$ 0.2 (1.7–2.2)	n/a (1.8–1.8)	n/a (1.8–2.0)	1.7 $\pm$ 0.2 (1.4–1.9)
TEY	1.4 $\pm$ 0.2 (1.0–1.7)	1.0 $\pm$ 0.2 (0.7–1.4)	1.1 $\pm$ 0.2 (0.8–1.3)	0.9 $\pm$ 0.1 (0.6–1.0)	n/a (1.3–1.7)	0.9 $\pm$ 0.1 (0.8–1.0)	1.3 $\pm$ 0.0 (1.2–1.3)	n/a (0.9–1.0)	n/a (1.1–1.3)	0.7 $\pm$ 0.1 (0.5–0.9)
SHK	23.4 $\pm$ 0.9 (21.3–25.2)	19.2 $\pm$ 0.9 (16.7–21.0)	22.7 $\pm$ 0.8 (21.5–24.4)	19.0 $\pm$ 0.4 (18.3–19.6)	n/a (24.0–24.5)	17.7 $\pm$ 0.9 (16.2–18.4)	23.3 $\pm$ 1.8 (20.9–25.1)	n/a (19.6–19.9)	n/a (21.1–22.4)	18.4 $\pm$ 0.9 (18.3–19.5)
TGH	21.0 $\pm$ 1.3 (16.9–22.7)	17.3 $\pm$ 1.2 (13.2–19.1)	19.9 $\pm$ 0.9 (18.2–21.6)	16.5 $\pm$ 1.0 (14.4–17.6)	n/a (21.2–22.0)	16.3 $\pm$ 0.8 (14.9–16.9)	21.2 $\pm$ 0.7 (20.6–22.2)	n/a (17.3–18.0)	n/a (18.5–19.9)	16.5 $\pm$ 1.1 (15.1–17.7)
HND	12.3 $\pm$ 0.9 (10.1–13.6)	9.7 $\pm$ 0.6 (7.9–10.9)	11.6 $\pm$ 0.6 (10.8–13.0)	9.3 $\pm$ 0.3 (8.9–9.8)	n/a (11.8–12.0)	8.7 $\pm$ 0.9 (7.3–9.6)	12.0 $\pm$ 0.7 (11.2–12.7)	n/a (9.1–10.0)	n/a (11.1–12.6)	9.3 $\pm$ 0.4 (8.9–9.7)
FTL	19.7 $\pm$ 1.4 (16.6–22.1)	16.2 $\pm$ 1.2 (13.5–18.4)	19.3 $\pm$ 0.8 (17.9–20.8)	16.0 $\pm$ 0.6 (15.1–17.1)	n/a (18.4–19.1)	14.3 $\pm$ 0.7 (13.1–15.1)	20.5 $\pm$ 0.7 (19.9–21.4)	n/a (16.6–17.2)	n/a (18.6–18.7)	15.8 $\pm$ 0.7 (15.0–16.8)

positions of NTF3, GRM2, and BDNF; third codon positions of SOCS5 and CHST1; third codon positions of GRM2, rhodopsin and tyrosinase; and third codon positions of RAG1, NCX1, and SLC8A3.

Bayesian inference (BI) was performed on the partitioned four-gene dataset using MrBayes 3.2.7a (Ronquist et al. 2012) on the Cyberinfrastructure for Phylogenetic Research (CIPRES) Science Gateway version 3.3 (Miller et al. 2010). In each of four independent analyses, four chains were run for 20 million generations using the default priors, trees were sampled every 4,000 generations, and the first 25% of trees were discarded as ‘burn-in’. The resulting trace plots were viewed using Tracer v.1.7 (Rambaut et al. 2018). A 50% majority-rule consensus of the post burn-in trees was constructed to calculate the posterior probabilities of nodes. Nodes with posterior probabilities  $\geq 0.95$  were considered to be supported.

Maximum likelihood (ML) analysis was performed on both the four-gene (rooted) and 13-gene (unrooted) datasets using raxml GUI 2.0 (Edler et al. 2021). The GTR+I+G model was applied to the nine and 15 partitions, respectively, selected by PartitionFinder 2. Nodal support values were estimated by the thorough bootstrap with 1,000 pseudoreplicates. Nodes with bootstrap values  $\geq 70$  were considered to be supported.

A genetic network was constructed from the 13-gene dataset to visualize patterns of genetic divergence using the NeighborNet algorithm implemented in SplitsTree v. 4.15.1 (Huson and Bryant 2006).

## Morphological measurements and analyses

Morphological data were obtained from 175 vouchered specimens (146 adults, 29 tadpoles) from 21 localities across Laos and Vietnam (Table 1; Fig. 1; Table S1), including the holotype and paratype of *A. cremnobatus*. For adults, thirteen continuous morphological characters were measured to the nearest 0.1 mm with digital calipers: snout-vent length (SVL), head length (HDL), head width (HDW), eye to tip of snout distance (SNT), eye diameter (EYE), inter-orbital distance (IOD), inter-nares distance (IND), tympanum diameter (TMP), tympanum to eye distance (TEY), shank length (SHK), thigh length (TGH), hand length, measured from proximal margin of palm to tip of longest digit (HND), and foot length, measured from proximal margin of foot to tip of longest digit (FTL). Sexual maturity was determined by the presence of nuptial pads (males) or eggs or enlarged oviducts (females). Male and female measurements were analyzed separately due to apparent sexual size dimorphism. Principal components analysis (PCA) was used to find the best low-dimensional representation of variation in the data to determine whether morphological variation formed the basis of detectable group structure. PCA was applied to the residuals of the linear regressions between SVL and the other 12 measured variables (Vitt et al. 2000; Funk et al. 2008), and performed using the prcomp function in R v4.0.3 (R Core Team 2020). The first two ma-



**Table 2.** Mean  $\pm$  SD (range) of tadpole body measurements (mm) for available *Amolops* examined in the present study. For ease of comparison, we present data on all tadpoles of each species, as well as those at or below Gosner stage (S) 30.

	<i>A. tanfuiianae</i> sp. nov. “A”		<i>A. cremnobatus</i> “B”		<i>A. sengae</i> sp. nov. “C”		<i>A. kottelati</i> sp. nov. “D”
	All	$\leq$ S30	All	$\leq$ S30	All	$\leq$ S30	All ( $\leq$ S30)
<i>n</i>	16	9	5	4	5	4	3
BL	15.9 $\pm$ 2.8 (12.9–21.5)	14.1 $\pm$ 1.3 (12.9–16.3)	15.3 $\pm$ 1.7 (13.3–17.0)	14.8 $\pm$ 1.6 (13.3–16.4)	11.3 $\pm$ 4.7 (7.7–18.2)	9.6 $\pm$ 3.1 (7.7–14.1)	17.0 $\pm$ 0.2 (16.8–17.1)
BW	9.8 $\pm$ 1.7 (7.9–13.2)	8.8 $\pm$ 0.3 (7.9–10.3)	9.7 $\pm$ 1.4 (8.3–11.8)	9.2 $\pm$ 1.0 (8.3–10.1)	7.1 $\pm$ 2.6 (4.7–11.1)	6.1 $\pm$ 1.6 (4.7–8.4)	10.8 $\pm$ 0.1 (10.8–10.9)
IND	2.9 $\pm$ 0.4 (2.5–3.9)	2.7 $\pm$ 0.2 (2.5–3.1)	2.8 $\pm$ 0.3 (2.4–3.2)	2.7 $\pm$ 0.3 (2.4–2.9)	2.3 $\pm$ 0.8 (1.6–3.4)	2.0 $\pm$ 0.6 (1.6–2.9)	3.1 $\pm$ 0.1 (3.1–3.2)
IP	4.9 $\pm$ 1.0 (3.7–6.6)	4.3 $\pm$ 0.6 (3.7–5.3)	4.9 $\pm$ 1.1 (3.9–6.7)	4.4 $\pm$ 0.5 (3.9–4.8)	3.5 $\pm$ 1.5 (2.4–5.7)	3.0 $\pm$ 1.0 (2.4–4.6)	5.4 $\pm$ 0.3 (5.1–5.7)
RND	3.5 $\pm$ 0.7 (2.5–4.7)	3.3 $\pm$ 0.4 (2.9–4.2)	3.2 $\pm$ 0.6 (2.5–4.1)	3.0 $\pm$ 0.4 (2.5–3.3)	2.6 $\pm$ 0.9 (1.9–4.0)	2.2 $\pm$ 0.6 (1.9–3.1)	3.7 $\pm$ 0.4 (3.5–4.1)
RED	5.6 $\pm$ 0.9 (4.4–7.2)	5.3 $\pm$ 0.6 (4.9–6.6)	5.6 $\pm$ 0.8 (4.6–6.7)	5.3 $\pm$ 0.5 (4.6–5.8)	4.2 $\pm$ 1.9 (3.6–6.3)	4.3 $\pm$ 1.3 (3.6–6.3)	6.0 $\pm$ 0.5 (5.7–6.6)
TMW	4.9 $\pm$ 0.8 (3.8–6.3)	4.5 $\pm$ 0.6 (3.8–5.7)	5.1 $\pm$ 1.0 (4.1–6.5)	4.7 $\pm$ 0.6 (4.1–5.4)	3.6 $\pm$ 1.8 (2.3–6.2)	3.0 $\pm$ 1.2 (2.3–4.8)	5.8 $\pm$ 0.1 (5.6–5.9)
SDL	11.6 $\pm$ 2.1 (8.9–15.4)	10.3 $\pm$ 0.8 (8.9–12.2)	10.8 $\pm$ 1.6 (9.0–13.0)	10.3 $\pm$ 1.2 (9.0–11.7)	8.3 $\pm$ 3.3 (5.8–13.3)	7.1 $\pm$ 2.0 (5.8–10.1)	12.9 $\pm$ 0.2 (12.7–13.2)
ODW	7.0 $\pm$ 1.5 (5.1–10.1)	6.1 $\pm$ 0.8 (5.1–7.8)	7.1 $\pm$ 0.6 (6.4–7.7)	7.0 $\pm$ 0.6 (6.4–7.7)	5.0 $\pm$ 1.8 (3.6–7.7)	4.3 $\pm$ 1.1 (3.6–6.0)	7.4 $\pm$ 0.3 (7.2–7.7)
IB	0.5 $\pm$ 0.1 (0.3–0.7)	0.5 $\pm$ 0.1 (0.3–0.6)	0.6 $\pm$ 0.1 (0.4–0.6)	0.5 $\pm$ 0.1 (0.4–0.6)	0.5 $\pm$ 0.2 (0.3–0.7)	0.4 $\pm$ 0.1 (0.3–0.6)	0.7 $\pm$ 0.1 (0.6–0.7)
BH	5.9 $\pm$ 1.1 (4.6–8.0)	5.3 $\pm$ 0.7 (4.6–6.4)	6.8 $\pm$ 1.2 (5.7–8.7)	6.4 $\pm$ 0.7 (5.7–7.2)	4.4 $\pm$ 2.1 (2.7–7.7)	3.5 $\pm$ 1.3 (2.7–5.4)	7.1 $\pm$ 0.4 (6.8–7.5)
R_BH	n/a	–0.28 $\pm$ 0.41 (–0.90–0.23)	n/a	0.45 $\pm$ 0.38 (–0.07–0.82)	n/a	0.03 $\pm$ 0.14 (–0.17–0.15)	0.20 $\pm$ 0.33 (–0.10–0.56)
ED	2.2 $\pm$ 0.5 (1.5–3.1)	1.9 $\pm$ 0.2 (1.5–2.3)	1.8 $\pm$ 0.3 (1.5–2.3)	1.6 $\pm$ 0.2 (1.5–1.8)	1.5 $\pm$ 0.2 (1.0–2.6)	1.3 $\pm$ 0.4 (1.0–1.9)	2.4 $\pm$ 0.1 (2.2–2.5)
TMH	4.6 $\pm$ 0.7 (3.7–5.9)	4.4 $\pm$ 0.7 (3.7–5.5)	5.4 $\pm$ 1.3 (4.4–7.6)	4.9 $\pm$ 0.5 (4.4–5.5)	3.8 $\pm$ 2.1 (2.3–6.9)	3.1 $\pm$ 1.3 (2.3–5.0)	5.7 $\pm$ 0.2 (5.5–5.9)
MTH	6.5 $\pm$ 1.1 (4.9–8.0)	6.0 $\pm$ 1.1 (4.9–7.9)	6.4 $\pm$ 1.4 (5.1–8.6)	5.8 $\pm$ 0.7 (5.1–6.6)	5.7 $\pm$ 3.1 (3.4–7.8)	4.6 $\pm$ 2.1 (3.4–7.8)	7.3 $\pm$ 0.3 (7.0–7.6)
UF	2.0 $\pm$ 0.3 (1.6–2.6)	1.8 $\pm$ 0.3 (1.6–2.4)	2.1 $\pm$ 0.4 (1.8–2.7)	2.0 $\pm$ 0.2 (1.8–2.2)	1.8 $\pm$ 1.0 (1.1–3.4)	1.4 $\pm$ 0.6 (1.1–2.3)	2.1 $\pm$ 0.1 (2.0–2.3)

major principal components (PCs) were plotted with 95% confidence ellipses using ggbiplot. Following PCA, discriminant analyses of principal components (DAPC) was performed using the dapc function in R v4.0.3.

