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Two new syntopic species of wolf snakes (genus *Lycodon* H. Boie in Fitzinger, 1826) from an imperiled ecosystem in the Song Giang River Valley of southern Vietnam (Squamata: Colubridae)

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http://zoobank.org/E1F61203-222C-418A-A8AC-100CE72604A5

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Academic editor Uwe Fritz | **Received** 13 February 2021 | **Accepted** 30 May 2022 | **Published** 14 June 2022

Citation: Nguyen AT, Duong TV, Wood PL Jr, Grismer LL (2022) Two new syntopic species of wolf snakes (genus *Lycodon* H. Boie in Fitzinger, 1826) from an imperiled ecosystem in the Song Giang River Valley of southern Vietnam (Squamata: Colubridae). Vertebrate Zoology 72 371–384. https://doi.org/10.3897/vz.72.e82201

Abstract

An integrative taxonomic analysis of species in the colubrid genus *Lycodon* Fitzinger, 1826 recovered two new syntopic species of the *L. rufozonatus* complex from the imperiled Song Giang River valley in Khan Hoa Province, of Southern Vietnam. Although *L. truongi* **sp. nov.** and *L. anakradaya* **sp. nov.** are syntopic, they are not particularly closely related and can be differentiated from each other and all other species in the *L. rufozonatus* complex on the basis of meristics, morphometrics, color pattern, and uncorrected pairwise genetic distance based on the mitochondrial gene cytochrome *b*. The discovery of these two new range-restricted species and a previously described range-restricted gekkonid in the genus *Cyrtodactylus* Gray, 1828 from the same valley, underscores the necessity of continued field work in the Song Giang River valley so as to catalog the unrealized herpetological diversity in this area and establish research-based conservation programs.

Keywords

Colubrid, conservation, integrative taxonomy, Khanh Hoa Province, Southeast Asia

Introduction

The colubrid snake genus *Lycodon* Fitzinger, 1826 comprises a large radiation of at least 71 non-venomous, terrestrial to arboreal, nocturnal species that occupy a range of forested habitats extending from the Caspian Sea to Sulawesi (Wallach et al. 2014; Uetz et al. 2021). Having such a wide distribution that crosses so many well-established biogeographic borders, it is not surprising that this genus manifests a broad array of species with trenchant morphological differences and species groups bearing deep phylogenetic substructuring (Siler et al. 2013; Wang et al. 2020).

Recent field work in the imperiled riparian habitats of the Song Giang River Valley in Khanh Hoa Province of Southern Vietnam (Fig. 1) has already begun to report the discovery of a new range-restricted endemic species (Nguyen et al. 2021) and here we report the discovery of two new syntopic species of wolf snakes, *Lycodon.* All three specimens have dorsoventrally flattened heads; vertically elliptical pupils; large nostrils; strongly arched upper maxillary bones whose anterior sections angle inwards; curved, enlarged, anterior maxillary teeth followed by a diastema; 17 or 18 anterior, 17 midbody, and 15 posterior rows of dorsal scales bearing weakly keeled vertebral rows; and rounded, weakly notched, ventral scales (Lanza 1999)—characters that place them in the genus *Lycodon*. Furthermore, sequences of the new specimens were added to a cytochrome *b* (cyt *b*) phylogenetic data set from Wang et al. (2021) and were recovered as two different species nested within a larger clade of Indochinese species. Additionally, these specimens differ from each other and all other species of *Lycodon* in the Indochinese clade by having a unique suite of morphological and color pattern characters. We therefore consider them new species and describe them below.

Materials and Methods

Species delimitation and concept

The general lineage concept (GLC: de Queiroz 2007) adopted herein proposes that a species constitutes a population of organisms evolving independently from other such populations owing to a lack of, or limited gene flow. By "independently," it is meant that new mutations arising in one species cannot spread readily into another species (Barraclough et al. 2003; de Queiroz 2007). Under the GLC implemented herein, molecular phylogenies recovered monophyletic mitochondrial lineages of individuals (populations) used to develop initial species-level hypotheses—the grouping stage of Hillis (2019). Discrete color pattern data and morphological data were then used to search for unique suites of characters consistent with the tree-designated species-level hypotheses—the construction of boundaries representing the hypothesis-testing step of Hillis (2019)—thus providing independent diagnoses to complement the molecular analyses. It is important to note, that delimiting species (phylogeny) and diagnosing species (taxonomy) are independent but overlapping operations that should not be conflated (Frost and Hillis 1990; Frost and Kluge 1994; Hillis 2019).

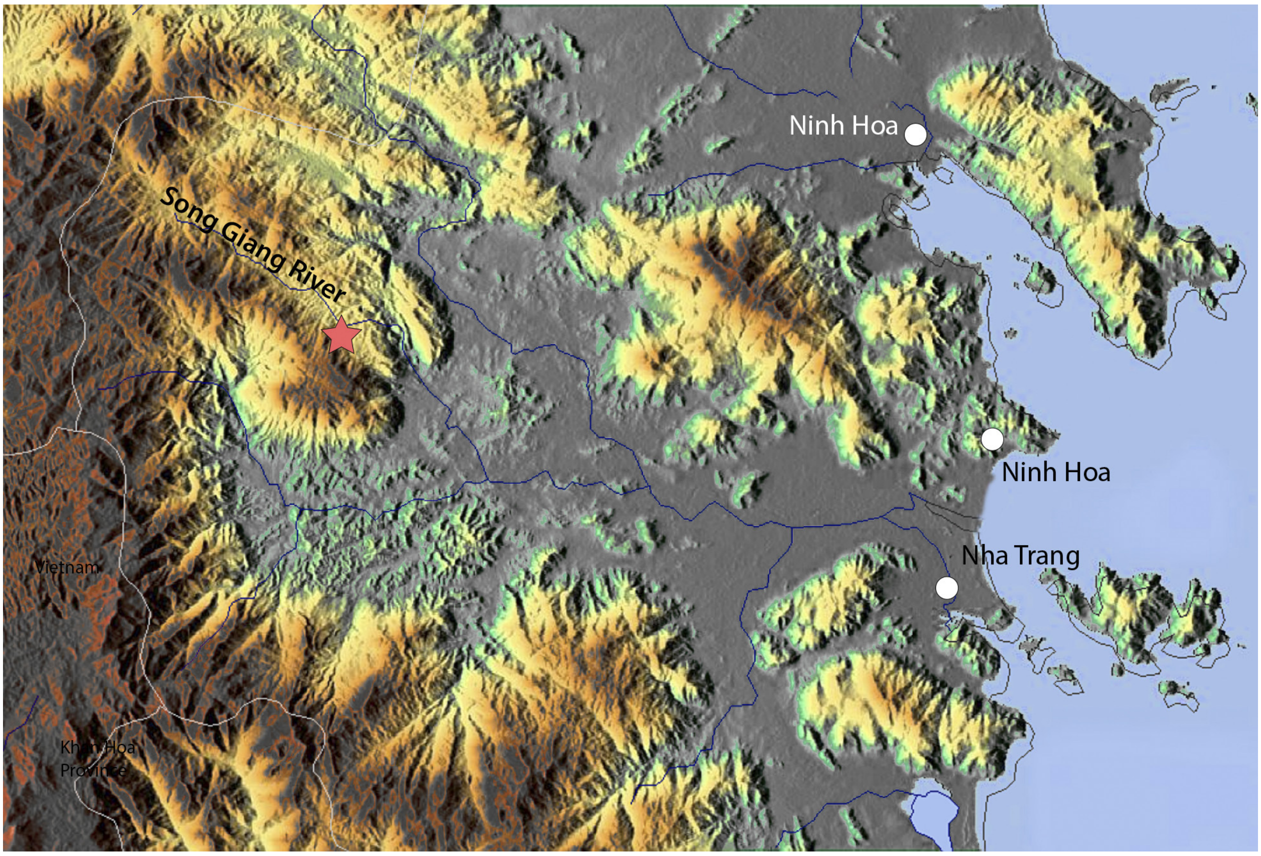

Figure 1. Location of the type locality of *Lycodon anakradaya* **sp. nov.** and *L. truongi* **sp. nov.** in the Song Giang River valley, Khanh Hoa Province, Vietnam.

