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
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
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*Limnonectes* Fitzinger is a specious genus of frog. This primarily Asian radiation is distributed from southern and eastern China, northeast India, southern Japan, mainland southeast Asia, the Sunda islands, the Philippines, East Timor and Papua New Guinea (Frost 2024). The Banna fanged frog (*Limnonectes bannaensis*) was described as a new species to science in 2007 and belongs to the “*L. kuhlii*” species group (Group I *sensu* Suwannapoom *et al.* 2016). *Limnonectes bannaensis* is assessed as Least Concern by the IUCN (IUCN SSC Amphibian Specialist Group 2022). The species is associated with swamps and streams in tropical forest at elevations between 320 and 1100 m (IUCN SSC Amphibian Specialist Group 2022) and has been reported from southern and western Yunnan Province and southern Guangxi Province in China, Houaphanh and Phongsaly provinces in Lao People's Democratic Republic, Nan Province in Thailand and Vinh Phuc, Quang Tri, Thanh Hoa, Cao Bang, Phu Yen, Quang Ninh, Lai Chau and Thua Thien Hue provinces in Vietnam (Jestrzemski *et al.* 2013; Luu *et al.* 2013; Gawor *et al.* 2016; Pham *et al.* 2017, 2018; Luong *et al.* 2021; Suwannapoom *et al.* 2021). However, it has been suggested that *L. bannaensis* is a complex of cryptic species and that populations from the southern part of the reported range may represent an undescribed species (IUCN SSC Amphibian Specialist Group 2022). It breeds by larval development and tadpoles at Gosner Stages 32–35 were described in the original species description (Ye *et al.* 2007); later, tadpoles from Nghe An Province in north-central Vietnam were described based on larvae at Gosner Stage 25 (Cao *et al.* 2020). During field work in Thua Thien Hue Province we encountered several *Limnonectes* tadpoles and their distinct colouration did not match the current descriptions of larval *L. bannaensis*.

We present a description of the tadpole *L. bannaensis* from the extreme south of their known range based on 12 specimens (ITBCZ 3636–47) collected at night in evergreen forest in Loc Tien Commune, Phu Loc District, Thua Thien Hue Province, Vietnam (N16.23319, E107.99256, 134 m asl; Fig. 1A) on the 08<sup>th</sup> April, 2019. At the time of collection, the air temperature was 27.0°C, water temperature 25.4°C and ambient humidity was 89.8% (measured by a Kestrel 3500 weather meter). The tadpoles were collected from a small pool beside a large stream. The puddle had a 0.5 m radius, and a maximum depth of 0.2 m. At the time of collection, approximately 15 tadpoles were active and feeding. The substrate consisted of rocks and stone and a layer of submerged leaf litter (Fig. 1B). Adult *L. bannaensis* were not seen at the collection site. A further 8 specimens (ITBCZ 3628–35) collected at night in evergreen forest in Sao La A Luoi Nature Reserve, A Luoi District, Thua Thien Hue Province, Vietnam (N16.11176, E107.47611, 458 m asl; Figs. 1A and 1B) on the 15<sup>th</sup> of January 2024 at 21:15. At the time of collection the air temperature was 20.1 °C, the water temperature was 19.2 °C and ambient humidity was 99.2% (measured by Kestrel 3500 weather meter). The tadpoles were collected from a puddle in a dry stream bed. The puddle had a 0.5 m radius, and a maximum depth of 0.2 m. At the time of collection, approximately 11 tadpoles were active and feeding. The substrate consisted of rocks and stone and a layer of submerged leaf litter (Fig. 1B). Adult *L. bannaensis* were not seen at the collection site.

Specimens were photographed in life before being euthanised using a 20% solution of benzocaine which was dissolved in stream water held in food grade plastic bags of water containing live tadpoles. Tissue samples (tail clips) for molecular analyses were extracted from freshly euthanised specimens and stored in absolute ethanol prior to fixation of specimens with 10% formalin for 24 hours and subsequent storage in absolute ethanol. Specimens were subsequently deposited at the Institute of Tropical Biology Zoological Collection (ITBCZ), Ho Chi Minh City, Vietnam.