We performed ANOVA on raw SVL data for females of Clades A, B, and D, and for males of Clades B, C, and E, the clades for each sex with  $n > 3$ . Because male SVL in Clades B, C, and E did not differ significantly (ANOVA  $F$ -stat = 1.77,  $df = 2$ ,  $p$ -value = 0.20), we conducted ANOVA on raw size data of the features listed above in order to provide more detailed information on how clades differed morphologically.

Preserved larvae were available for four of the five mt clades (below), including from the type locality of *A. cremnobatus* (Table 2). Only tadpoles that were sequenced (Clade A) or that were collected at the same locality as sequenced adults (Clades A–D) were included in the analyses. As no geographic overlap among clades was found (below), these clade assignments of larvae should be robust. Tadpoles were staged following Gosner (1960) and keratodont formulae followed Altig (2007). Larvae were photographed in standardized positions

and 15 measurements were taken to the nearest 0.1 mm using Image-J (Rasband 1997–2018): maximum body length (BL), maximum body width (BW), maximum body height (BH), interpupilar distance (IP), internarial distance (IND), rostral distance (RND), horizontal eye diameter (ED), rostral-eye distance in dorsal view (RED), oral disk width (ODW), distance from tip of snout to posterior edge of sucker disc (SDL), inter-beak distance (IB), height of caudal muscle minus fin at base of tail (TMH), width of caudal muscle at base (TMW), maximum tail height (MTH), and maximum height of upper fin (UF; Table 2). PCA was applied to the residuals of the linear regressions between body length (BL) and the other 14 measured variables (Funk et al. 2008; Vitt et al. 2000). We used the residuals of body height on body length (R\_BH) for inter-specific comparisons (Table 2). Because the largest number of tadpoles were at or below Gosner stage (S) 30 (Gosner 1960), and because one clade had no tadpoles above this stage, only tadpoles at or below S30 were included in analyses. PCA and DAPC were performed on the larval measurements as described for adults.

## Results

The four-gene dataset (two mt, two nu) contained 3,669 characters and 106 taxa. The BI analysis resulted in a standard deviation of split frequencies of 0.006409 among the four runs, and the Estimated Sample Sizes (ESS) of parameters were  $\geq 2,019$ . The ML analysis recovered a single best tree with log likelihood of  $-16571.641905$  and had a very similar topology to the BI analysis. Analyses of the four-gene dataset recovered five major clades within *A. cremnobatus* (labeled A–E in Fig. 2), all strongly supported with BI posterior probabilities = 1.00 and ML bootstrap values  $\geq 99$  (Fig. 2). Most of the samples belonged to Clade A, a geographically widespread clade encompassing approximately the northern half of the range that extended from Luang Phabang Province, Laos eastward into Nghe An and Thanh Hoa Provinces, Vietnam (Figs 1, 2). Clade B consisted of samples encompassing the southeastern portion of the range in Bolikhamxay and Khammouan Provinces, Laos, and Ha Tinh and Quang Binh Provinces, Vietnam, including localities surrounding the type locality of *A. cremnobatus* (Figs 1, 2). Clade C consisted of samples encompassing the western portion of the range, from Vientiane Province, Laos westward across the Mekong River into Xaignabouli Province, Laos, and Nan Province, Thailand (Figs 1, 2). Clade D was represented by samples from a small area of northern Laos in western Bolikhamxay and eastern Xaysomboun Provinces. Clade E was represented by samples from a small area in eastern Bolikhamxay and Xieng Khouang Provinces of northern Laos, extending eastward into southern Nghe An Province, Vietnam (Figs 1, 2). Mt divergences were relatively high among clades, with uncorrected pairwise distances (p) of 16S ranging from 4.9–8.5% between clades.

The 13-nu gene dataset contained 9,450 characters and 15 taxa. The ML analysis recovered a single best tree with log likelihood of  $-13876.757112$ . The SplitsTree network had a very similar clustering pattern to the ML analysis. Clades B, D, and E were each recovered as reciprocally monophyletic (or as distinct clusters in the SplitsTree network), but Clades A and C grouped together without separation (Fig. 3).

For males, PCA showed separation between some clades, but high overlap among others in PC1, which accounted for 27.0% of the variance (Fig. 4A), while the DAPC revealed better separation among the clades (Fig. 5A). For females, PCA showed little separation between clades along PC1, which accounted for 29.2% of the variance (Fig. 4B), while the DAPC showed good separation among clades, with especially strong separation of Clade D from Clades A and B (Fig. 5B). For tadpoles, there was some separation of Clade B from other clades along PC1, which accounted for 36.8% of the variance (Fig. 4C), while the DAPC showed strong separation among the four analyzed clades (Fig. 5C).

ANOVA showed significant size differences among females of Clades A, B, and D (ANOVA F-stat = 6.02,

df = 2, p-value = 0.004), with females of Clade A significantly larger than those of Clades B (t-stat = 3.53, df = 25, p-value = 0.002) and D (t-stat = 2.74, df = 20, p-value = 0.01). Males of Clades B, C, and E differed significantly in measures of SNT, EYE, TEY, SHK, and FTL (Table 3).

## Taxonomy

Five clades of *A. cremnobatus* are readily diagnosed in mt DNA, larval or adult morphology (quantitative and qualitative characters), and with the exception of two clades, in nu DNA. On the bases of these corroborated lines of evidence, we hypothesize that *A. cremnobatus* represents at least five species across its geographic range. These are formally described as follows.

### *Amolops cremnobatus* Inger & Kottelat, 1998

Figure 6A

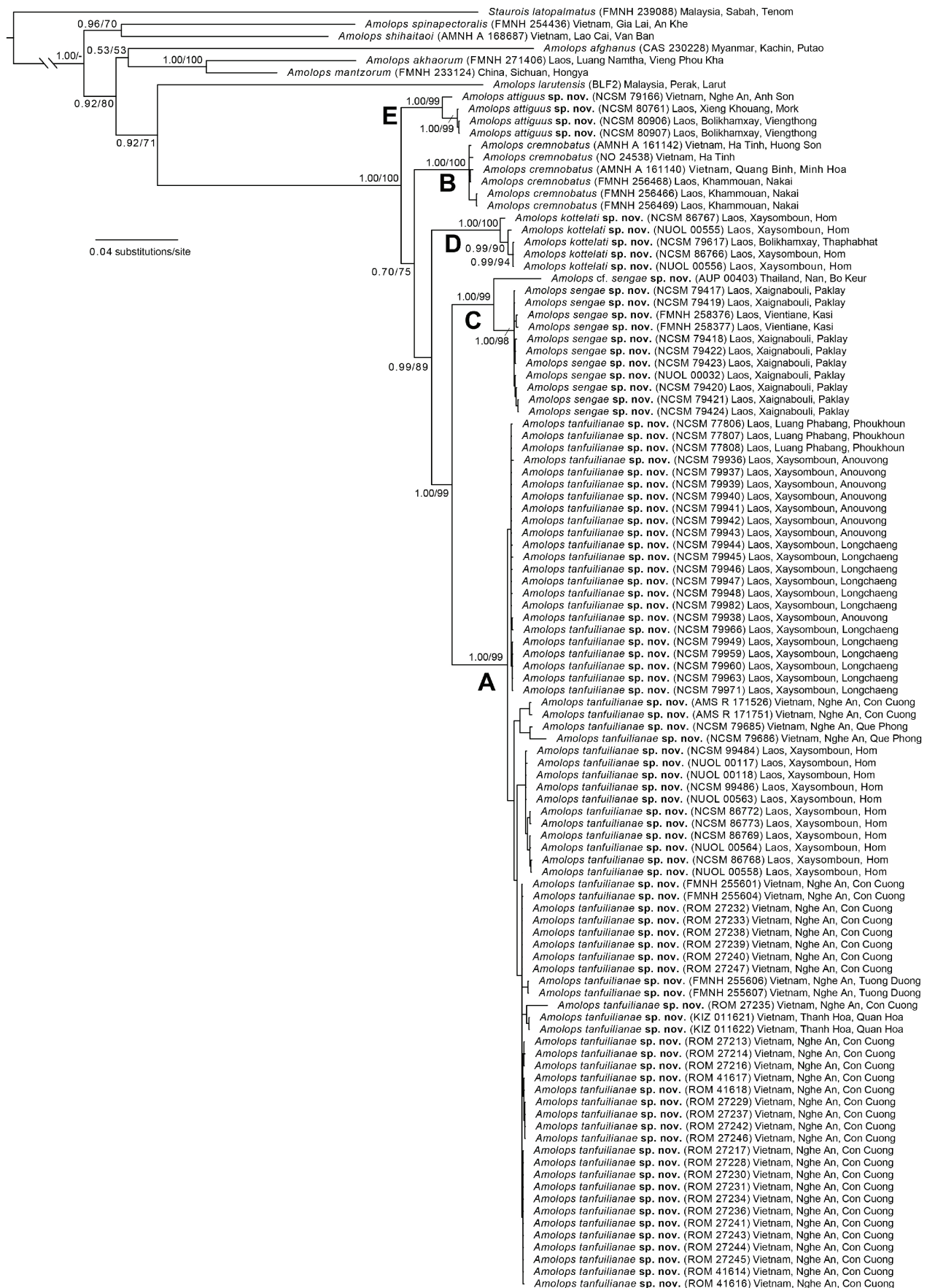
“Clade B”

**Chresonymy.** *Amolops cremnobatus* – Inger and Kottelat (1998: 30), Stuart (1999: 45), Bain and Nguyen (2001: 269), Orlov et al. (2002: 86, part), Stuart (2005: 476, part), Bain et al. (2007: 109, part), Cai et al. (2007: 51), Matsui et al. (2006: 661), Nguyen et al. (2009: 121, part), Luu et al. (2013: 295), Poyarkov et al. (2021: 44, part).

**Types.** FMNH 252861, adult male holotype, FMNH 252862, adult male paratype (both examined). The type locality was given by Inger and Kottelat (1998) as “Laos, Khammouan Prov., Nam Phao River, just downstream from border post on Lak Sao/Vinh Road (18° 23'N/105° 09'20"E).” This portion of the Nam Phao straddles the borders of Khammouan and Bolikhamxay Provinces, and the site described by Inger and Kottelat (1998) is actually on the Bolikhamxay side of that boundary. The type locality is amended here to Laos, Bolikhamxay Province, Khamkeut District, Nam Phao River, just downstream from border post on Lak Sao/Vinh Road, 18.38267N 105.15758 E.