Morphological analysis

Morphological and color pattern data were taken from three specimens from Song Giang River Valley (12.37079° N, 108.83643° E; at elevation 500 m a.s.l.), Khanh Trung Commune, Khanh Vinh District, Khanh Hoa Province, Vietnam and from literature on *Lycodon banksi* Luu, Bonkowski, Nguyen, Le, Calame and Ziegler, 2018; *L. cathaya* Wang, Qi, Lyu, Zeng, and Wang, 2020; *L. chapaensis* (Angel and Bourret, 1933); *L. flavozonatus* (Pope, 1928); *L. futsingensis* (Pope, 1923); *L. meridionalis* (Bourret, 1935); *L. rufozonatus* Cantor, 1842; and *L. septentrionalis* (Günther, 1875) of the *L. rufozonatus* complex (Luu et al. 2018, 2019; Jansen et al. 2019; Wang et al. 2020, 2021). Scale counts and scale nomenclature follow Wang et al. (2021). Given the small sample sizes (n=1 and n=2), standard statistical analyses would not be viable. We are well-aware that the acquisition of new material could render some of these diagnostic characters invalid just as it may validate others and/or recover new characters not listed. Nonetheless, as mentioned above, species diagnoses (i.e. morphological differences) and species delimitation (i.e. phylogenetic relationships) are different operations with different endpoints that stand independent of one another and should not be conflated.

All body measurements were made to the nearest millimeter. Morphometric data include snout-vent length (SVL), tail length (TaL), total length (ToL), head length, width, and height (HL, HW, and HH, respectively), eye diameter (ED), snout length (SnL), eye to narial distance (EN), and internarial distance (IND). Meristic data include maxillary teeth (MT); supralabial and infralabial scales (SL and IL, respectively); of SL contacting the eye (SL-E), loreals (LoR); LoR contacting the eye (LoR-E); preoculars (PrO); postoculars (PtO); anterior temporals (aTMP); posterior temporals (pTMP); dorsal scale rows one head length posterior to the head, at midbody, and one head length anterior to the vent presented in that order; ventral scales (VEN); subcaudal scales (SC); light-colored body bands (BB); and light-colored tail bands (TB). Discrete characters evaluated were body scale texture, cloacal plate divided or single, adult head color pattern, presence or absence of a wide nuchal-occipital collar in adults and/or juveniles, dorsal ground color, body bands thin (1–3 scales rows) or wide (> three scales rows), color of body bands, ventrolateral body pattern, and ventral pattern. The institutional acronym SIEZC refers to the Zoological collection of the Southern Institute of Ecology in Hochiminh City, Vietnam.

Molecular analysis

Sequence data from a 1,114 base pair fragment of the cytochrome *b* gene (cyt *b*) was obtained from 149 specimens comprising 40 species in GenBank and three specimens (SIEZC 20247–48, and SIEZC 20249) from Song Giang. *Ahaetulla prasina* (Boie, 1827), *Boiga dendrophila* (Boie, 1827), *Gonyosoma oxycephalum* (Boie, 1827, and *Oligodon chinensis* Günther (1888) were used as outgroups to root the tree following (Liu et al. 2020 in part). Genbank accession numbers for SIEZC 20247–49 are OM674283, OM674284, and OM674282, respectively.

Genomic DNA was isolated from muscle tissue stored in 95% ethanol following Nguyen et al., 2021. A fragment of the 5'- end of michondrial Cyt *b* gene was amplified using a double-stranded Polymerase Chain Reaction (PCR) under the following conditions: 1.0 µl genomic DNA (~ 35 µg of DNA), 0.75 µl forward primer (10µM) cytbL14910 5'–GAC CTG TGA TMT GAA AAC CAY CGT TGT–3' or CytbL14919 5'-AAC CAC CGT TGT TAT TCA ACT–3', 0.75 µl reverse primer (10µM) CytbH16064 5'- CTT TGG TTT ACA AGA ACA ATG CTT TA-3 (Burbrink et al. 2000), 12.5 µl of Master Mix 2x (CWBIO, China), and 10 µl ultra-pure H2O. PCR reactions were completed using a Bio-Rad T100™ gradient thermocycler with the following reaction conditions: initial denaturation at 94°C for 5 mins, second denaturation at 94°C for 1 min, annealing at 50°C for min followed by an extension cycle at 72°C for 1 min per cycle for 35 cycles and the final extension step 72 °C for 10 mins. PCR products were electrophoresed on 2.0% agarose gel, and then target band was cut for purification using the GeneJET Gel Extraction Kit (Thermo Fisher Scientific Co., USA). The purified DNA fragments were sent to National Key Laboratory of Gene Technology (Institute of Biotechnology, VAST, Hanoi, Vietnam) for sequencing using the forward primers of the amplification step. The sequences were viewed by DNA Baser v.5, then uncertain nucleotides at both ends were removed and exported under fasta format. Sequences were aligned by MAFFT version online and checked by eye using Bioedit v.7.0.5.2 to ensure the correct amino acid reading frame.

Phylogenetic analysis

Maximum likelihood (ML) and Bayesian inference (BI) were used to estimate the phylogenetic relationships among the sampled species in our sequence alignment. An ML phylogeny was estimated using the IQ-TREE webserver (Nguyen et al. 2015; Trifinopoulos et al. 2016) preceded by the selection of substitution models using the Bayesian Information Criterion (BIC) in ModelFinder (Kalyaanamoorthy et al. 2017), which supported TPM2+F+I+G4 as the best fit model for codon position one, TIM3+F+I+G4 for position 2, and TIM2+F+I+G4 for position 3. One-thousand bootstrap pseudoreplicates via the ultrafast bootstrap (UFB; Hoang et al. 2018) approximation algorithm were employed and nodes having UFB values of 95 and above were considered highly supported (Minh et al. 2013). A Bayesian inference (BI) phylogenetic analysis was carried out in MrBayes 3.2.3. (Ronquist et al. 2012) on XSEDE using the CIPRES Science Gateway (Cyberinfrastructure for Phylogenetic Research; Miller et al. 2010) employing default priors and models of evolution that most closely approximated those selected by the BIC and used in the ML analysis. Two independent Markov chain Monte Carlo (MCMC) analyses were performed, each with four chains, three hot