Total genomic DNA was extracted from ethanol-preserved tissues using a DNeasy® Blood and Tissue Kit (QIAGEN GmbH, Hilden, Germany), following the manufacturer's protocols for purification of genomic DNA from animal tissues. We amplified a section (550 bp) of 16S (mtDNA) using the primers 16SL2021 (5' CCTACCGAGCTTAGTAATAGCTGGT-3') modified from Hedges (1994) 16SH1 (5'-CTCCGGTCTGAACTC AGATCACGTAGG-3') by Hedges & Maxson (1993). PCR amplification was carried out in 25-μL reactions volume, including 12.5-μL 2 x ES Master Mix (CW BIO, China), 0.75-μL of each primer (10 pmol/μL), 1-μL of cDNA template, and 10-μL H<sub>2</sub>O. Negative controls were included in each PCR batch. Thermocycling was performed on an Eppendorf Mastercycler EpS (Eppendorf, Hamburg, Germany) under the following conditions: initial denaturation 94°C (5 mins), followed by 35 cycles of 94°C (1 min) denaturation, 55°C (1 min) annealing and 72°C (1 min) extension, followed by a final extension step at 72°C (10 mins). All PCR products were purified using ExoSap-IT™ (USB Corporation, Ohio USA), and sequenced in both 5' and 3' directions at Macrogen (Seoul, South Korea). Sequence chromatograms were edited and checked by eye for quality using BioEdit V. 7.0.5.3 (Hall 1999). The new sequences were then checked on BLAST (The National Center for Biotechnology Information) (Altschul *et al.* 1990) to verify their approximate identity and sequences were deposited in GenBank. Tadpoles at both sites were assigned to *L. bannaensis* based upon mitochondrial 16S RNA sequences taken from the tails of ITBCZ 3628 (GenBank accession number PQ757240) collected from Sao La A Luoi Nature Reserve and ITBCZ 3638 (GenBank accession number PQ757247) collected from Loc Tien Commune. Uncorrected p-distances of 16S mtDNA between these two specimens was 0.19%. Uncorrected p-distances of 16S mtDNA between the 2 tadpoles collected from Thua Thien Hue Province and from *L. bannaensis* collected from their approximate type locality 1350 km to the northwest, in Xishuangbanna, Yunnan Province, China (KIZ011728, GenBank accession number KU599852) differed by 1.7–1.9%. Uncorrected p-distance (with partial deletion of gaps and missing data) was calculated using MEGA 11 (Tamura *et al.* 2021).