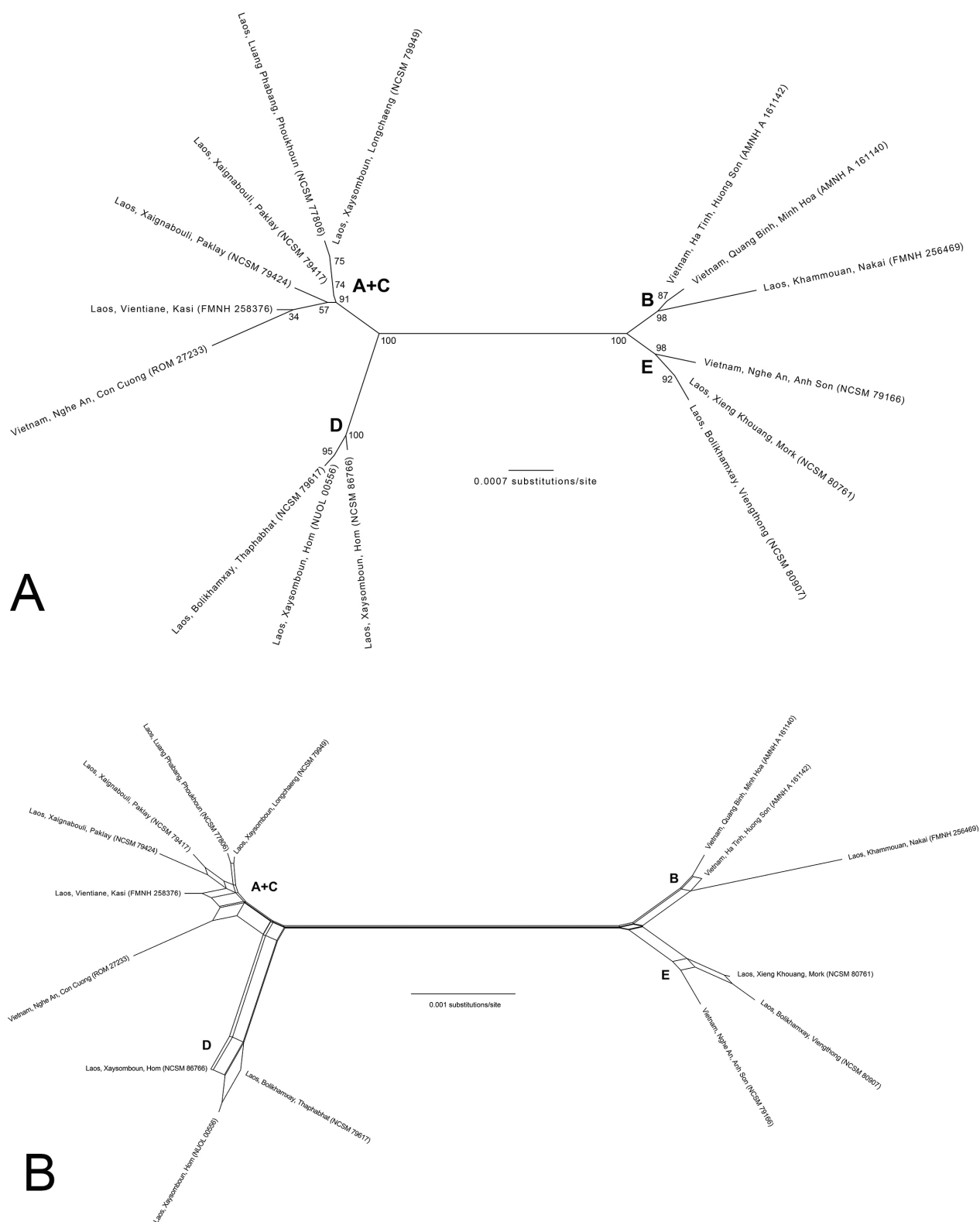
**Suggested Common Names.** Inger’s Lao torrent frog (English), ຂຽດເກາະຜາລາວອິງເກີ (Khat Korpha Lao Inger; Lao).

**Expanded diagnosis.** A member of the *Amolops larutensis* group having the combination of 2–3 faint vomerine teeth, sometimes absent; relative finger lengths I<II<IV<III; mean  $\pm$  SE SVL of adult males  $31.0 \pm 1.3$  mm (range 29.6–34.2 mm; n = 12) and of adult females  $38.6 \pm 1.7$  mm (range 35.3–40.4 mm; n = 15); and tadpoles with BL  $15.3 \pm 1.1$  mm (range 13.3–17.0 mm; n = 5).



**Figure 2.** Fifty percent majority-rule consensus phylogram resulting from partitioned Bayesian analysis of 3,669 aligned characters of the mitochondrial 16S, mitochondrial ND2 and flanking tRNAs, nuclear NCX1, and nuclear tyrosinase genes from *Amolops* frogs. Trees were rooted with *Staurois latopalmatus*. Numbers at nodes are Bayesian posterior probabilities (left) and bootstrap values  $\geq 50$  from a separate maximum likelihood analysis (right). Additional voucher and locality data for samples are provided in Table S2 and Table S3.

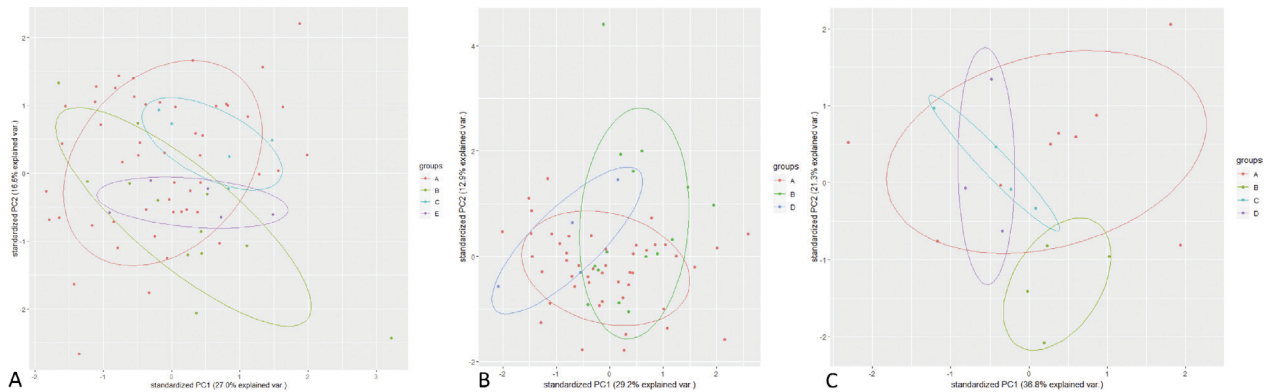




**Figure 3.** The best unrooted maximum likelihood tree (**A**) and genetic network using the NeighborNet algorithm implemented in SplitsTree (**B**) of 9,450 aligned characters of 13 nuclear genes from frogs allied to *Amolops cremnobatus*. Additional voucher and locality data for samples are provided in Table S2 and Table S3.

**Remarks.** Our examinations of the holotype and paratype agreed closely with the thorough description of Inger and Kottelat (1998) and the description is not repeated here beyond the addition of relative finger lengths I<II<IV<III [given only as “fingers short, first much shorter than second” in Inger and Kottelat (1998)].

**Distribution and natural history.** This species is verified to occur in portions of Bolikhamxay and Khammouan Provinces, Laos, and Ha Tinh and Quang Binh Provinces, Vietnam (Fig. 1; Table S1 and Table S2).



**Figure 4.** Principal components analysis, showing 95% confidence ellipses. **A** Males, **B** females, **C** tadpoles. For all panels, groups A–E represent *Amolops tanfuiliana* sp. nov., *A. cremnobatus*, *A. sengae* sp. nov., *A. kottelati* sp. nov., and *A. attiguus* sp. nov., respectively.

### *Amolops tanfuiliana* sp. nov.

<https://zoobank.org/0653C571-E9FF-4580-B07F-D3A586986E3D>

Figure 7

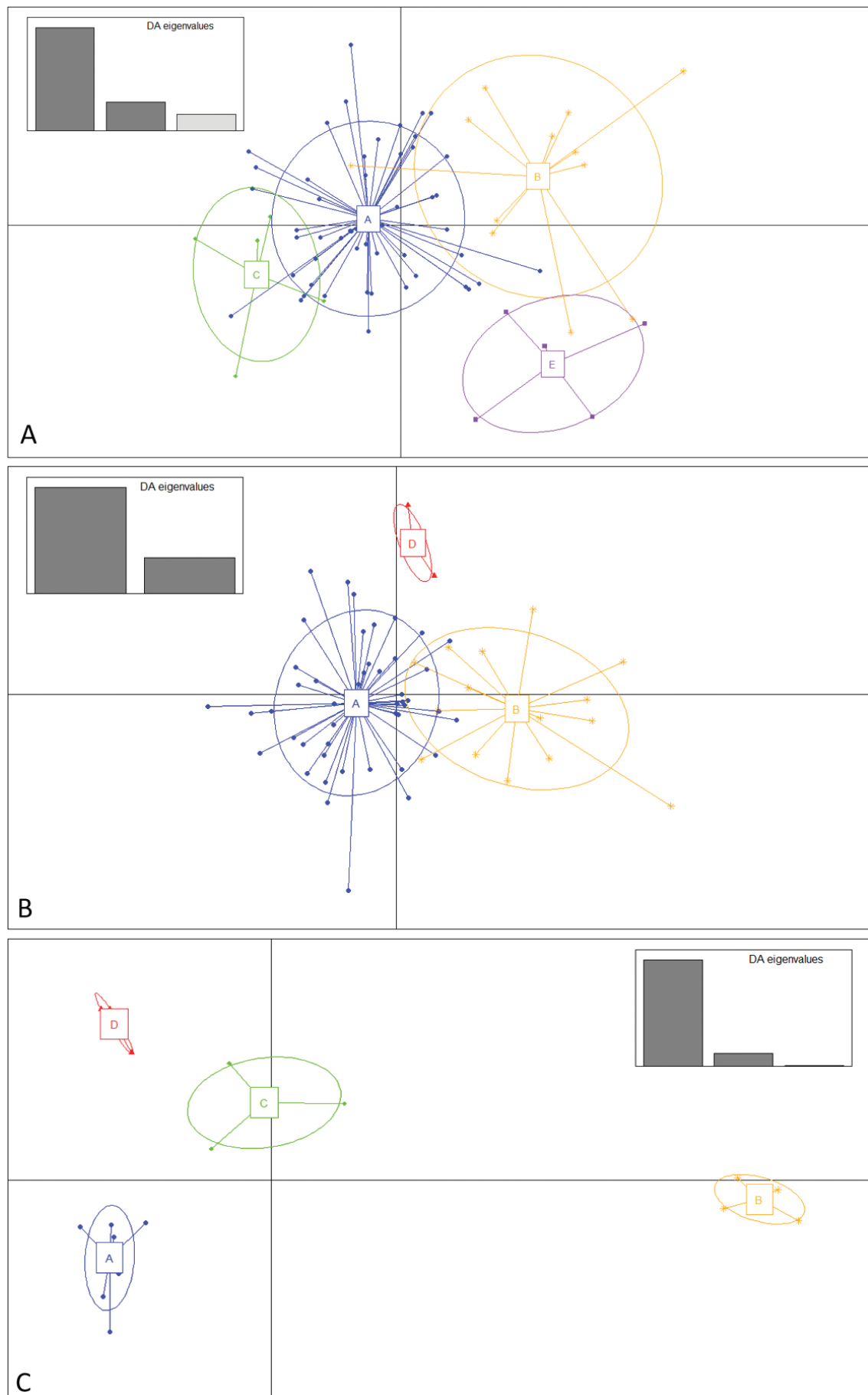
“Clade A”

**Chresonymy.** *Amolops cremnobatus* Orlov et al. (2002: 86, part), Chen et al. (2005: 234), Ngo et al. (2006: 84), Stuart (2008: 51), Nguyen et al. (2009: 121, part), Pham et al. (2015: 111), Pham et al. (2016: 40), Wu et al. (2020: 5, part), Brakels et al. (2021: 159), Pham et al. (2022: 171).

**Holotype.** NCSM 79949 (field number BLS 15368), adult male, Laos, Xaysomboun Province, Longchaeng District, 19.01807°N, 102.87633°E, 490 m elev., coll. 2045 h on a plant leaf 0.3 m above <1 m wide steep rocky seep flowing into the Nam Ngum River in disturbed semi-evergreen forest on 10 May 2012 by Bryan L. Stuart, Somphouthone Phimmachak, and Niane Sivongxay.