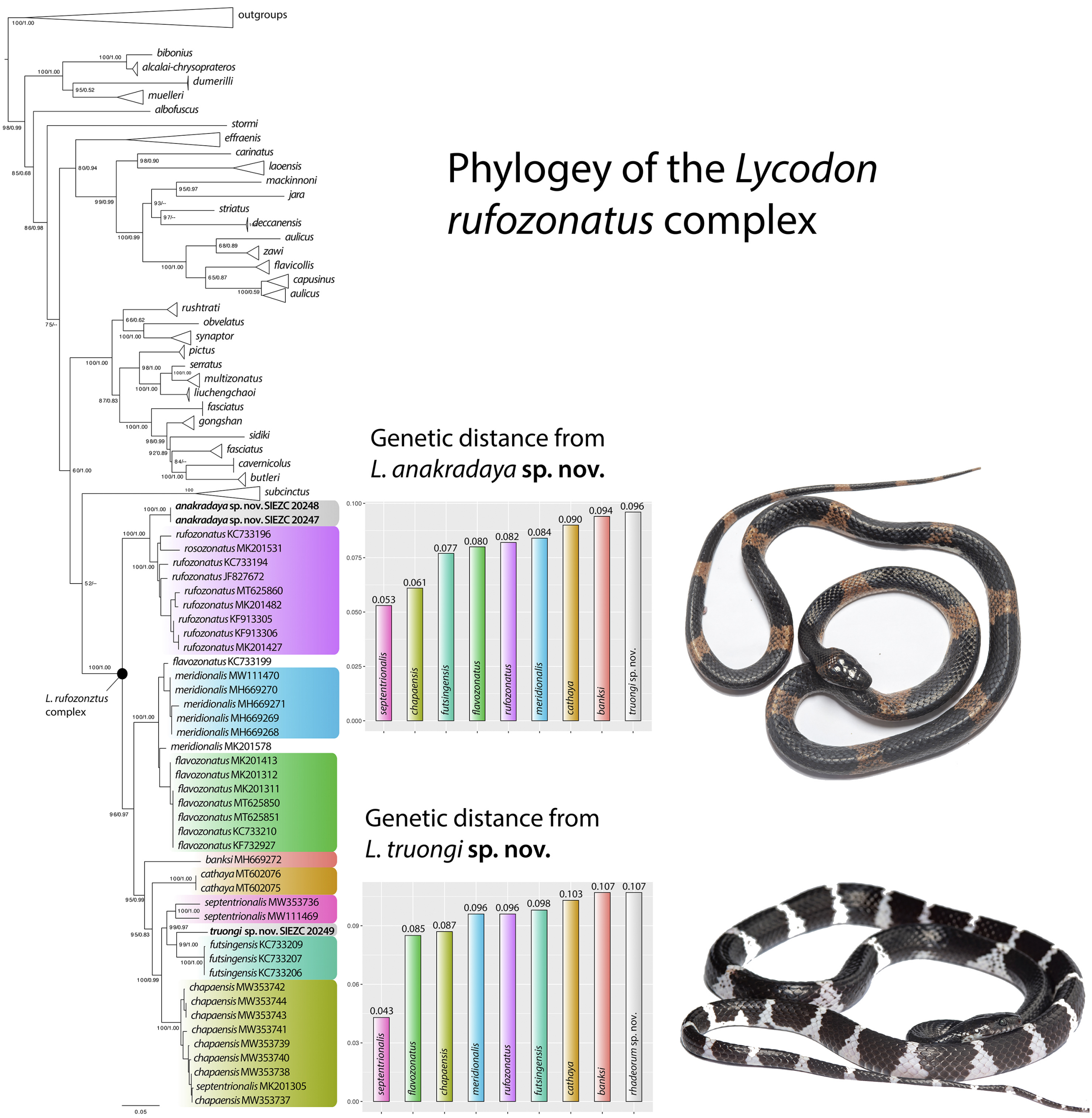

Figure 2. Majority-rule consensus tree from 1000 ML bootstrap pseudoreplicates of species in the genus *Lycodon* based on 1114 bp of Cyt*b* with UFB and BPP support values at the nodes, respectively and associated uncorrected pairwise genetic distance barplots. Photographs by Anh The Nguyen.

and one cold. The MCMC simulation ran for 100 million generations, was sampled every 10,000 generations, and the first 10% of each run were discarded as burn-in. Convergence and stationarity of all parameters from both runs were checked in Tracer v1.6 (Rambaut et al. 2014) to ensure effective sample sizes (ESS) were well-above 200. Post-burn-in sampled trees from both runs were combined and a 50% majority-rule consensus tree was constructed. Nodes with Bayesian posterior probabilities (BPP) of 0.95 and above were considered highly supported (Huelsenbeck et al. 2001; Wilcox et al. 2002). After removing the outgroups and other species not part of the *Lycodon rufozonatus* complex (Fig. 2), MEGA7 (Kumar et al. 2016) was used to calculate uncorrected pairwise sequence divergences among and within species using the complete deletion option which removes missing data and gaps.

Results

Both the ML and BI analyses recovered trees with the same general topology with high nodal support (Fig. 2). Both analyses placed SIEZC 20247–48 and SIEZC 2049 within a clade referred to here as the *Lycodon rufozonatus* complex comprised of *L. banksi*, *L. cathaya*, *L. chapaensis*, *L. flavozonatus*, *L. futsingensis*, *L. meridionalis*, *L. rufozonatus*, and *L. septentrionalis*. The analyses also demon-

Table 1. Uncorrected pairwise sequence divergence among species of the *Lycodon rufozonatus* complex based on 1114 base pairs of the mitochondrial gene cytochrome b. Bold numbers are intraspecific differences. *** indicates a sample size of one.

	banksi	*cathaya*	*chapaensis*	*flavozonatus*	*futsingensis*	*meridionalis*	*truongi* **sp. nov.**	*anakradaya* **sp. nov.**	*rufozonatus*	*septentrionalis*
banksi	***									
cathaya	0.098	**0.000**								
chapaensis	0.078	0.070	**0.008**							
flavozonatus	0.090	0.094	0.063	**0.008**						
futsingensis	0.085	0.098	0.063	0.112	0.000					
meridionalis	0.094	0.092	0.069	0.023	0.104	**0.007**				
***truongi* sp. nov.**	0.094	0.090	0.061	0.080	0.077	0.084	***			
***anakradaya* sp. nov.**	0.107	0.103	0.087	0.085	0.098	0.096	0.107	**0.000**		
rufozonatus	0.112	0.082	0.090	0.072	0.111	0.082	0.096	0.042	**0.026**	
septentrionalis	0.081	0.056	0.040	0.061	0.047	0.053	0.043	0.064	0.067	**0.034**

Table 2. Diagnostic characters of *Lycodon truongi* **sp. nov.** and *L. anakradaya* **sp. nov.** that differentiate them from each other and all other species of the *L. rufizonatus* complex. Green cells denote characters that separate *L. truongi* **sp. nov.** and *L. anakradaya* **sp. nov.** from the other species. Orange cells denote characters separating *L. anakradaya* **sp. nov.** from all other species. Blue cells denote characters separating *L. truongi* **sp. nov.** from all other species. Data for previously described species come from Jansen et al (2019), Luu et al (2018, 2019), Wang et al (2020, 2021), and Song (2021).