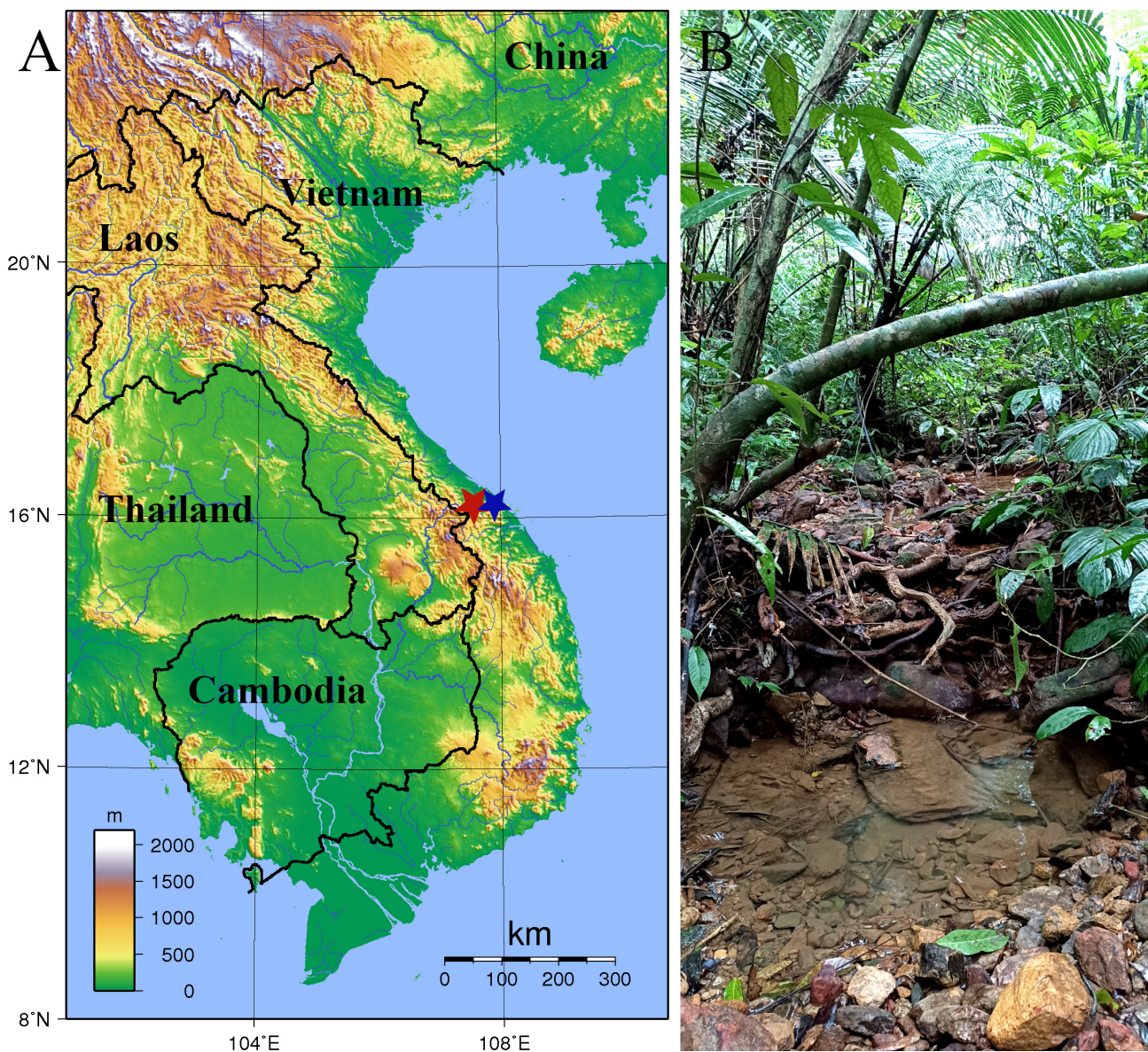
We used ImageJ 1.49 (Schneider *et al.* 2012) to measure preserved tadpoles from photographs. Staging followed the Gosner (1960) table and we used Altig & McDiarmid (1999) for the terminology for tadpole description. Measurements followed McLeod (2008) and include: body length (BL), tail length (TL), total length (ToL), body width (BW), body height (BH), eye diameter (LED), interorbital distance (LIO), internarial distance (LIN), naris–snout distance (LNS), eye–naris distance (LEN), spiracle tube width (SW), tail muscle height at body junction (TMH1) and mid length (TMH2), and tail height (TH).