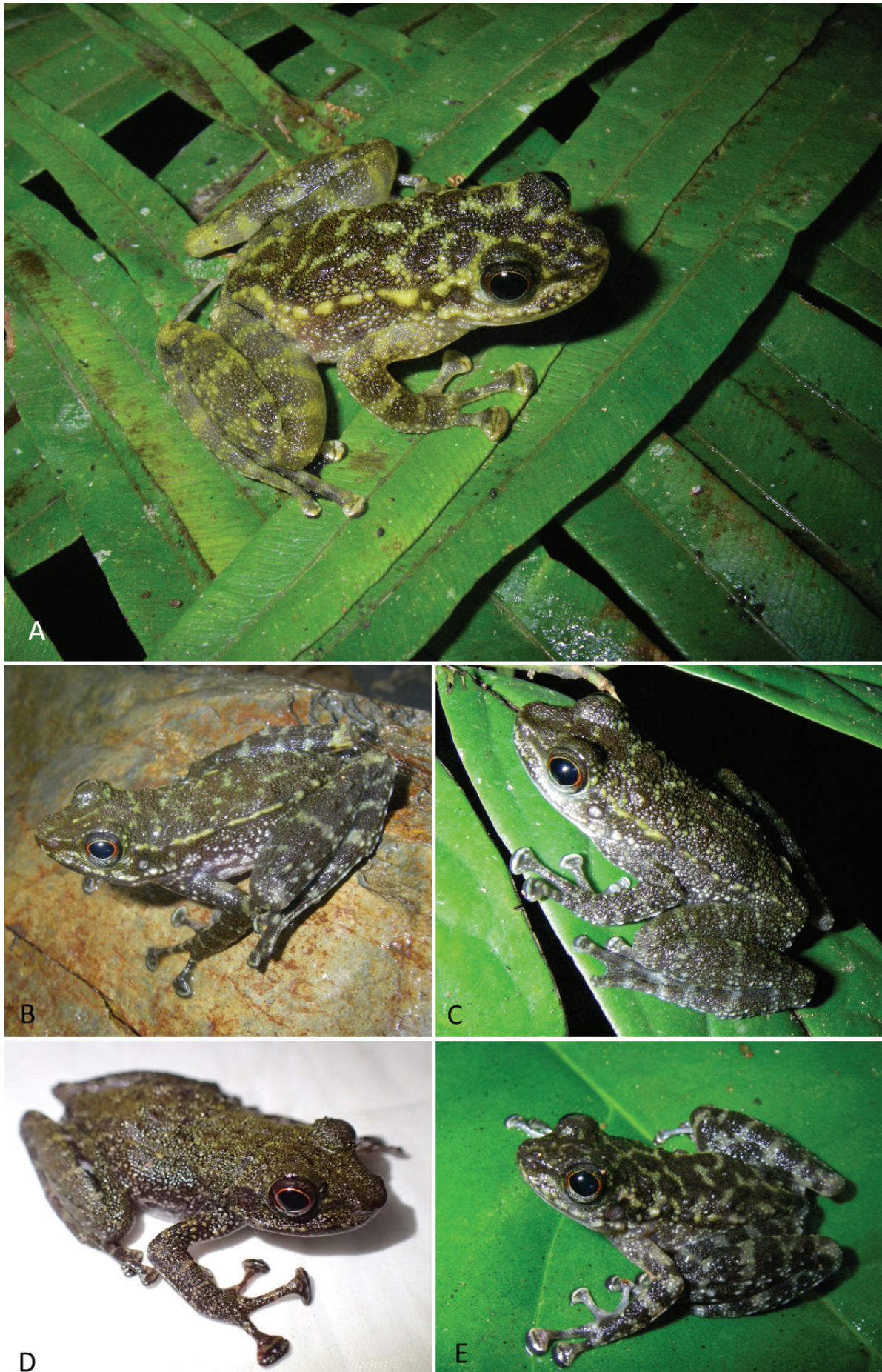
**Paratypes.** Laos, Luang Phabang Province, Phoukhoun District: FMNH 280104, NCSM 77803–04, NCSM 77806 (four adult males), FMNH 280108, NCSM 77805, NCSM 77807 (three adult females), 19.30189°N, 102.57361°E, 1269 m elev., coll. 5 January–9 June 2009 by Somphouthone Phimmachak; NCSM 77809 (one adult male), 19.30103°N, 102.57581°E, 1250 m elev., coll. 8 April 2009 by Somphouthone Phimmachak. — Laos, Xaysomboun Province, Anouvong District, tributary of Nam Ngum River: NCSM 79936–37 (two adult males), NCSM 79940 (one adult female), 18.96260°N, 102.86817°E, 1065 m elev., coll. 23 May 2012 by Bryan L. Stuart, Somphouthone Phimmachak, and Niane Sivongxay; NCSM 79938, NUOL 00030 (two adult females), 18.96346°N, 102.86800°E, 1044 m elev., coll. 23 May 2012 by Bryan L. Stuart, Somphouthone Phimmachak, and Niane Sivongxay; NCSM 79939 (one adult male), 18.96286°N, 102.86802°E, 1054 m elev., coll. 23

May 2012 by Bryan L. Stuart, Somphouthone Phimmachak, and Niane Sivongxay; NCSM 79941, NCSM 79943 (two adult males), NCSM 79942 (one adult female), 18.96227°N, 102.86815°E, 1074 m elev., coll. 23 May 2012 by Bryan L. Stuart, Somphouthone Phimmachak, and Niane Sivongxay. — Laos, Xaysomboun Province, Hom District: NCSM 86769, NCSM 86772, NUOL 00558, NUOL 00560 (four adult males), NCSM 86768, NCSM 86771, NUOL 00559 (three adult females), Phou Samsao, 18.77802°N, 103.28593°E, 1545 m elev., coll. 16–17 July 2015 by Somphouthone Phimmachak and Sengvilay Seateun; NCSM 86773 (one adult male), NUOL 00563–64 (two adult females), 18.77854°N, 103.28757°E, 1594 m elev., coll. 18 July 2015 by Somphouthone Phimmachak and Sengvilay Seateun; NCSM 99484 (one adult male), Phou Samliam, 18.89780°N, 103.40057°E, 960 m elev., coll. 21 April 2018 by Somphouthone Phimmachak, Sengvilay Seateun, Sinyasone Souvannasy, and Nidarphone Kouangvilaykhoun; NCSM 99486 (one adult male), 18.87535°N, 103.41138°E, 560 m elev., coll. 24 April 2018 by Somphouthone Phimmachak, Sengvilay Seateun, Sinyasone Souvannasy, and Nidarphone Kouangvilaykhoun; NCSM 99485, NUOL 00115–19 (six adult females), 18.89857°N, 103.39857°E, 926 m elev., coll. 22 April 2018 by Somphouthone Phimmachak, Sengvilay Seateun, Sinyasone Souvannasy, and Nidarphone Kouangvilaykhoun. — Laos, Xaysomboun Province, Longchaeng District: NCSM 79948 (one adult male), NCSM 79944–47 (four adult females), 18.96546°N, 102.84858°E, 790 m elev., coll. 24 May 2012 by Bryan L. Stuart, Somphouthone Phimmachak, and Niane Sivongxay; NCSM 79960 (one adult male), 19.04444°N, 102.88918°E, 538 m elev., coll. 14 May 2012 by Bryan L. Stuart, Somphouthone Phimmachak, and Niane Sivongxay; NCSM 79961 (one adult female), 19.09203°N, 102.89792°E, 883 m elev., coll. 16 May 2012 by Bryan L. Stuart, Somphouthone Phimmachak, and Niane Sivongxay; NCSM 79963–66, NCSM 79968–70, NCSM 79975, NUOL 00034–35 (ten adult males), NCSM 79962, NCSM 79967, NUOL 00033 (three adult females), 19.09100°N, 102.89607°E, 885 m elev., coll. 16–18 May 2012 by Bryan L. Stuart, Somphouthone Phimmachak, and Niane Sivongxay; NCSM 79971 (one



**Figure 5.** Discriminant analyses of principal components, showing 95% confidence ellipses. **A** Males, **B** females, **C** tadpoles. Within panels, groups A–E represent *Amolops tanfuiianae* **sp. nov.**, *A. cremnobatus*, *A. sengae* **sp. nov.**, *A. kottelati* **sp. nov.**, and *A. attiguus* **sp. nov.**, respectively.





**Figure 6.** Photos in life. **A** *Amolops cremnobatus*, **B** *A. tanfuiliana* sp. nov., **C** *A. sengae* sp. nov., **D** *A. kottelati* sp. nov., **E** *A. atiguus* sp. nov.



**Table 3.** ANOVA and paired t-test results comparing sizes of males across clades. **B** *Amolops cremnobatus*, **C** *A. sengae* **sp. nov.**, and **E** *A. attiguus* **sp. nov.** Sample sizes are given in parentheses next to clade heading. All ANOVA df = 2. Bolded values are significantly different from other values in the same row.

	<b>B (12)</b>	<b>C (5)</b>	<b>E (5)</b>	<b>F-stat; p-value</b>	<b>t-stat, df; p-value (clades)</b>	<b>t-stat, df; p-value (clades)</b>
SVL	31.0±1.3	29.8±1.5	30.2±0.9	1.77; 0.20	n/a	n/a
SNT	4.3±0.3	<b>4.9±0.3</b>	4.1±0.1	7.16; 0.005	3.13; 8; 0.01 (C–B)	3.66; 8; 0.003 (C–E)
EYE	4.4±0.3	4.3±0.1	<b>4.7±0.3</b>	3.01; 0.07	–2.51; 15; 0.02 (B–E)	–4.12; 8; 0.003 (C–E)
TEY	0.9±0.1	0.9±0.1	<b>0.7±0.1</b>	3.94; 0.04	2.49; 10; 0.03 (B–E)	3.17; 8; 0.01 (C–E)
SHK	<b>19.0±0.4</b>	17.7±0.9	18.4±0.9	7.45; 0.004	–3.19; 5; 0.02 (C–B)	2.02; 5; 0.21 (B–E)
FTL	16.0±0.6	<b>14.3±0.7</b>	15.8±0.7	12.84; <0.001	–4.65; 6; <0.01 (C–B)	03.14; 8; 0.01 (C–E)



**Figure 7.** *Amolops tanfuilianae* **sp. nov.** NCSM 79949 in life (top row) and in preservative (bottom row).

adult female), 19.08988°N, 102.89599°E, 887 m elev., coll. 17 May 2012 by Bryan L. Stuart, Somphouthone Phimmachak, and Niane Sivongxay. — Vietnam, Nghe An Province, Con Cuong District, Pu Mat National Park: FMNH 255604–05 (two adult females), 18.9333 N, 104.7500 E, 300 m elevation, coll. 8 September 1998 by Bryan L. Stuart; ROM 27213–15, ROM 27217, ROM 27229, ROM 27232, ROM 27236–37, ROM 27246 (nine adult males), ROM 27231, ROM 27233–34, ROM 27239–41, ROM 27243–44, ROM 27247 (nine adult females), 18.93833°N, 104.88361°E, 308 m elev., coll. 5–6 June 1995 by Robert W. Murphy et al.; ROM 41614 (one adult male), 18.94167°N, 104.80972°E, 308 m elev., coll. 24 October 1994 by Robert W. Murphy et al. — Vietnam, Nghe An Province, Que Phong District, Pu Hoat Proposed Nature Reserve: NCSM 79685–86 (two adult males), 19.70865°N, 104.74938°E, 834 m elev., coll. 10 June 2010 by Jodi J. L. Rowley, Trung Tien Cao, Vinh Quang Dau, Huong Thu Phung, Tuan Ngoc Le, Luong Thi Nguyen, Trung Danh Hoang, and Thang Thanh Le. — Vietnam, Nghe An Province, Tuong Duong District,

Pu Mat National Park: FMNH 255608 (one adult male), FMNH 255606–07 (two adult females), 19.05000°N, 104.61667°E, 600 m elev., coll. 13 September 1998 by Bryan L. Stuart.

**Referred larvae.** Laos, Xaysomboun Province, Longhaeng District: NCSM 79982 (sequenced; nine larvae), 19.09100°N, 102.89607°E, 885 m elev., coll. 16 May 2012 by Bryan L. Stuart, Somphouthone Phimmachak, and Niane Sivongxay. — Laos, Xieng Khouang Province, Phoukout District: NCSM 80179 (two larvae), 19.48167 N, 102.71222 E, 884 m elevation, coll. 27 February 2012 by Maurice Kottelat. — Vietnam, Nghe An Province, Con Cuong District, Pu Mat National Park: AMS R 171526 (sequenced; two larvae), 18.80028°N, 104.84694°E, 647 m elev., coll. 20 June 2008 by Jodi J. L. Rowley, Trung Tien Cao, Vinh Quang Dau, Thu Thi Le, Son Van Chu, and Toan Van Do. — Vietnam, Nghe An Province, Que Phong District, Pu Hoat Proposed Nature Reserve: AMS R 176252 (sequenced; four larvae), 19.69859°N, 104.73171°E, 1352 m elevation, 7 June 2010 by Jodi J. L.

Rowley, Trung Tien Cao, Vinh Quang Dau, Huong Thu Phung, Tuan Ngoc Le, Luong Thi Nguyen, Trung Danh Hoang, and Thang Thanh Le.

**Etymology.** The specific epithet is a matronym for Tan Fui Lian, Malaysian herpetologist, illustrator of the holotype of *A. cremnobatus* (figure 1 of Inger and Kottelat 1998), and wife of the late Robert F. Inger who led the description of *A. cremnobatus*. Fui Lian's scientific contributions and her generous hospitality to us and innumerable visitors to the Field Museum of Natural History have had immeasurable positive impacts on Southeast Asian herpetology.