	anakra-daya **sp. nov.**	*truongi* **sp. nov.**	*meri-dionalis*	*rufozo-natus*	*flavo-zonatus*	*banksi*	*cathaya*	*cha-paensis*	*septen-trionalis*	*futsin-gensis*
Maximun SVL (mm)	790	700	1295	980	901	415	730	890	990	663
DSR	18–17–15	17–17–15	17–17–15	17/19–17–15	17–17–15	17–17–15	17–17–15	17-17-15	17	17–16/17–15
MT	12	14	11	11–13	13	?/	10	11 or 12	8	12–15
SPL	8	8	8	8	8	8	8	7 or 8	8	7–8
SPL-E	3rd–5th	3rd–5th	3rd–5th	3rd–5th	3rd–5th	3rd–5th	3rd–5th	4th–5th	2nd–3rd	2–4; 3/4–5; 4–6
IFL	9 or 10	9	10	9–10	10	10	9	8–10	9	9–11
PrO	1	1	1	1	1	1	1	1	1	1
PtO	2	2	2	2	2	2	2	2	2	2–3
Lor	1	1	1	1	1	1	1	1	1	1
Lor-E	no	no	no	no	no	no	no	no	no	no
aTMP	2	2	2	2	2	2	2	2	2	1–2
pTMP	3	2	3	3	2–3	3	3	2 or 3	3	2–3
VEN	225–232	200	227–240	184–225	211–221	241	199 or 200	200–225	207–212	193–208
SC	87	91	96–106	53–98	80–88	26 (bro-ken tail)	78	74–84	78	72–87
PrC	divided	divided	divided	entire	entire / divided	entire	entire	entire	entire	entire
BSC	5 keeled vertebral rows from midbody to vent	15 keeled vertebral rows from midbody to vent	10–12 medial rows distinctly keeled	weakly keeled posteri-orly	7 keeled medial rows	6 central scales of posterior 1/3 weak-ly keeled	smooth	posterior vertebral row weak-ly keeled	keeled	smooth
Adult head pattern	uniform dark-brown/ black	uniform dark-brown/ black	black w/ yellow sutures	dark-brown w/ reddish sutures	black w/ yellow markings	uniform dark-grey	uniform dark-brown to black	uniform black	uniform black	greyish brown
Dorsal ground color	dark-brown/ black	black	black	dark-brown/ black	black	dark-brown/ black	black	black	dark-brown/ black	dark-brown

	anakra-daya **sp. nov.**	*truongi* **sp. nov.**	*meri-dionalis*	*rufozo-natus*	*flavo-zonatus*	*banksi*	*cathaya*	*cha-paensis*	*septen-trionalis*	*futsin-gensis*
Ventrolateral body pattern	banded	banded	reticulated	reticulated	reticulated	reticulated	reticulated	banded	banded	banded
Ventral pattern	orangish anteriorly grading to brown posteri-orly	white anteriorly black and white banded posteri-orly	/	/	/	unicolor grey-cream			/	/
Wide nuchal-occipi-tal band in adults	absent	absent	absent	absent	absent	absent	present	absent	absent	present
Wide nuchal-occipi-tal band in juveniles	absent	/	absent	absent	absent	/	present	absent	present	present
Light-colored BB	12 or 13	19	84–115	44–52	51–78	87	31–35	28	33–35	19–33
Body bands narrow or wide	wide	thin	thin	thin	thin	thin	thin	thin	thin	wide
Color BB in adults	orangish	white	yellow (adult)	reddish	yellow	yellow	light-rose	white	white	light-rose
Light-colored TB	5–7	13	25–35	20	17–24	15	13–16	11	19	8–19

strated that not only were SIEZC 20247–48 and SIEZC 2049 not conspecific, they were not even closely related. SIEZC 20247–48 was recovered as the strongly supported (UFB=100/BPP=1.00) sister species to *L. rufozonatus* and SIEZC 2049 as the strongly supported (99/1.00) sister species to *L. futsingensis*. The uncorrected pairwise sequence divergence between SIEZC 20247–48 and all other species of the *L. rufozonatus* complex ranged from 4.3–10.7% and for SIEZC 2049 the range was 5.3%–9.6% (Table 1). The morphological comparisons are in accordance with the phylogenetic analyses and indicate that each new population from Song Giang bears a unique suite of diagnostic characters separating them from each other and all other species of the *L. rufozonatus* complex (Table 2). As such, each is described below as a new species. The phylogenies also suggest that *Lycodon flavozonatus* (GenBank accession number KC733199) from Guo et al. (2013) is a misidentification of *L. meridionalis* and that *L. meridionalis* (GenBank accession number MK201578) from Li et al. (2020) is a misidentification of *L. flavozonatus*.

Taxonomy

Lycodon truongi sp. nov.

Figures 3, 4

http://zoobank.org/4A2ADC11-3FE6-4E8C-8303-7775CE52-9F40

Suggested common name. Truong's wolf snake — Rắn khuyết Trường.

Holotype. Adult male (SIEZC 20249) collected on 22 December 2020 by Anh The Nguyen from Song Giang River Valley (12.37079° N, 108.83643° E; at elevation 500 m a.s.l.), Khanh Trung Commune, Khanh Vinh District, Khanh Hoa Province, Vietnam.

Diagnosis. *Lycodon truongi* **sp. nov.** is separated from all other species of the *L. rufozonatus* complex by having the combination of a maximum SVL length of 700 mm; tail length 195 mm; 17–17–15 dorsal scale rows; 14 maxillary teeth; eight supralabials with the third–fifth contacting the eye; nine infralabials; one preocular; two postoculars; an elongate loreal not contacting the eye; two anterior temporals; two posterior temporals; 200 ventral scales; 91 paired subcaudal scales; a divided precloacal plate; 15 keeled vertebral scale rows from midbody to vent; uniform dark-brown to black adult head pattern; no wide light-colored nuchal-occipital collar in the adult; black dorsal ground color; 19 narrow white body bands; 13 white caudal bands; white dorsal bands on ventrolateral section of body as opposed to a reticulated pattern; anterior one-half of venter white, and posterior of venter bearing white and broken black bands. These characters are scored across all species of the *L. rufozonatus* complex in Table 2.

Description of holotype (Figs 3, 4; Table 3). Head flattened slightly sloping anteriorly, distinct from the neck, HL 16.0 mm, HW 12.9 mm, HH 7.6 mm, snout somewhat elongate, SnL 6.3 mm, EN 4.8 mm; nostril oval, large, in the middle of the nasal, IND 5.6 mm; eye moderately sized, ED 2.5 mm, with a vertically elliptic pupil; rostral triangular, hardly visible from above; nasal vertically divided by a furrow along posterior margin of nostril; two square internasals, in wide, medial contact, and in contact with two large, subrectangular prefrontals posteriorly; single, azygous, subpentagonal frontal, longer than wide; two large, elongate parietals, contacted laterally by upper anterior and posterior temporals and a larger paraparietal; 1/1 wide, elongate supraocular; 1/1 small