**TABLE 1.** Measurements (in mm) of *Limnnectes bannaensis* tadpole specimens: range (average).

Character	Stage 26 (N=2)	Stage 27 (N=4)	Stage 28 (N=1)	Stage 31 (N=3)	Stage 32 (N=2)	Stage 34 (N=3)	Stage 35 (N=4)	Stage 41 (N=1)
BL	8.3–8.8 (8.6)	8.5–9.6 (8.8)	9.2	10.5–12.2 (11.2)	11.6–12.2 (11.9)	12.3–12.9 (12.6)	12.6–13.9 (13.0)	14.2
TL	13.9–15.2 (14.6)	14.1–15.4 (14.9)	15.7	19.2–22.0 (20.7)	21.6–22.6 (22.1)	25.5–27.7 (26.6)	24.9–28.3 (26.1)	33.5
ToL	22.0–24.0 (23.0)	22.6–25.0 (23.6)	24.9	31.4–32.9 (31.9)	33.8–34.2 (34.0)	37.8–40.0 (38.8)	37.5–42.2 (39.1)	47.7
BW	4.9–5.0 (5.0)	4.7–5.5 (5.0)	5.1	6.4–6.7 (6.6)	7.0–7.1 (7.1)	6.9–7.4 (7.1)	4.3–7.8 (6.8)	8.8
BH	2.6–2.7 (2.7)	2.7–3.1 (2.9)	2.7	3.2–3.7 (3.4)	3.5–3.8 (3.7)	3.7–3.8	2.4–4.3 (3.6)	4.4
LED	0.9 (-)	0.9–1.0 (0.9)	0.9	0.9–1.2 (1.0)	1.1–1.2 (1.2)	0.9–1.1 (1.1)	1.2–1.3 (1.3)	1.2
LIO	1.6 (-)	1.5–1.7 (1.6)	1.8	2.1–2.2 (2.2)	2.1–2.2 (2.2)	2.1–2.2 (2.1)	1.2–2.6 (2.0)	2.5
LIN	1.5 (-)	1.5–1.6 (1.5)	1.3	1.8–1.9 (1.8)	1.9 (-)	1.4–1.9 (1.7)	0.9–2.9 (1.7)	1.7
LNS	1.2 (-)	1.0–1.3 (1.2)	0.8	1.3 (-)	1.3–1.4 (1.4)	0.9–1.4 (1.1)	0.8–1.5 (1.2)	1
LEN	0.8–0.9 (0.9)	0.8–0.9 (0.9)	1.0	1.1–1.3 (1.2)	1.2–1.3 (1.3)	1.1–1.5 (1.3)	0.9–1.8 (1.4)	1.5
SW	0.2 (-)	0.2–0.3 (0.3)	0.3	0.3 (-)	0.3 (-)	0.2–0.3 (0.2)	0.2–0.3 (0.3)	0.3
TMH1	2.0–2.1 (2.1)	2.2–2.4 (2.3)	2.4	2.4–3.0 (2.7)	2.7 (-)	2.9–3.0 (3.0)	2.9–3.0 (3.0)	3.5
TMH2	1.7–1.8 (1.8)	1.7–1.9 (1.8)	2.0	2.1–2.9 (2.4)	2.4 (-)	2.7–3.1 (2.9)	3.0–3.2 (3.1)	3.4
TH	3.4–3.8 (3.6)	3.6–4.0 (3.9)	4.0	4.7–5.2 (5.0)	5.6–5.9 (5.8)	6.6–6.9 (6.8)	6.7–7.0 (6.8)	6.5



The description of tadpole morphology is based on 20 specimens collected in Sao La A Luoi Nature Reserve and Phu Loc District (Figs. 1–4, Table 1) at Stages 26–41. Body elongated (BW 31.0–62.0% BL), and depressed (BH 46.0–61.0% of BW); snout rounded (Figs. 2–4); nares anterodorsally positioned, typically closer to eyes than to tip of snout, rim not raised; eyes positioned dorsolaterally, oriented laterally, comparatively small (LED 7.0–11.0% of BL); conical tube shaped sinistral spiracle, positioned anteriorly to the widest part of the body, closer to eye than to end of body, fused to body with a free short distal portion; tail muscle long, tail length approximately twice body length (TL 157.0–236.0% of BL) pointed tail tip, tail musculature distinct, muscle height greatest at tail base and gradually tapers towards tail tip, tail fins prominent, both upper and lower tail fins reach maximum height approximately half way along tail length; cup-like, emarginated oral disc, anteroventrally positioned, fringed with single row of short, lobed papillae interrupted in a wide dorsal gap and a short ventral gap. Two upper rows of keratinised labial teeth with medial gap in 2<sup>nd</sup> row; three lower rows of keratinised labial teeth with a medial gap in 1<sup>st</sup> row [labial tooth row formula 2(2)/3(1)]; jaw sheaths black, robust; upper jaw sheath broadly curved, with distinctly serrated edges and a distinct medial convexity, lower jaw sheath developed with distinctly serrated edges (Fig. 4).