**Suggested Common Names.** Fui Lian's Lao torrent frog (English), ຂຽດເກາະຜາລາວຟຸຍໝູນ (Khiat Korpha Lao Fui Lian; Lao).

**Diagnosis.** A member of the *Amolops larutensis* group having the combination of 3–4 vomerine teeth reliably present; relative finger lengths I<II<IV<III; mean  $\pm$  SE SVL of adult males  $32.2 \pm 1.5$  mm (range 28.0–35.3 mm; n = 51) and of adult females  $40.0 \pm 1.8$  (range 35.0–43.1; n = 47); tadpoles with glands near groin in individuals above S30; tadpoles with BL  $15.9 \pm 2.8$  mm (range 12.9–21.5 mm); and tadpoles with BH  $5.9 \pm 1.1$  mm (range 4.6–8.0; n = 16).

**Description of holotype.** Habitus moderately slender. Head width approximately equal to head length. Snout weakly pointed in dorsal view. Snout projecting slightly beyond lower jaw in lateral view, sloping obliquely back to lip. No humeral glands apparent. Nostril lateral, nearer to tip of snout than to eye. Canthus rostralis distinct. Lores oblique and slightly concave. Eye diameter roughly equal to snout. Pineal gland absent. Tympanum distinct, round, less than 1/2 of eye diameter, with rim slightly elevated. Rictal glands small, slightly oval. Vomerine teeth closer to each other than to choanae and obliquely angled. Distance between vomerine teeth approximately equal to tooth row width; 3–4 teeth on each side. Tongue cordiform, notched posteriorly. Vocal sac opening. No gular pouch, but some extra skin at corners of lower jaw is present.

Forelimbs moderately slender. Tips of all four fingers expanded into wide discs, with the disc on Finger I notably smaller than discs on the other fingers. Fingers slender. Relative finger lengths I<II<IV<III. No webbing on hands but very minimal basal webbing on outer fingers present. No skin fringe on arms. Subarticular tubercles conspicuous, surfaces rounded, formula 1, 1, 2, 2. One supernumerary tubercle at base of fingers 2–4. Irregular palmar tubercle present. Velvety, well-developed nuptial pads present on dorsal surface of Finger I to distal edge of subarticular tubercle.

Hindlimbs moderately long and slender. Toes slender. Tips of toes expanded into discs with circummarginal grooves. Disc on outermost toe narrower than those on other toes, and barely wider than the toe. Toes fully webbed to base of disc. Thin flap of skin along inner edge of Toe I and outer edge of Toe V. Subarticular tubercles

conspicuous, surfaces rounded; formula 1, 1, 2, 2, 2. Inner metatarsal tubercle oval, outer metatarsal tubercle small, round.

Dorsal skin surface with spinose glands and glandular dorsolateral fold. Flanks glandular with few spinose glands just beneath dorsolateral fold. Spinose glands behind tympanum extending to top of arm. Vent glandular.

Measurements of holotype (mm): SVL 33.5, HDL 11.8, HDW 11.6, SNT 4.6, EYE 5.0, IOD 3.5, IND 3.9, TMP 1.9, TEY 0.8, SHK 20.0, TGH 18.6, HND 10.7, FTL 16.9.

**Coloration.** In preservative, dorsal coloration dark brown with beige mottling. Dorsal surface of thighs and arms with pale bars on dark brown background. Posterior surface of thighs smooth (not glandular), dark brown with variegated beige pattern. Ventral surface uniformly creamy beige across chin, chest, belly, and underside of arms and legs. Ventral surfaces of feet dark brown, and ventral surfaces of hands dark beige.

In life, from photo of specimen prior to preservation (Fig. 7), dorsal surfaces greenish-brown with green mottling. Dorsal surface of thighs and arms with gray bars on greenish brown background. Eye mottled gray on bottom, mottled yellow on top, and orange at anterior and posterior one-quarter. Posterior surface of thighs smooth (not glandular), but with glands near vent extending ventrally and distally. Posterior surface of thighs mainly black with gray mottling, lacking the greenish tinge present on remaining dorsal surfaces. Ventral surface bright white on chin, chest, and belly, with underside of arms and legs transparent. Ventral surfaces of feet dark brown to black, and ventral surfaces of hands dark brown.

**Larvae.** Based on a larva at S34 from series NCSM 79982, which includes tadpoles of S25–40, that are assigned to this species based on molecular data (Table S2; Fig. 2). Tadpoles of *A. tanfuilianae* **sp. nov.** belong to the exotrophic, lotic, gastromyzophorus larval type (McDiarmid and Altig 1999), with large oral discs ventrally.

HB oval, broadly rounded, wider anteriorly than posteriorly, with weak constriction near line through nares. HB width 57–64% of HBL, widest anterior to eyes. HB flat below with large abdominal sucker. Eyes dorsolateral, pointed laterad. Nares dorsolateral, closer to eyes than tip of snout. Spiracle low on side, tube free of body wall. Tail lanceolate, margins tapering in distal third to narrowly rounded tip; muscle deeper than fins in proximal half. Dorsal fin origin behind HB, origin of ventral fin distal to origin of dorsal fin. HB without spinules. Glands postocular, no glands in fins. Distinct glands ventrally near groin in tadpoles  $\geq$ S32.

Oral disc nearly as wide as HB, ventral; labial teeth 9–10(5–9; 5–10)/6(1). A1 at margin of upper lip, short, approximately one-third length of A2, with very small denticles compared to other tooth rows. Papillae short, thick, in single row, absent from middle third of upper lip, but present across entire lower lip. Jaw sheaths with outer surface smooth, upper sheath divided, gap between





**Figure 8.** Tadpoles in preservative, showing dorsal (left) and ventral (right) views. **A** *Amolops tanfuiliana* **sp. nov.**, NCSM 79982; **B** *A. cremnobatus*, FMNH 252863; **C** *A. kottelati* **sp. nov.**, NCSM 87612; **D** *A. sengae* **sp. nov.** NUOL 01596.

black halves about equal to depth of keratinized portions; lower jaw sheath in single piece. Jaw sheaths with fine serrae.

Color in preservative of HB brown dorsally and laterally, white ventrally without spots. Caudal muscle brown, with increasing amounts of lighter mottling distally,

and white stripe ventrally. Upper fin with melanophores throughout, and lower fin with melanophores in distal half.

**Sexual dimorphism.** Males ( $n = 51$ ) differ from females ( $n = 47$ ) in being smaller ( $32.2 \pm 1.5$  mm SVL vs  $40.0 \pm 1.8$  mm; Table 1), and in having paired vocal slits and distinct nuptial pads at base of first finger.

**Variation.** Pineal gland sometimes present. Distance between vomerine teeth varies from almost nothing to about the width of one tooth row apart. Males have spinose glands dorsally, but sometimes only weakly spinose (NCSM 81003), and females are glandular but not usually spinose, though occasionally females will be spinose or weakly spinose (NCSM 79971 and 80134, NUOL 00033, 00116, 00118, 00559, 00563). Glandular dorsolateral fold sometimes very faint. When spinose glands are present on the flanks, they are restricted to the upper third to quarter of the flank. Spinose glands behind tympanum extending to top of arm present in all females, and present but sometimes weak in males. Dorsal coloration ranges from dark brown to black with beige mottling. All individuals have more dark than light patches, and almost no individuals have thick beige patches. Ventral surfaces may be entirely beige or may be beige under thighs and arms but creamy white on chin, chest, and belly. Chin and chest may lack dark markings, or may have some dark stippled reticulations, but the entire ventral surface is never dark or marked.

**Distribution and natural history.** This species is known from clear streams with torrents in Luang Phabang, Xieng Khouang and Xaysomboun Provinces of northern Laos and Nghe An and Thanh Hoa Provinces of northern Vietnam.

**Comparisons.** *Amolops tanfuiianae* **sp. nov.** differs from *A. cremnobatus*, *A. sengae* **sp. nov.**, and *A. attiguus* **sp. nov.** by having a larger number (3–4) of vomerine teeth (2–3 in *A. cremnobatus*, *A. sengae* **sp. nov.**, and *A. attiguus* **sp. nov.**) that are reliably present (faint or sometimes absent in *A. cremnobatus*, *A. sengae* **sp. nov.**, and *A. attiguus* **sp. nov.**); and females with larger SVL (Table 1). *Amolops tanfuiianae* **sp. nov.** differs from *A. kottelati* **sp. nov.**, *A. sengae* **sp. nov.**, and *A. attiguus* **sp. nov.** by having relative finger lengths  $I < II < IV < III$  ( $I < IV < II < III$  in *A. kottelati* **sp. nov.**, *A. sengae* **sp. nov.**, and *A. attiguus* **sp. nov.**). *Amolops tanfuiianae* **sp. nov.** further differs from *A. sengae* **sp. nov.** by having spinose glands above the arm usually present (absent in *A. sengae* **sp. nov.**). *Amolops tanfuiianae* **sp. nov.** further differs from *A. cremnobatus*, *A. sengae* **sp. nov.**, and *A. kottelati* **sp. nov.** by having tadpoles with relatively smaller BH (ANOVA  $F = 4.16$ ,  $df = 3$ ,  $P = 0.02$ ; Table 2). *Amolops tanfuiianae* **sp. nov.** further differs from *A. kottelati* **sp. nov.** by having tadpoles with smaller BL (Table 2). *Amolops tanfuiianae* **sp. nov.** further differs from *A. cremnobatus* by having tadpoles with opaque glands on the ventral surface near the vent, proximal to the legs, in tadpoles at S32 or greater (Inger and Kottelat 1998; Fig. 8).

## *Amolops kottelati* **sp. nov.**

<https://zoobank.org/69F194E1-959A-4F0C-A357-A88C-913CA88E>

Figure 9

“Clade D”

**Holotype.** NUOL 00556 (field number SP 00745), adult male, Laos, Xaysomboun Province, Hom District, Ban Nam Yuak, Houay Kator,  $18.72669^{\circ}\text{N}$ ,  $103.35481^{\circ}\text{E}$ , 405 m elev., coll. 1932 h on a rock 0.5 m above a stream torrent on 26 June 2015 by Somphouthone Phimmachak and Sengvilay Seateun.

**Paratypes.** Laos, Bolikhamxay Province, Thaphabhat District, Phou Khao Khouay National Protected Area, Tad Leuk: NCSM 79617 (one adult male),  $18.23727^{\circ}\text{N}$ ,  $103.04321^{\circ}\text{E}$ , 214 m elev., coll. 9 May 2012 by Sengvilay Seateun, Misan Keooudone, and Poklavanh Khounthavong. — Laos, Xaysomboun Province, Hom District: NCSM 86766–67, NUOL 00557 (three adult females), same data as holotype; NUOL 00555 (one adult female), Ban Nong (=Ban Sob Youak), Houay Tadmud,  $18.64285^{\circ}\text{N}$ ,  $103.46059^{\circ}\text{E}$ , 987 m elev., coll. 23 June 2015 by Somphouthone Phimmachak and Sengvilay Seateun.

**Referred larvae.** Laos, Xaysomboun Province, Hom District: NCSM 87612 (three larvae), Houay Kolong, first stream crossing road from Ban Houaysey to Ban Nam Yuak,  $18.72556^{\circ}\text{N}$ ,  $103.35556^{\circ}\text{E}$ , 415 m elev., coll. 14 February 2014 by Maurice Kottelat.

**Etymology.** The specific epithet is a patronym for Maurice Kottelat, Swiss ichthyologist and specialist of the Indochinese fish fauna, collector and co-describer of the types of *A. cremnobatus*, and collector of the larvae of the new species. Kottelat’s larval collections proved invaluable for the present study, exemplified by *A. kottelati* **sp. nov.** being most readily diagnosed by its larval characters.

**Suggested Common Names.** Kottelat’s Lao torrent frog (English); ຂຽດເກາະຟາລາວກົດເຕີລັດ (Khat Korpha Lao Kottelat; Lao).

**Diagnosis.** A member of the *Amolops larutensis* group having the combination of 3–4 vomerine teeth reliably present; relative finger lengths  $I < IV < II < III$ ; mean  $\pm$  SE SVL of adult males  $33.3\text{--}35.0$  mm ( $n = 2$ ) and of adult females  $39.0 \pm 1.8$  (range  $38.0\text{--}41.7$  mm;  $n = 4$ ); and tadpoles with BL  $17.0 \pm 0.2$  mm (range  $16.8\text{--}17.1$  mm;  $n = 3$ ).