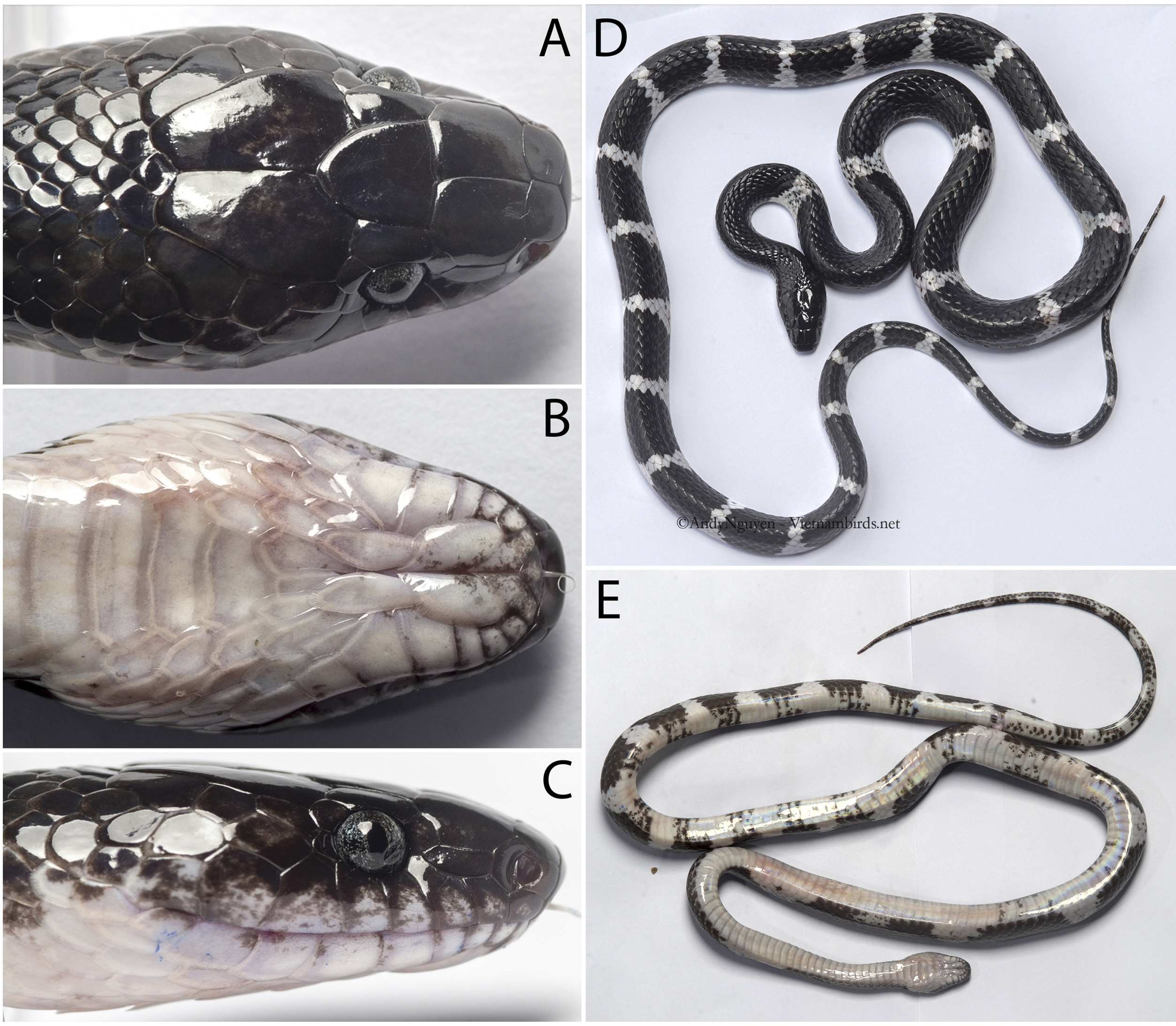

Figure 3. Holotype of *Lycodon truongi* **sp. nov.** SIEZC 20249 from the Song Giang River Valley Khanh Trung Commune, Khanh Vinh District, Khanh Hoa Province, Vietnam. **A** Dorsal view of head. **B** Gular region. **C** Lateral view of head. **D** Dorsal view of body. **E** Ventral view of body. Photographs by Anh The Nguyen.

preocular, located above the posterior portion of loreal; 2/2 postoculars of similar size; 1/1 narrow, elongate loreal not entering orbit, in contact with second, third, and fourth supralabials ventrally, the prefrontal and preocular dorsally, the nasal posteriorly; 8/8 supralabials all higher than wide except last scale in the series; first and second supralabials in contact with nasal; third, fourth, and fifth, supralabials contact eye; seventh supralabial largest; two elongate anterior temporals, lower temporal the largest; two square posterior temporals of similar size; 9/9 infralabials; first pair infralabials contact medially forming a deep, medial groove; first five infralabials in contact with first pair of chinshields; anterior and posterior pair of chinshields elongate, posterior pair smaller, bearing a deep, medial grooves contiguous with groove separating first pair of infralabials; and 14 maxillary teeth.

Body elongate, somewhat laterally compressed; SVL 700 mm; TaL 195 mm; ToL 895 mm; 200 ventrals, 91 paired subcaudals; cloacal shield divided; dorsal scales in 17–17–15 rows with 15 keeled vertebral rows from midbody to vent; vertebral row not enlarged; no apical pits.

Coloration in life (Figs 3, 4). Head, body and tail nearly uniformly black; body bearing 19 thin white body bands extending to ventral scales, 13 thin white caudal bands encircling tail; anterior half of venter white, posterior half bearing mottled black bands; subcaudal region banded.

Etymology. The specific epithet "*truongi*" is a patronym honoring Professor Dr. Quang Truong Nguyen for his long-standing extensive contributions to the herpetology of Vietnam and his broad international collaborations.

Natural history (Fig. 7). The Song Giang River and its riparian habitat course through a forested river valley formed by east-west tending mountains in the northwestern portion of Khanh Hoa Province in the vicinity of the Song Giang Hydropower Station. The surrounding mountains form the northeastern slopes of Langbian Plateau and are covered with polydominant montane evergreen tropical forest that are dissected by a rich network

Figure 4. A. Holotype of *Lycodon anakardaya* **sp. nov.** SIEZC 20247. **B** Holotype of *Lycodon truongi* **sp. nov.** SIEZC 20248. **C** Paratype of *Lycodon ankardaya* **sp. nov.** SIEZC 20248. Photographs by Anh The Nguyen.

of small streams and rivulets that feed into the Song Giang River. The specimen was found while foraging on the ground, in a relatively flat forest floor habitat surrounded by small rivulets and was taking refuge in the hollow of a tree.

Comparisons (Table 2). *Lycodon truongi* **sp. nov.** could not be separated statistically from other species of the *L. rufozonatus* complex because of its small sample size (n=1). Therefore, the comparisons below are based on discrete differences some of which are color pattern characters tradionally used to separate species (Jansen et al. (2019), Luu et al. (2018, 2019), Wang et al. (2020, 2021), and Song (2021). *Lycodon truongi* **sp. nov.** differs from *L. chapaensis* and *L. septentrionalis* in the third, fourth, and fifth supralabials contacting the eye as opposed to the fourth and fifth in the former and second or third in the latter. *Lycodon truongi* **sp. nov.** differs from *L. cathaya*, *L. chapaensis*, and *L. anakradaya* **sp. nov.** by having 14 as opposed to 10–12 maxillary teeth and it differs from *L. septentrionalis* which has eight. *Lycodon truongi* **sp. nov.** differs from all other species of the *L. rufozonatus* complex by having 19 light-colored dorsal bands as opposed to 12 (*L. anakradaya* **sp. nov.**, see below) or 19–115 collectively in the remaining species. *Lycodon truongi* **sp. nov.** is further separated from *L. anakradaya* **sp. nov.** by having two as opposed to three posterior temporals. The divided cloacal shield differentiates *L. truongi* **sp. nov.** from *L. banksi*. *L. cathaya*, *L. chapaensis*, *L. futsingensis*, and *L. rufozonatus*. Having keeled dorsal scales differentiates it from *L. cathaya* and *L. futsingensis*. It is further separated from *L. meridionalis*, *L. anakradaya* **sp. nov.**, and *L. banksi* by having 200 ventral scales versus 225–241, collectively. Its uniform black head pattern differentiates it from *L. futsingensis*, *L. flavozonatus*, *L. meridionalis*, and *L. rufozonatus.* It is separated from *L. banksi*, *L. cathaya*, *L. flavozonatus*, *L. meridonalis*, and *L. rufozonatus* by having the ventral portion of the white body bands form the pattern on the ventrolateral side of the body as opposed to a light-colored reticulum. It differs from *L. banksi* and *L. anakradaya* **sp. nov.** by having a partially banded venter as opposed to a orangish-brown or uniform grey-colored venter, respectively. It differs further from *L. cathaya* and *L. futsingnesis* by not having a wide light-colored nuchal-occipital collar. Having thin white body bands separates *L. truongi* **sp. nov.** from *L. anakradaya* **sp. nov**. and *L. futsingensis* which have wide body bands. Furthermore, having white body bands separates it from all other species except *L. chapaensis* and *L. septentrionalis*. Having 13 light-colored caudal bands separates it from *L. anakradaya* **sp. nov.** with 5–7 and *L. chapaensis* with 11 and all other species except *L. cathaya* and *L. futsingensis* which collectively have 15–35 bands.