**FIGURE 1.** (A) Map of Vietnam, Cambodia, northeast Thailand, Laos and southern China. The red star represents the tadpole collection site in Sao La A Luoi Nature Reserve, Thua Thien Hue Province. The blue star represents the tadpole collection site in Loc Tien Commune, Thua Thien Hue Province; (B) microhabitat of *Limnonectes bannaensis* tadpoles in Sao La A Luoi Nature Reserve, Thua Thien Hue Province, Vietnam.





**FIGURE 2.** *Limnionectes bannaensis* tadpole (preserved, ITBCZ 3633, Stage 27) in (A) lateral, (B) dorsal, and (C) ventral views. Scale bar = 5 mm.

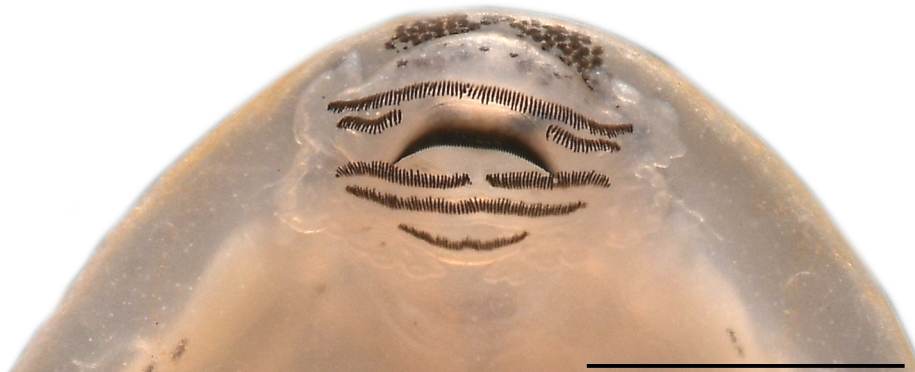


**FIGURE 3** *Limnionectes bannaensis* tadpoles in life: lateral, dorsal, and ventral views of tadpole ITBCZ 3628, Stage 31 (A–C) and ITBCZ 3629, Stage 28 (E–F). Scale bar = 5 mm.

**Colouration.** In preservative (Fig. 2), body and tail cream with dark brown flecks which increase in density on the dorsal surface of body posterior to eyes, these flecks form a brown bar running from anterior edge of eye to snout; tail fin similar to colouration in life; pupil milky, iris black. In life (Fig. 3), light brown body with dark brown flecks which increase in density on dorsal surface of body posterior to eyes, these flecks form a brown bar running from anterior edge of eyes, joining at tip of snout; oral disc translucent; whitish neuromasts arranged in two mirroring lines running from snout between nares and eyes, running dorsolaterally along body before continuing along lateral surfaces of tail muscle, whitish neuromasts surround each eye; pupil black and round; iris orange, speckled with black dots; internal gills distinctly reddish; tail beige-brown, upper and lower tail fins cream, tail fins

with two equally spaced, bold, broad, black bars, stripes and in some individuals, blotches; one saddle at body tail junction, other immediately after it. These saddles extend to stripes on latter half of tail; translucent skin on ventral surface of body, gut coil clearly visible.