**Description of holotype.** Habitus moderately slender. Head width slightly greater than head length. Snout weakly pointed in dorsal view, projecting slightly beyond lower jaw in lateral view, sloping obliquely back to lip. Nostril lateral, nearer to tip of snout than to eye. Canthus rostralis distinct. Lores oblique and slightly concave. Eye



**Figure 9.** *Amolops kottelati* sp. nov., NUOL 00556, in preservative.

diameter subequal to snout length. Pineal body absent. Tympanum distinct, round, greater than one-third diameter of eye, slightly depressed relative to skin of temporal region, tympanic rim elevated relative to tympanum. Ricinal glands small and oval, but very indistinct. Vomerine teeth ( $n = 4$ ) obliquely angled, closer to each other than to choanae. Tongue cordiform, notched posteriorly. Vocal slit opening near corner of jaw very small and hard to find. No gular pouch but some extra skin at corner of jaw.

Forelimbs moderately slender. Tips of all four fingers expanded into wide discs with circummarginal grooves. Fingers slender. Relative finger lengths  $I < IV < II < III$ . No webbing on hands other than very minimal basal webbing on outer fingers. Subarticular tubercles conspicuous, surfaces rounded, formula 1, 1, 2, 2. One supernumerary tubercle at base of fingers 2–4. Irregular palmar tubercle. Velvety well-developed nuptial pads on dorsal surface of Finger I, to level of distal end of subarticular tubercle.

Hindlimbs moderately long and slender. Toes slender. Tips of toes expanded into discs, with circummarginal

grooves. Outermost toe disc barely wider than toe. Web on all toes to base of disc. Subarticular tubercles conspicuous, surfaces rounded, formula 1, 1, 2, 2. Disc of longest toe narrower than discs of all fingers except thumb.

Skin with fine spinose glands dorsally. No supra-tympanic fold. Glandular dorsolateral fold. Flanks with small spinose glands on upper one-third to one-quarter.

Measurements of holotype (mm): SVL 35.0, HDL 10.9, HDW 11.8, SNT 5.0, EYE 4.8, IOD 3.2, IND 3.7, TMP 1.8, TEY 0.9, SHK 19.9, TGH 18.0, HND 10.0, FTL 17.2.

**Coloration.** In preservative, dorsal coloration dark brown with beige markings. Dorsal surface of thighs and arms with pale beige bars on dark brown background. Posterior surface of thighs smooth, with light beige mottling on dark brown background. Ventral surfaces of chin, chest, belly, and underside of thighs and arms uniformly creamy beige. Ventral surfaces of feet are dark gray-brown, and ventral surfaces of hands are dark beige.



In life (photograph of NUOL 00555), dorsal surfaces greenish-brown with green mottling. Dorsal surfaces of thighs with green bars on dark green background. Eye mottled gray on bottom one-third, and mottled orange on remaining area. No photographs available of posterior surface of thighs, or ventral surface.

**Larvae.** Based on a larva at S29 from the series NCSM 87612, which includes tadpoles between S25–29. Tadpoles are assigned to this species because they were collected at the type locality and their morphology largely agrees with the tadpole description of *A. cremnobatus* by Inger and Kottelat (1998), including a divided upper jaw sheath and high number of labial tooth rows [9–10(5–9, 5–10)/6(1)]. Tadpoles of *A. kottelati* **sp. nov.** belong to the exotrophic, lotic, gastromyzophorus larval type (McDiarmid and Altig 1999), with large oral discs ventrally.

HB oval, broadly rounded, wider anteriorly than dorsally, with weak constriction near line through nares. HB width 63–65% of HBL, widest anterior to eyes. HB flat below with large abdominal sucker. Eyes dorsolateral, pointed laterad. Nares dorsolateral, closer to eyes than tip of snout. Spiracle low on side, tube free of body wall. Tail lanceolate, margins tapering in distal third to narrowly rounded tip; muscle deeper than fins in proximal half. Dorsal fin origin behind HB, origin of ventral fin distal to origin of dorsal fin. HB without spinules. Glands postocular, no glands in fins.

Oral disc nearly as wide as HB, ventral; labial teeth 10(5–10)/6(1). A1 at margin of upper lip, short, approximately one-third length of A2, with very small denticles compared to other tooth rows. Papillae short, thick, in single row, absent from middle third of upper lip, but present across entire lower lip. Jaw sheaths with outer surface smooth, upper sheath divided, gap between black halves slightly greater than depth of keratinized portions; lower jaw sheath in single piece. Jaw sheaths with fine serrae.

Color in preservative of HB brown dorsally and laterally, cream ventrally without spots. Caudal muscle brown dorsally and laterally, cream ventrally. Upper fin with melanophores throughout, and lower fin with melanophores in distal half.

**Sexual dimorphism.** Males ( $n = 2$ ) differ from females ( $n = 4$ ) in being smaller (33.3–35.0 mm SVL vs  $39.0 \pm 1.8$  mm SVL; Table 1), and in having paired vocal slits and distinct nuptial pads at base of first finger.

**Variation.** Pineal body sometimes present (NUOL 00555), but often absent. Rictal glands small and indistinct in nearly all individuals. Oval thenar tubercle present in females. Skin of males with fine spinose glands dorsally, while dorsal skin of females is glandular but not spinose. Flanks of males have small spinose glands, and flanks of females are glandular but not spinose. All females except NUOL 00557 with spinose glands above arm, posterior to tympanum. Female NUOL 00555 is very dark compared to the others with much less of the pale mottling dorsally, and all individuals except this one

have uniformly cream chin, chest, belly, and underside of thighs and arms. NUOL 00555 has dark stippling on a beige background on the chin and chest, becoming less stippled on the belly, ventral surface of legs, and arms.

**Distribution and natural history.** This species is known from clear streams with torrents in western Bolikhamxay and eastern Xaysomboun Provinces of northern Laos.

**Comparisons.** *Amolops kottelati* **sp. nov.** differs from *A. cremnobatus*, *A. sengae* **sp. nov.**, and *A. attiguus* **sp. nov.** by having a larger number (3–4) of vomerine teeth (2–3 in *A. cremnobatus*, *A. sengae* **sp. nov.**, and *A. attiguus* **sp. nov.**) that are reliably present (faint or sometimes absent in *A. cremnobatus*, *A. sengae* **sp. nov.**, and *A. attiguus* **sp. nov.**). *Amolops kottelati* **sp. nov.** further differs from *A. cremnobatus* and *A. tanfuiianae* **sp. nov.** by having relative finger lengths  $I < IV < II < III$  ( $I < II < IV < III$  in *A. cremnobatus* and *A. tanfuiianae* **sp. nov.**). *Amolops kottelati* **sp. nov.** further differs from *A. sengae* **sp. nov.** by having spinose glands above the arm usually present (absent in *A. sengae* **sp. nov.**). *Amolops kottelati* **sp. nov.** differs from *A. tanfuiianae* **sp. nov.** and further differs from *A. cremnobatus*, and *A. sengae* **sp. nov.** by having larvae with much larger BL (Table 2).

### *Amolops sengae* **sp. nov.**

<https://zoobank.org/D79CD056-AD9C-4478-A53C-78A0B-22CC3B5>

Figure 10

“Clade C”

**Chresonymy.** *Amolops cremnobatus* Stuart (2005: 476, part), Stuart et al. (2010: 57), Stuart et al. (2013: 102), Wu et al. (2020: 5, part).

**Holotype.** NCSM 79424 (field number BLS 15273), adult male, Laos, Xaignabouli Province, Paklay District, Pak Maat, 18.82588°N, 101.84090°E, 242 m elev., coll. 1942h on wet rock face above chute in 1–2 m wide steep rocky stream in disturbed semi-evergreen forest on 25 March 2012 by Bryan L. Stuart, Sengvilay Seateun, and Khampong Thanonkeo.

**Paratypes.** Laos, Vientiane Province, Kasi District, Ban Namon: FMNH 258376–77 (two adult females), 19.07750°N, 102.14556°E, 200–300 m elev., coll. 1 September 1999 by Michael Baltzer. — Laos, Xaignabouli Province, Paklay District, Pak Maat: NCSM 79417, NCSM 79422–23, NUOL 00032 (four adult males), same data as holotype.

**Referred larvae.** Laos, Vientiane Province, Feuang District, Ban Naxeng, Houay Kang Thang: NUOL 01596 (five larvae), 18.86951°N, 102.12005°E, 410 m elev., coll. 17 December 2014 by Fongfany Libounyasao.



**Figure 10.** *Amolops sengae* sp. nov., NCSM 79417, in life (top row), and NCSM 79424 in preservative (bottom row).

**Etymology.** The specific epithet is a matronym for Sengvilay (“Seng”) Seateun of the National University of Laos, co-collector of much of the new material described here, major contributor to the herpetology of Laos, native of Xaignabouli Province that is home to the type locality of the new species, and cherished friend and colleague of the authors.

**Suggested Common Names.** Seng’s Lao torrent frog (English), ຂງຄເກາະຜາລາວແສງ (Khiat Korpha Lao Seng; Lao).

**Diagnosis.** A member of the *Amolops larutensis* group having the combination of 2–3 vomerine teeth, sometimes faint or absent; relative finger lengths  $I < IV < II < III$ ; spinose glands above the arm absent; mean  $\pm$  SE SVL of adult males  $29.8 \pm 1.5$  mm (range 27.2–31.2 mm;  $n = 5$ ) and of females  $39.2\text{--}39.6$  mm ( $n = 2$ ); SNT  $4.9 \pm 0.3$  mm (range 4.4–5.2 mm) in males and 5.2–5.8 mm in females; FTL  $14.3 \pm 0.7$  mm (range 13.1–15.1 mm) in males and 18.4–19.1 mm in females; HND  $8.7 \pm 0.9$  mm (range 7.3–9.6 mm) in males and 11.8–12.0 mm in females; and SHK  $17.7 \pm 0.9$  mm (range 16.2–18.4 mm) in males and 24.0–24.5 mm in females.

**Description of holotype.** Habitus moderately slender. Head width approximately equal to head length. Snout weakly pointed in dorsal view. Snout projecting slightly beyond lower jaw in lateral view, sloping obliquely back to lip. Nostril lateral, nearer to tip of snout than to eye. Canthus rostralis distinct. Lores oblique and slightly concave. Eye diameter sub-equal to snout. Pineal body present. Tympanum distinct, round, roughly one-third eye diameter, slightly depressed relative to skin of temporal re-

gion. Rictal glands very small and round. Vomerine teeth obliquely angled, closer to each other than to choanae, and extremely small and indistinct. Tongue cordiform notched posteriorly. Vocal slit opening near corner of jaw. No gular pouch, but extra skin at corners of lower jaw.