Table 3. Meristic, morphometric, and discrete color pattern and scale morphology characters of the type specimens of *Lycodon truongi* **sp. nov.** and *L. anakradaya* **sp. nov.** All measurements are in millimeters.

	***anakradaya* sp. nov.**		***truongi* sp. nov.**
	NAT21-11 holotype	NAT21-8 paratype	
SVL	790	230	700
TaL	190	55	195
TL	980	285	895
HL	27.8	11.8	16.0
HW	18.0	7.1	12.9
HH	11.8	4.6	7.6
ED	4.0	2.3	2.5
SnL	8.2	3.5	6.3
EN	5.2	2	4.8
IND	6.0	2.6	5.6
MT	12	12	14
DSR	18–17–15	17–17–15	17–17–15
Keeled vertebral rows	3	0	15 starting at midbody
VEN	225	232	200
SC	87		91
Cloacal plate	divided	divided	divided
SL (right and left)	8	8	8
SL-L	2nd–3rd	2nd–3rd	2nd–3rd
SL-E	3rd–5th	3rd–5th	3rd–5th
LoR	1	1	1
LoR-E	0	0	0
PrO	1	1	1
PtO	2	2	2
aTMP	2	2	2
pTMP	3	3	2
IL (right and left)	10	9	9
BB	13	12	19
TB	7	5	13
Head pattern	uniform dark-brown	dark-brown plates edged with white	uniform dark-brown
Wide nuchal-occipital band in juveniles	absent	absent	absent
Dorsal ground color	dark-brown	black	black
Ventral pattern	orangish anteriorly grading to brown posteriorly	white anteriorly grading to dark-brown banding posteriorly	white anteriorly, black and white banded posteriorly
Ventrolateral body pattern	banded	banded	banded
Wide nuchal-occipital band in adults and juveniles	absent	absent	absent
Body bands narrow or wide	wide	wide	thin
Color of body bands	orange	white	white

Lycodon anakradaya, sp. nov.

Figures 4, 5

http://zoobank.org/FF1EA2E5-923B-4FA6-BF92-BECBEF-7B824E

Suggested common name. Rhade wolf snake – Rắn khuyết Ê đê.

Holotype. Adult male (SIEZC 20247) collected on 21 December 2020 by Anh The Nguyen from Song Giang River Valley (12.37079° N, 108.83643° E; at elevation 493 m a.s.l.), Khanh Trung Commune, Khanh Vinh District, Khanh Hoa Province, Vietnam.

Paratype. Juvenile (SIEZC 20248) collected on 21 August 2020 by Anh The Nguyen from Song Giang River Valley (12.37079° N, 108.83643° E; at elevation 580 m a.s.l.), Khanh Trung Commune, Khanh Vinh District, Khanh Hoa Province, Vietnam.

Diagnosis. *Lycodon anakradaya* **sp. nov.** is separated from all other species in the *L. rufozonatus* complex by having the combination of a maximum SVL length of 790 mm; TaL 190 mm; 17 or 18–17–15 dorsal scale rows; 12 maxillary teeth; eight supralabials with the 3rd–5th contacting

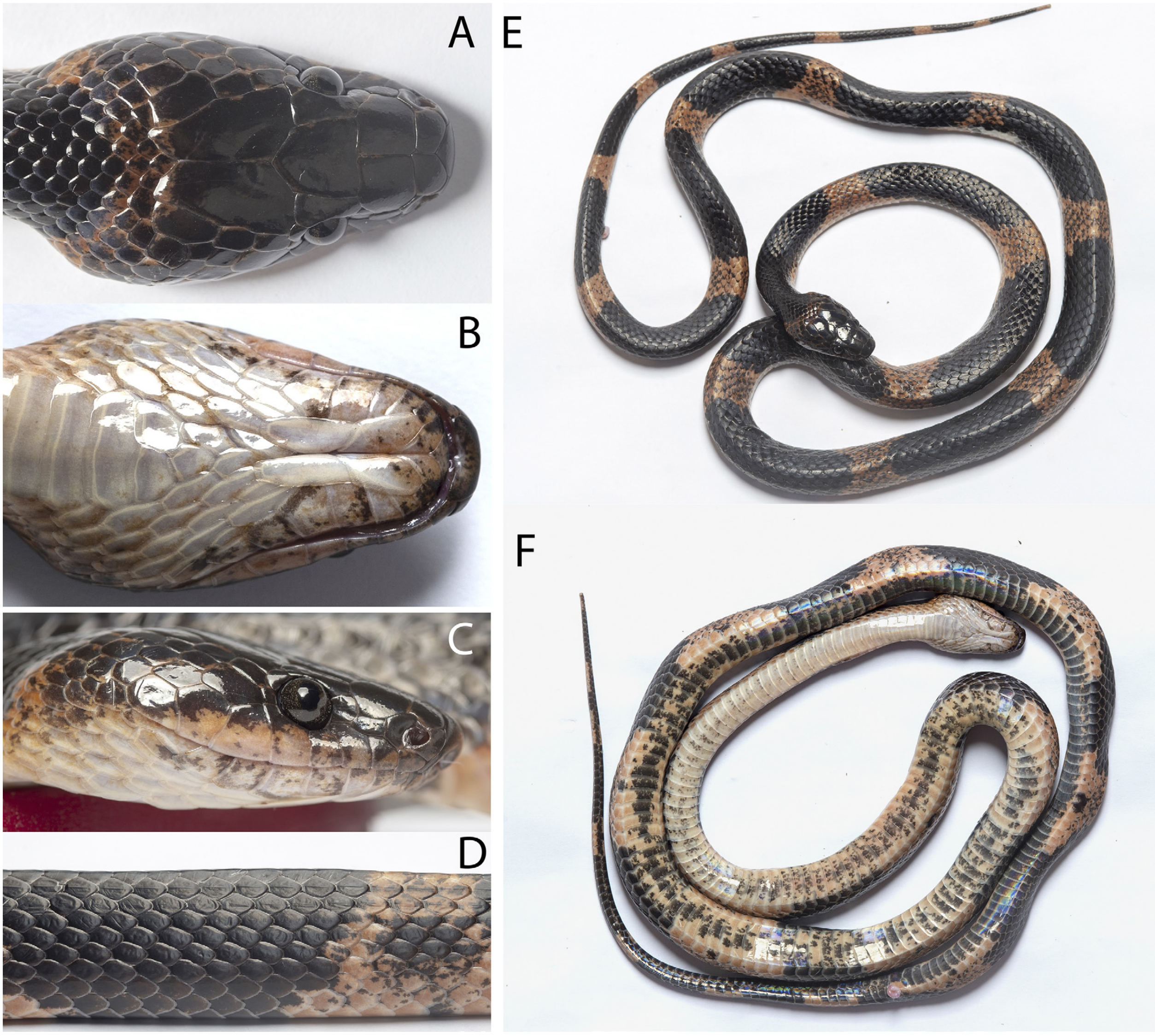

Figure 5. Holotype of *Lycodon anakradaya* **sp. nov.** SIEZC 20247 from the Song Giang River Valley Khanh Trung Commune, Khanh Vinh District, Khanh Hoa Province, Vietnam. **A** Dorsal view of head. **B** Gular region. **C** Lateral view of head. **D** Lateral view of a portion of the body. **E** Dorsal view of body. **F** Ventral view of body. Photographs by Anh The Nguyen.