Variation. Tail tips missing in ITBCZ 3639 and ITBCZ 3642. The colouration of *L. bannaensis* tadpoles collected from Thua Thien Hue Province in this study differs from those collected further north from their type locality and from Nghe An Province. The equally spaced, bold, broad, black bars and blotches are absent in these populations (Ye *et al.* 2007; Cao *et al.* 2020). In the original species description, tadpoles we assigned to *L. bannaensis* without associated molecular data and only the second half of the tadpole tail is reported as being black (Ye *et al.* 2007); however this is not apparent in the associated figure (Fig. 2B of Ye *et al.* 2007). Cao *et al.* (2020), who confirmed species identification with molecular data, report 4–5 black stripes on the tail fins but the associated figure (Fig. 6) shows a tadpole at Stage 25 with diffuse black vertical markings rather than bold, broad, black bars and blotches. Tadpoles we collected in this study have a wide dorsal gap and a short ventral gap in the papillae which fringe the oral disc; the short ventral gap is not present in the figure of the oral disc in Cao *et al.* (2020).



**FIGURE 4.** Oral disc of *Limnonectes bannaensis* tadpole ITBCZ 3628 in life, Stage 31. Scale bar = 2 mm.

We compared the tadpole of *L. bannaensis* with other *Limnonectes* tadpoles reported from mainland southeast Asia where species identity has been confirmed with molecular data. The tadpole of *L. bannaensis* differs from the tadpole of *L. dabanus* (Smith) by the presence of bold black vertical stripes on the tail (absent in *L. dabanus* at Gosner Stages 29 and 31; Rowley *et al.* 2014); from *L. isanensis* by the presence of bold black vertical stripes on the tail (vs. absent in *L. isanensis* at Gosner Stage 38; Ampai *et al.* 2015); from *L. megastomias* McLeod by the presence of bold black vertical stripes on the tail (vs. absent in *L. megastomias* at Gosner Stage 40; McLeod 2008); from *L. nguyenorum* McLeod, Kurlbaum & Hoang by having a larval tooth row formula 2(2)/3(1) [vs. 1/3(1–2) in *L. nguyenorum* at Gosner Stage 28; Ziegler *et al.* 2015] and the presence of bold black vertical stripes on the tail (absent in *L. nguyenorum* at Gosner Stages 25–29); and from *L. selatan* Matsui, Belabut & Ahmed by a short ventral gap in marginal papillae (no ventral gap in *L. selatan* at Gosner Stage 40) and by the presence of bold black vertical stripes on the tail (vs. dark bands in *L. selatan*).

This is the only *Limnonectes* species currently known to exhibit such bold black striped tail colouration, although dark bars are present in *L. selatan* and tadpoles putatively assigned to *L. utara* Matsui, Belabut & Ahmed. A similar pattern has been reported in other tadpoles, such as in the hylids *Pseudis platensis* (Dixon *et al.* 1995), *Litoria micromembrana* (Altig & Channing 1993) and *Xenohyla truncata* (Dias *et al.* 2023). The bold colouration on the tails of tadpoles has been suggested to attract the attention of potential predators to less vital regions of the body (e.g., Thibaudeau & Altig 2012), to confuse predators, or to provide camouflage in linearly arranged habitats (Altig & Channing 1993). Elucidating the function of the bold black vertical bars on the tail of *L. bannaensis* tadpoles would require field experiments.

We are sincerely grateful to the management board of Sao La Thua Thien Hue NR for issuing the relevant permissions to undertake field work and the staff of Sao La Thua Thien Hue NR (Ky Tran and Hoa Nguyen for their support during fieldwork. Luan Nguyen would like to thank Ba Dinh Vo, Manh Van Le, Sang Ngoc Nguyen for their assistance in the field in Suoi Voi (Hue), and Cleveland Metro Parks Zoo, USA (CMZ) for Asian Turtle Program (ATP) of Indo Myanmar Conservation (IMC).

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