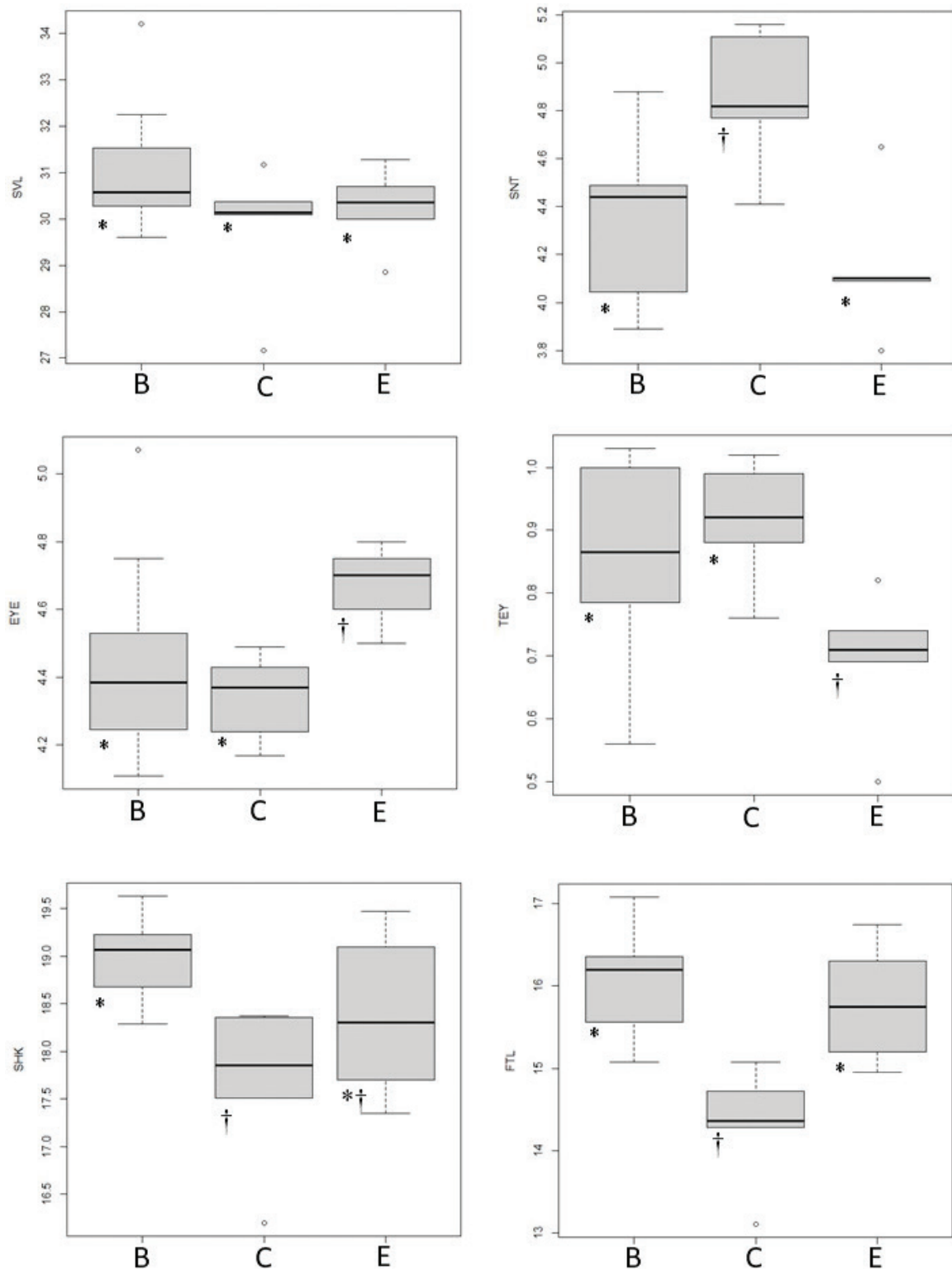
Forelimb moderately slender. Tips of all four fingers expanded into wide discs, with circummarginal grooves. Fingers slender. Relative finger lengths  $I < IV < II < III$ . No webbing on hands. Subarticular tubercles conspicuous, surfaces rounded, formula 1, 1, 2, 2. One supernumary tubercle at base of fingers 2–4. Irregular palmar tubercle. Well-developed nuptial pad on Finger I, on dorsal surface to level of distal end of subarticular tubercle.

Hindlimbs moderately long and slender. Toes slender. Tips of toes expanded into wide discs, with circummarginal grooves. Web on all toes to base of disc. Outermost toe disc small, barely wider than finger. Widest toe disc narrower than all finger discs except for that of thumb. Subarticular tubercles conspicuous, surfaces rounded; formula 1, 1, 2, 2. Inner metatarsal tubercle oval; outer metatarsal tubercle small, round.

Dorsal skin surface glandular and spinose. No supratympanic fold. Glandular dorsolateral fold. Flanks with spinose glands at dorsal edge, but no spinose glands above arm. Glands surrounding vent. Ventral surface slightly glandular.

Measurements of holotype (mm): SVL 30.1, HDL 10.7, HDW 10.9, SNT 4.8, EYE 4.5, IOD 3.0, IND 3.4, TMP 1.6, TEY 0.8, SHK 17.5, TGH 16.5, HND 8.7, FTL 14.4.

**Coloration.** In preservative, dorsal coloration very dark brown-black with minimal beige spotting. Pale bars on dorsal surface of thighs and some pale spots on arms, but no distinct bars. Posterior surface of thighs smooth and



**Figure 11.** Box and whisker plots of measurements (mm) of morphological features of males of *Amolops cremnobatus* (B; n = 12), *A. sengae* sp. nov. (C; n = 5), and *A. attiguis* sp. nov. (E; n = 5). Measurements are snout-vent length (SVL), snout length (SNT), eye diameter (EYE), tympanum-eye distance (TEY), tarsus length (SHK), and foot length (FTL). Boxes show interquartile range and median (thick black line). Whiskers represent range up to 1.5 × interquartile range (IQR); dots show range beyond 1.5 × IQR. Clusters with the same symbol (\*) and †) are not significantly different, as determined by paired two-tailed t-tests following ANOVA.

dark with some light beige coloration. Ventral surfaces creamy beige, but with some dark veining under chin and along posterior margin of chin. Ventral surface of feet are dark brown to black, and ventral surfaces of hands are dark beige to brown.

In life, dorsal surface greenish-brown with bright green markings. Dorsal surface of legs paler in color than dorsum. Eye mottled gray on lower one-third, mottled yellowish on upper one-third, with orange on anterior and posterior portions. No ventral photos exist for this



species in life, but ventral surface of recently euthanized NCSM 79417 shows chin and belly to be bright white with some dark mottling, and underside of arms and thighs to be translucent.

**Larvae.** Based on a larva NUOL 01596.2 at S28. Tadpoles are assigned to this species because they were collected at the type locality and their morphology largely agrees with the tadpole description of *A. cremnobatus* by Inger and Kottelat (1998), including a divided upper jaw sheath and high number of labial tooth rows [9–10(5–9, 5–10)/6(1)]. Tadpoles of *A. sengae* **sp. nov.** belong to the exotrophic, lotic, gastromyzophorus larval type (McDiarmid and Altig 1999), with large oral discs ventrally.

Head-body oval, broadly rounded, wider anteriorly than posteriorly, with weak constriction near line through nares. Body width approximately 60% of BL, widest anterior to eyes. HB flat below with large abdominal sucker. Eyes dorsolateral, pointed laterad. Nares dorsolateral, closer to eyes than tip of snout. Spiracle low on side, tube free of body wall. Tail lanceolate, margins tapering in distal third to narrowly rounded tip; muscle deeper than fins in proximal half. Dorsal fin origin behind HB, origin of ventral fin distal to origin of dorsal fin. HB without spinules. Glands postocular, no glands in fins. Oral disc nearly as wide as head-body, ventral; labial teeth 9–10(4 or 5–10)/6(1). A1 at margin of upper lip, short, approximately one-half length of A2, with very small denticles compared to other tooth rows. A2 approximately two-thirds length of A3. Papillae short, thick, in single row, absent from middle third of upper lip, but present across entire lower lip. Jaw sheaths with outer surface smooth, upper sheath divided, gap between black halves slightly greater than depth of keratinized portions; lower jaw sheath in single piece. Jaw sheaths with fine serrae.

Color in preservative of head-body brown dorsally and laterally, cream ventrally without spots. Caudal muscle brown dorsally and laterally, cream ventrally. Upper fin with melanophores throughout, and lower fin with melanophores in distal half to two-thirds.

**Sexual dimorphism.** Males ( $n = 5$ ) differ from females ( $n = 2$ ) in being smaller ( $29.8 \pm 1.5$  mm SVL vs  $39.2$ – $39.6$  mm; Table 1), possessing an obvious pineal body (indistinct in females), as well as having paired vocal slits and distinct nuptial pads at base of first finger. Dorsal coloration very dark in males, mostly black with very minimal beige spotting or mottling. Females dark brown with beige mottling/reticulation.

**Variation.** Pineal body sometimes absent, sometimes present, and seems to be more obvious in males than in females. Rictal glands indistinct in one female (FMNH 258377). Roof of mouth of females is much rougher than that of males. Outer metatarsal tubercle nearly indistinguishable on one female (FMNH 258377). No webbing on hands, but in some males there appears to be very minimal basal webbing. Oval thenar tubercle in females. Outermost toe disc extremely small in all males (about the width of the toe itself) but slightly wider in females.

Outer metatarsal tubercle much more difficult to see on FMNH 258377. Dorsal skin of two males (NCSM 79417 and 79422) as well as females glandular but not spinose. Flanks with spinose glands at dorsal edge in individuals that have spinose glands dorsally, all others have glandular skin on flanks without spines. Females dark brown with beige mottling/reticulation. Rear of thighs of females with irregular pale mottling. Females and most males have some dark mottling under chin, and most males have dark mottling on chest, but male NCSM 79422 lacks any dark spots/markings on chin, chest, or belly.

**Distribution and natural history.** This species is known from clear streams with torrents from Vientiane Province, Laos westward across the Mekong River into Xaignabouli Province, Laos. A population from Nan Province, Thailand, is provisionally referred to this species.

**Comparisons.** *Amolops sengae* **sp. nov.** differs from *A. cremnobatus*, *A. tanfuiianae* **sp. nov.**, *A. kottelati* **sp. nov.**, and *A. attiguus* **sp. nov.** by lacking spinose glands above the arm (usually present in *A. cremnobatus*, *A. tanfuiianae* **sp. nov.**, *A. kottelati* **sp. nov.**, and *A. attiguus* **sp. nov.**). *Amolops sengae* **sp. nov.** further differs from *A. tanfuiianae* **sp. nov.** and *A. kottelati* **sp. nov.** by having a smaller number (2–3) of vomerine teeth (3–4 in *A. tanfuiianae* **sp. nov.** and *A. kottelati* **sp. nov.**). *Amolops sengae* **sp. nov.** further differs from *A. tanfuiianae* **sp. nov.** by having males with smaller SVL of  $29.8 \pm 1.5$  ( $32.2 \pm 1.5$  in *A. tanfuiianae* **sp. nov.**; t-stat 3.3, two-tailed p-value = 0.02). *Amolops sengae* **sp. nov.** further differs from *A. cremnobatus* and *A. tanfuiianae* **sp. nov.** by having relative finger lengths I<IV<II<III (I<II<IV<III in *A. cremnobatus* and *A. tanfuiianae* **sp. nov.**). *Amolops sengae* **sp. nov.** further differs from *A. cremnobatus* and *A. attiguus* **sp. nov.** by having larger SNT and smaller FTL, and from *A. cremnobatus* by having smaller SHK (Table 3; Fig. 11). *Amolops sengae* **sp. nov.** further differs from *A. tanfuiianae* **sp. nov.** and *A. kottelati* **sp. nov.** by having tadpoles with smaller BL. *Amolops sengae* **sp. nov.** further differs from *A. cremnobatus* by having tadpoles with smaller relative ODW and larger relative IP and RND (Table 2).

### *Amolops attiguus* **sp. nov.**

<https://zoobank.org/27659B24-8B32-4D0A-B0B9-AECBEF-B954AC>

Figure 12

“Clade E”

**Holotype.** NCSM 80907 (field number BLS 15852), adult male, Laos, Bolikhamxay Province, Viengthong District, Nam Kading National Protected Area, Nam Xouang,  $18.42676^{\circ}\text{N}$ ,  $104.39136^{\circ}\text{E}$ , 343 m elev., coll. 2200 h on wet rock face next to series of waterfalls over exposed sandstone bedrock with potholes on 1 March



**Figure 12.** *Amolops attiguus* sp. nov. NCSM 80907 in life (top row) and in preservative (bottom row).

2013 by Bryan L. Stuart, Jennifer A. Sheridan, Sengvilay Seateun, and Niane Sivongxay.

**Paratypes.** Laos, Xieng Khouang, Mork District, tributary of Nam Mork: NCSM 80761 (one adult male), 19.07075°N, 103.95870°E, 454 m elev., coll. 12 April 2012 by Somphouthone Phimmachak. — Vietnam, Nghe An Province, Anh Son District, Pu Mat National Park, Cao Ven: AMNH A 191846 (one adult male), 18.81050°N, 104.95150°E, 273 m elev., coll. 16 May 2010 by David A. Kizirian, Truong Quang Nguyen, and Sinh Van Nguyen; AMNH A 191848 (one adult male), AMNH A 191849, NCSM 79166 (two adult females), coll. 18 May 2010 by David A. Kizirian, Truong Quang Nguyen, and Sinh Van Nguyen.

**Etymology.** The specific epithet taken from *attigua* (L.) for neighboring or adjacent, in reference to the new species' nested geographic distribution between its morphologically similar relatives *A. tanfuiiana* sp. nov. and *A. kottelati* sp. nov. (Fig. 1).