the eye; nine or 10 infralabials; one preocular; two postoculars; an elongate loreal not contacting the eye; two anterior temporals; three posterior temporals; 223–232 ventral scales; 87 paired subcaudal scales; a divided precloacal plate; five keeled vertebral scale rows from midbody to vent; uniform dark-brown to black adult head pattern; no wide light-colored nuchal-occipital collar; dark-brown/black dorsal ground color; 12 wide, orangish body bands; 5–7 orangish caudal bands; orangish dorsal bands on ventrolateral section of body as opposed to a reticulated pattern; and anterior one-half of venter orangish grading into a dark-brown posteriorly. These characters are scored across all species of the *L. rufozonatus* complex in Table 2.

Description of holotype (Figs 4, 5; Table 3). Head flattened slightly sloping anteriorly, distinct from the neck, HL 27.8 mm, HW 18.0 mm, HH 11.8 mm, snout somewhat elongate, SnL 8.2 mm, EN 5.2 mm; nostril oval, large, in the middle of the nasal, IND 6.0 mm; eye moderately sized, ED 4.0 mm, with a vertically elliptic pupil; rostral triangular, hardly visible from above; nasal vertically divided by a furrow along posterior margin of nostril; two square internasals in wide, medial contact, and in contact with two large, square prefrontals posteriorly; followed by a single, azygous, subpentagonal frontal, nearly as wide as long; two large, elongate parietals, contacted laterally by upper anterior and posterior temporals and a larger paraparietal; 1/1 wide, elongate supraocular; 1/1 small preocular, contacting third supralabial; 2/2 postoculars, upper slightly larger than lower; 1/1 elongate loreal not contacting eye, in contact with second and third supralabials ventrally, the prefrontal dorsally, preocular posteriorly, the nasal anteriorly; 8/8 supralabials, second to sixth higher than wide; first and second supralabials in contact with nasal; third, fourth, and fifth, supralabials contacting eye; sixth and seventh supralabials largest; two elongate anterior temporals, lower temporal largest; three square posterior temporals of similar size; 10/10 infralabials; first pair of infralabials contact medially forming a deep, medial groove; first

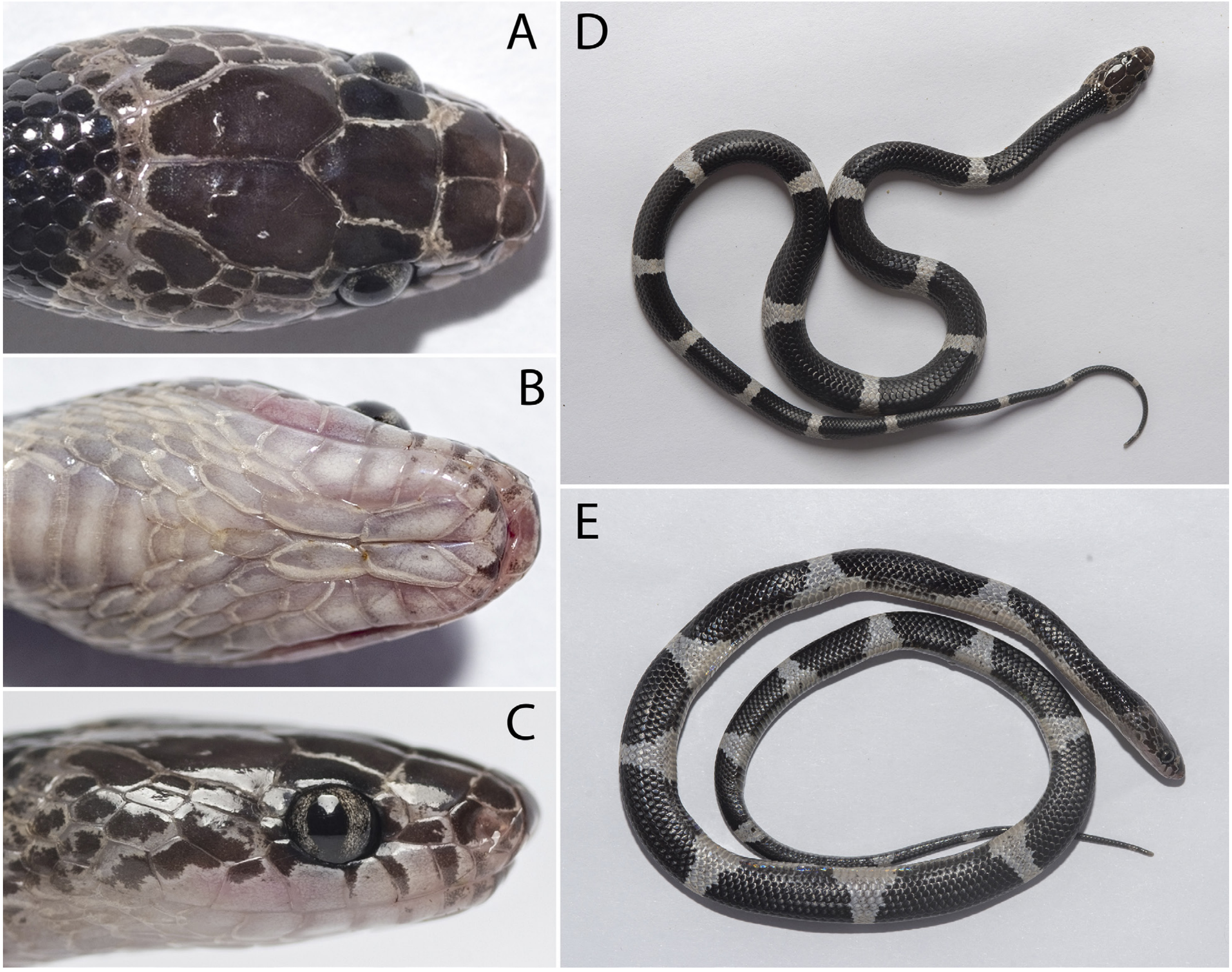

Figure 6. Paratype of *Lycodon anakradaya* **sp. nov.** SIEZC 20248 from the Song Giang River Valley Khanh Trung Commune, Khanh Vinh District, Khanh Hoa Province, Vietnam. **A** Dorsal view of head. **B** Gular region. **C** Lateral view of head. **D** Dorsal view of body. **E** Lateral view of body. Photographs by Anh The Nguyen.

five infralabials in contact with first pair of chinshields; similarly sized anterior and posterior pair of elongate chinshields, bearing deep, medial grooves contiguous with groove separating first pair of infralabials; 12 maxillary teeth.

Body elongate, somewhat laterally compressed; SVL 790 mm; TaL 190 mm; ToL 980 mm. 225 ventrals, 87 paired, subcaudals; cloacal shield divided; dorsal scales in 18–17–15 rows with three keeled vertebral rows; vertebral scale row not enlarged; no apical pits.