**Suggested Common Names.** Similar Lao torrent frog (English), ຂຸງດູກະຜາລາວແອດທິກຸສ (Khiat Korpha Lao Sumphan; Lao).

**Diagnosis.** A member of the *Amolops larutensis* group having the combination of 2–3 vomerine teeth, sometimes faint or absent; relative finger lengths I < IV < II < III; mean  $\pm$  SE SVL of adult males  $30.2 \pm 0.9$  mm (range 28.9–31.3 mm;  $n = 5$ ) and of females 39.2–39.4 mm ( $n = 2$ ); EYE  $4.7 \pm 0.3$  mm (range 4.1–4.8 mm) in males and 5.3–6.1 mm in females; TEY  $0.7 \pm 0.1$  mm (range 0.5–0.9 mm) in males and 1.1–1.3 mm in females; SNT  $4.1 \pm 0.1$  mm (range 4.5–4.7 mm) in males and 5.4–5.5 mm in females; and FTL  $15.8 \pm 0.7$  mm (range 15.0–16.8 mm) in males and 18.6–18.7 mm in females.

**Description of the holotype.** Habitus moderately slender. Head length subequal to head width. Snout weakly pointed in dorsal view. Snout projecting slightly beyond lower jaw in lateral view, sloping obliquely back to lip. Nostril lateral, nearer to tip of snout than to eye. Canthus rostralis distinct. Lores obliquely and slightly concave. Eye diameter approximately equal to snout. No pineal body visible. Tympanum distinct, round, approximately one-third of eye diameter, slightly depressed relative to skin of temporal region, tympanic rim elevated relative to tympanum. Rictal glands slightly oval and pronounced. Vomerine teeth obliquely angled, closer to each other than to choanae, and extremely small, with 2–3 teeth each. Tongue cordiform, notched posteriorly. Vocal slit opening near corner of jaw. No gular pouch, but with some extra skin at edge of jaw.

Forelimb moderately slender. Tips of all four fingers expanded into wide discs with circummarginal grooves. Fingers slender. Relative finger lengths I < IV < II < III. Minimal basal webbing on hands. Subarticular tubercles conspicuous, surfaces rounded, formula 1, 1, 2, 2. One supernumerary tubercle at base of fingers 2–4. Irregularly-shaped palmar and oval thenar tubercles present. Well-developed nuptial pad on Finger I, on dorsal surface to level of distal end of subarticular tubercle.

Hindlimbs moderately long and slender. Toes slender. Tips of toes expanded into wide discs with circummarginal grooves. Web on all toes to base of disc. Subarticular tubercles conspicuous, surfaces rounded, formula 1, 1, 2, 2, 2. Inner metatarsal tubercle oval. No outer metatarsal tubercle. Discs of toes smaller than that of finger, but larger than or equal to that of outermost finger. Outermost toe disc slightly wider than toe. Widest toe disc narrower than all finger discs except for that of thumb. Subarticular tubercles conspicuous, surfaces rounded; formula 1, 1, 2, 2, 2. Inner metatarsal tubercle oval; outer metatarsal tubercle barely visible, small, round.

Dorsal surface with weakly spinose glands. No supra-tympanic fold. Glandular dorsolateral fold. Flanks glandular. Spinose glands behind tympanum above arm. Glands around vent. Ventral surfaces weakly granular.

Measurements of holotype (mm): SVL 30.4, HDL 10.3, HDW 11.0, SNT 4.7, EYE 4.6, IOD 3.2, IND 3.6, TMP 1.7, TEY 0.8, SHK 19.1, TGH 17.2, HND 9.7, FTL 16.8.

**Coloration.** In preservative, dorsal coloration black-brown with paler markings. Pale bars on dorsal surface of thighs and arms. Posterior surface of thighs with irregular pale mottling or reticulation on dark surface. Ventral surfaces cream with very fine dark mottling. Ventral surface of feet dark grey-brown, ventral surface of hands dark beige.

In life (from photos of NCSM 80906, Fig. 1), dorsal surface greenish-grey with bright green markings. Dorsal surface of legs paler in color than dorsum. Eye mottled gray on lower one-third, mottled yellowish on upper one-third, with orange on anterior and posterior portions. No ventral photos exist for this specimen in life, but photos of this species recently euthanized (NCSM 80907, Fig. 12) show chin and chest to be creamy white with dark mottling, belly to be white with very fine mottling, and underside of arms and thighs to be translucent.

**Sexual dimorphism.** Males ( $n = 5$ ) differ from females ( $n = 2$ ) in being smaller ( $30.2 \pm 0.9$  mm SVL vs 39.2–39.4 mm; Table 1), and in possessing paired vocal slits and distinct nuptial pads at base of first finger.

**Variation.** Pineal body distinguishable in NCSM 80761, and clear and obvious in AMNH A 191845, 191846, 191848, and 191849, but not apparent in NCSM 79166. Rictal glands indistinct, bordering on absent in all paratypes. Vomerine teeth totally absent in AMNH A 191847; almost absent in AMNH A 191846 and A 191848; extremely small in NCSM 80761, and a bit more prominent in AMNH A 191845. Females have oval thenar tubercle. Outermost toe disc variable: in some individuals it is barely wider than toe, but in others appears wider.

Male AMNH A 191845 very slightly spinose. Dorsal surface of females glandular but not spinose. Glandular dorsolateral fold weak in females and in AMNH A 191845, 191846, and 191848. Spinose glands behind tympanum above arm, and in NCSM 79166 (female), coming down to skin in front of arm, though this feature is absent in AMNH A 191845, 191846, and 191848. In NCSM 79166, the pale dorsal markings are extremely sparse, giving the frog a dark appearance overall with few markings, while in the others, the dorsal surfaces are much more mottled with the paler markings. Pale bars on dorsal surface of thighs and arms less apparent in NCSM 79166 than in other individuals. Ventral coloration of NCSM 79166 creamy on the belly, and white on chin and chest with dark mottling. AMNH specimens A 191845, 191848, and 191849 have white chin and chest with yellow-cream belly (no dark mottling). AMNH A 19146 has white chin and chest with some dark mottling, and yellow-cream belly. Ventral surfaces of feet are dark brown

to black, and ventral surfaces of hands are dark beige to black.

**Distribution and natural history.** This species is known from clear streams with torrents in eastern Bolikhamxay and Xieng Khouang Provinces of northern Laos and southern Nghe An Province of northern Vietnam. Larvae of the new species remain unknown.

**Comparisons.** *Amolops attiguus* **sp. nov.** differs from *A. tanfuiianae* **sp. nov.** and *A. kottelati* **sp. nov.** by having a smaller number (2–3) of vomerine teeth (3–4 in *A. tanfuiianae* **sp. nov.** and *A. kottelati* **sp. nov.**). *Amolops attiguus* **sp. nov.** differs from *A. cremnobatus* and further differs from *A. tanfuiianae* **sp. nov.** by having relative finger lengths  $I < IV < II < III$  ( $I < II < IV < III$  in *A. cremnobatus* and *A. tanfuiianae* **sp. nov.**). *Amolops attiguus* **sp. nov.** further differs from *A. sengae* **sp. nov.** by having spinose glands above the arm usually present (absent in *A. sengae* **sp. nov.**). *Amolops attiguus* **sp. nov.** further differs from *A. cremnobatus* and *A. sengae* **sp. nov.** by having larger EYE and smaller TEY (Fig. 11). *Amolops attiguus* **sp. nov.** further differs from *A. sengae* **sp. nov.** by having smaller SNT and FTL (Fig. 11).

## Discussion

Our mitochondrial, morphological, and, in part, nuclear data support the hypothesis of Wu et al. (2020) that *A. cremnobatus* consists of more than a single species across its range. Specifically, our data support recognizing at least five species within this taxon, restricting *A. cremnobatus* sensu stricto to the southeastern portion of its former range in eastern Bolikhamxay and Khammouang Provinces, Laos, and Ha Tinh and Quang Binh Provinces, Vietnam. A recently discovered population in Thailand that we provisionally assigned to *A. sengae* **sp. nov.** was available to us only through mt sequences (Wu et al. 2020), but the revealed genetic divergence to Lao populations warrants further study of the Thai population, as it may represent an additional species.

*Amolops larutensis*, the sister taxon of *A. cremnobatus* (as one or five species), was recently partitioned into three species (*A. larutensis*, *A. australis*, and *A. gerutu*) in the Malay Peninsula, also based on corroborating lines of evidence in morphological, mt, and nu data (Chan et al. 2017; Chan et al. 2018), with notably greater representation of nu data than was used here consisting of genome-wide single-nucleotide polymorphisms (Chan et al. 2017). Chan et al. (2018) found considerable overlap in morphological variation among their three species, and therefore advocated for primarily using the tuberculation and pattern on the rear of the thighs to diagnose them morphologically. The morphological variation among their three species was further exacerbated by the findings of strong positive correlations between intraspecific body size and elevation, with populations from higher



elevations being considerably larger than those from lower elevations (Chan et al. 2018). Like *A. larutensis*, the species contained within the former *A. cremnobatus* are morphologically very similar to one another and are not always easily distinguished in the absence of molecular data. To our lament, tuberculation and pattern on the rear of the thigh did not aid our morphological diagnoses of members of the *A. cremnobatus* species complex. We also did not find an association between elevation and body size within *A. tanfuiiana* **sp. nov.**, the species for which we had the largest sample size. However, larval characteristics proved to be very useful in morphologically diagnosing some of the species, a finding that has been demonstrated in other Asian ranid taxa (Grosjean et al. 2015; Grosjean and Preininger 2020). Interestingly, vomerine teeth were found to be reliably present in adults of only two of the five species (*A. tanfuiiana* **sp. nov.** and *A. kottelati* **sp. nov.**). Although these are not sister species, they are the two largest in body sizes, supporting the hypothesis that vomerine teeth may be lost as frogs evolve smaller body sizes (Estrada and Hedges 1996).

Contemporary geographic features or elevational boundaries that might separate the five members of the *A. cremnobatus* species complex are not apparent to us. Samples from the southeastern portion of the range can be reliably assigned to *A. cremnobatus* sensu stricto, from the northern portion to *A. tanfuiiana* **sp. nov.**, and from the western portion of the range, but still east of the Mekong River, to *A. sengae* **sp. nov.** It is not known if the Mekong River serves as the genetic break between Lao populations of *A. sengae* **sp. nov.** and the provisionally assigned Thai population, as no populations are yet known from the intervening swath of territory on the western side of the Mekong River in Xaignabouli Province, Laos. It is also not known if the lack of nu distinction between *A. tanfuiiana* **sp. nov.** and *A. sengae* **sp. nov.** is a result of ongoing gene flow or incomplete lineage sorting. None of the five species were found in sympatry, but very close geographic proximity was found between *A. tanfuiiana* **sp. nov.** and *A. kottelati* **sp. nov.** in southeastern Xaysomboun Province, Laos, and between *A. tanfuiiana* **sp. nov.** and *A. attiguus* **sp. nov.** in southern Nghe An Province, Vietnam. Genome-wide single-nucleotide polymorphisms in the three members of the *A. larutensis* species complex revealed that interspecific diversification was largely driven by patterns of isolation-by-colonization (Chan and Brown 2019). Finer scale field sampling, including studies of natural history (Pham et al. 2015), advertisement call variation, and improved measures of gene flow (Chan and Brown 2019) may reveal the abiotic or biotic factors that maintain these evolutionary lineages of Indochinese torrent frogs.

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## Competing interests

The authors have declared that no competing interests exist.

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## Supplementary Material 1

### Table S1

**Authors:** Sheridan J, Phimmachak S, Sivongxay N, Stuart B (2023)

**Data type:** .xlsx

**Explanation note:** Specimens of the *Amolops cremnobatus* complex used in the morphological analyses. Latitude, longitude, and elevation in bold were estimated by the authors based on collection locality description because coordinates were not taken at time of collection.

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**Link:** <https://doi.org/vz.73.e102475.suppl1>

## Supplementary Material 2

### Table S2

**Authors:** Sheridan J, Phimmachak S, Sivongxay N, Stuart B (2023)

**Data type:** .xlsx

**Explanation note:** Samples of *Amolops* and the outgroup *Staurois* used in the four-gene dataset. An asterisk (\*) indicates the sample was also included in the 13-nuclear gene dataset (Table S3). Latitude, longitude, and elevation in bold were estimated by the authors because they were not taken at time of collection.

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**Link:** <https://doi.org/vz.73.e102475.suppl2>

## Supplementary Material 3

### Table S3

**Authors:** Sheridan J, Phimmachak S, Sivongxay N, Stuart B (2023)

**Data type:** .xlsx

**Explanation note:** Samples of the *Amolops cremnobatus* complex used in the 13-gene dataset. Latitude, longitude, and elevation in bold were estimated by the authors because they were not taken at time of collection.

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