Coloration in life (Figs 4, 5). Dorsal ground color of head, body and tail dark-brown to nearly uniformly black; body bearing 13 wide orangish body bands extending to ventral scales, seven wide orangish caudal bands incompletely encircling tail; anterior half of venter orangish with dark-brown mottling grading into a uniform dark-brown venter and subcaudal region.

Variation (Figs 4-6; Table 3). The paratype SIEZC 20248 resembles the holotype in overall color patter except the dorsal and caudal bands are white and the head plates are edged in white. Presumably the 12 dorsal and five caudal bands become orangish and the head will become unicolor dark-brown/black with increasing SVL. The paratype lacks keeled vertebral scales which also may become keeled with increasing SVL. Meristic differences are listed in Table 3.

Etymology. The specific epithet "*anakradaya*" is given in a reference to the Ede people ("*Anak Radaya*" in Ede language), an Austronesian ethnic group living in the upland forested areas of southern Vietnam including those that surround the Song Giang River valley.

Natural history (Fig. 7). The holotype was observed between 18:30–22:30 h swimming across a small, clear stream with a sandy bottom in an effort to escape detection. Once crossing the stream, it climbed up a steep river bank. The stream is at 600 m elevation and bordered by moss-covered granite boulders less than 4 m wide beneath a canopy of broad-leaved evergreen forest. The stream supports many small fishes (*Poropuntius* cf. *deauratus*, *Channa* cf. *gachua*, *Schistura* sp.) and various species of amphibians (*Hylarana* sp., *Odorrana* sp.). The juvenile paratype of *L. anakradaya* **sp. nov.** was found on a branch 30 cm above the surface of the water approximately 1 km upstream from the type locality.

Figure 7. Riparian habitat of a tributary of the Song Giang River where *Lycodon truongi* **sp. nov.** and *L. anakardaya* **sp. nov.** were collected. Photographs by Anh The Nguyen.

Comparisons (Table 2). *Lycodon anakradaya* **sp. nov.** could not be separated statistically from other species of the *L. rufozonatus* complex because of its small sample size (n=2). Therefore, the comparisons below are based on discrete differences some of which are color pattern characters tradionally used to separate species (Jansen et al. (2019), Luu et al. (2018, 2019), Wang et al. (2020, 2021), and Song (2021). Differences between *Lycodon anakradaya* **sp. nov.** and *L. truongi* **sp. nov.** are discussed above. *Lycodon anakradaya* **sp. nov.** differs from all other species of the *L. rufozonatus* complex by having 12 or 13 light-colored dorsal bands as opposed to 19–115 collectively in the remaining species. It differs further from all other species in that the body bands are orangish as opposed to white, reddish, yellow, or light-rose in adults. *Lycodon anakradaya* **sp. nov.** differs from *L. chapaensis* and *L. septentrionalis* in the third, fourth, and fifth supralabials contacting the eye as opposed to the fourth and fifth in the former and second or third in the latter. Having 5–7 light-colored caudal bands also separates it from all other species which collectively have 13–35 caudal bands. *Lycodon anakradaya* **sp. nov.** differs from *L. cathaya* by have 12 maxillary teeth as opposed to 10 and from *L. septentrionalis* which has eight. The divided cloacal shield differentiates *L. anakradaya* **sp. nov.** from *L. banksi*. *L. cathaya*, *L. chapaensis*, *L. rufozonatus*, and *L. septentrionalis*. Having keeled dorsal scales as adults differentiates it from *L. cathaya* and *L. futsingensis*. It is further separated from *L. meridionalis* by having 223–232 ventral scales versus 199–212 in *L. cathaya*, *L. futsingensis*, and *L. septentrionalis* and from *L. banksi* with 241ventral scales. Its uniform black head pattern differentiates it from *L. futsingensis*, *L. flavozonatus*, *L. meridionalis*, and *L. rufozonatus*. It is separated from *L. banksi*, *L. cathaya*, *L. flavozonatus*, *L. meridonalis*, and *L. rufozonatus* by having the ventral portion of the orangish body bands forming the pattern on the ventrolateral side of the body as opposed to a light-colored reticulum. It differs from *L. banksi* by not having an orangish brown or uniform grey-colored venter. It differs further from *L. cathaya* and *L. futsingnesis* by not having a wide light-colored nuchal-occipital collar. Having wide light-colored body bands separates *L. anakradaya* **sp. nov.** from *L. banksi*, *L. cathaya*, *L. chapaensis*, *L. flavozonatus*, *L. meridonalis*, and *L. rufozonatus* and *L. septentrionalis* which have narrow body bands.

Discussion

The discovery of *Lycodon truongi* **sp. nov.** and *L. anakradaya* **sp. nov.** brings the total of number of *Lycodon* in Vietnam to 18. Prior to these descriptions, the newest species to be described from the Vietnam was *L. pictus* Janssen, Pham, Ngo, Le, Nguyen, & Ziegler 2019 from Cao Bang Province, ~1.300 km overland distance to the north from the Song Giang River Valley. Other than that, the only addition of *Lycodon* to southern Vietnam was *L. cardamomensis* Daltry and Wüster 2002 that was reported from Phu Yen Province by Truong et al. (2017).

The description of the new species described here from southern Vietnam continue to underscore the fact that the mountainous areas of southern Vietnam are very likely to harbor additional new species to science. As noted by Nguyen et al. (2021), these recent discoveries along with that of *Cyrtodactylus raglai* from the same river valley is a clear indication that the true diversity of this area is unrealized. This creates an urgent need for continued field work in this region, and in particular, forested riparian areas where additional range-restricted endemics are likely to be discovered. To date, *C. raglai*, *L. truongi* **sp. nov.** and *L. anakradaya* **sp. nov.** are known only from a narrow area within the Song Giang River Valley. In 2014, a significant part of this valley was partially flooded after the construction of the Song Giang Hydropower Station. A second hydropower station on the Song Giang River is currently under construction and is expected to be completed in 2022. Economic growth in Khanh Hoa Province requires additional electricity, and the plans for further development of hydropower stations on the Song Giang River will likely have a deleterious impact on the hydrological regime of the river and the surrounding riparian ecosystems. Currently, this area has no legal protection. Therefore, the need for additional herpetological surveys in the Song Giang River Valley is not only urgent but crucial for estimating biodiversity and importance of this region for nature conservation in southern Vietnam. Given the relatively small estimated range of these three new species, the increasing threats to their habitat requires additional field work to clarify the extent of their distribution, population trends, and conservation status. Because these are the only known specimens of *L. truongi* **sp. nov.** and *L. anakradaya* **sp. nov.**, we suggest they should be categorized as Data Deficient (DD) according to the IUCN's Red List categories (IUCN Standards and Petitions Committee, 2017).

Acknowledgements

We thank the Bureau of Forestry, Ministry of Agriculture and Rural Development of Vietnam and of administration of Khanh Hoa Province for permitting the fieldwork. Anh The Nguyen thanks Doan Manh Tuan, Vu Long (Center for Biodiversity conservation and Endangered Species) and Bui Huu Manh for numerous support and assistance in during the field surveys. Sincere thanks go to Dr. Le Khac Quyet (Fauna & Flora International), Dr. Nguyen Thien Tao (Institute of Genome Research), Dr. Hoang Minh Duc, and Tran Van Bang (Southern Institute of Ecology). Tang Van Duong expresses his gratitude to Leaders of Vietnam National Museum of Nature (VNMN) for supporting his work in Lab. Molecular analysis of this research were supported by Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 18/2020/STS02.

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