

**FIRST RECORD OF *Limnonectes fastigatus* STUART, SCHOEN, NELSON,  
MAHER, NEANG, ROWLEY & MCLEOD, 2020 (ANURA:  
DICROGLOSSIDAE) FROM VIETNAM**

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**ABSTRACT**

We record *Limnonectes fastigatus* for the first time from Vietnam based on a new amphibian collection from Chu Mom Ray National Park, Kon Tum province. Morphological characteristics of the specimens from Chu Mom Ray National Park resemble those of the type series from Cambodia. The specimens from Vietnam differ from the type series from Cambodia by having a slightly larger size in males and a distinct colour pattern on the dorsum. Genetic divergences between sequences of the Vietnamese specimens and those of *Limnonectes fastigatus* from Cambodia available from GenBank are 2.0–2.1% (16S gene). In addition, we provide morphological data and natural history notes of the aforementioned species from Vietnam.

**Keywords:** *Limnonectes fastigatus*, morphology, molecular divergence, Chu Mom Ray National Park.

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## INTRODUCTION

Members of *Limnonectes* Fitzinger, 1843 have a wide distribution in Asia from eastern and southern China, eastwards to Japan, throughout Indochina and southwards to Malaysia, Indonesia, the Philippines, and New Guinea (Frost, 2021). *Limnonectes* is one of the most diverse groups of amphibians with 78 currently recognized species and 20 of which have been described in the last ten years (Frost, 2021). In Vietnam, four species of the *Limnonectes kuhlii* group were recently discovered, namely *L. nguyenorum* McLeod, Kurlbaum & Hoang, 2015 from Ha Giang province, *L. quangninhensis* Pham, Le, Nguyen, Ziegler, Wu & Nguyen, 2017 from Quang Ninh province, *L. kiziriani* Pham, Le, Ngo, Ziegler & Nguyen, 2018 from Quang Binh, Thua Thien Hue, and Gia Lai provinces, and *L. phuyenensis* Pham, Do, Le, Ngo, Nguyen, Ziegler & Nguyen, 2020 from Phu Yen province (McLeod et al., 2015; Pham et al., 2017, 2018, 2020).

Based on newly collected specimens from the Central Highlands in Vietnam, we herein record *Limnonectes fastigatus* Stuart, Schoen, Nelson, Maher, Neang, Rowley & McLeod, 2020 for the first time from Vietnam. This species was originally described from Virachey National Park, Ratanakiri province, Cambodia.

## MATERIALS AND METHODS

Two field surveys were conducted in Chu Mom Ray National Park, Sa Thay district, Kon Tum province in April 2014 and in September 2019 (Figs. 1, 2). Specimens were collected between 19:00 and 23:00. After taking photographs in life, specimens were euthanized in a closed vessel with a piece of cotton wool containing ethyl acetate (Simmons, 2002), fixed in 80% ethanol for five hours, and then transferred to 70% ethanol for permanent storage. Tissue samples were preserved separately in 70% ethanol prior to fixation. Voucher specimens referred to in this paper were deposited in the collection of the Institute of Ecology and Biological Resources (IEBR), Ha Noi, Vietnam.



Figure 1. Map showing the distribution of *Limnonectes fastigatus*. 1) The type locality in Cambodia, 2) New record in Vietnam

Morphological characters. Measurements were taken with a digital caliper to the nearest 0.1 mm. Morphological terminology followed McLeod (2008) and Matsui et al. (2010). The following abbreviations were used: SVL: snout-vent length, HL: head length (measured as a parallel line with the vertebral column from posterior margin of mandible to tip of snout), HW: maximum head width (at rictus), BL: body length, calculated as SVL minus HL, RL: rostral length (from anterior corner of orbit to tip of snout), NS: distance from nostril to tip of snout, EN: distance from anterior corner of orbit to nostril, IND: internarial distance, IOD: interorbital distance, ED: eye diameter, UEW: maximum width of upper eyelid, DAE: distance between anterior margins of orbits, MN: posterior margin of mandible to nostril, MFE: posterior margin of mandible to anterior margin of orbit, MBE: posterior margin of mandible to posterior margin of eye, DPE: distance between posterior margins of orbits, OH: odontoid

height (perpendicular distance between the ventral border of mandible and the tip of odontoid process), UAL: upper arm length (from axilla to elbow), FAL: fore arm length (from elbow to tip of third finger), FL1–4 (from inner to outer): finger length I–IV, OPT: outer palmar tubercle length, IPT: inner palmar tubercle length, NPL: nuptial pad length - finger I, FeL: femur length (from vent to knee), TbL: tibia length (from knee to tarsus), TbW: maximum tibia width, FoL: foot length (from tarsus to tip of fourth toe), TL 1–5: toe length I–V, IMT: inner metatarsal tubercle length. For the webbing formula, we followed Glaw & Vences (2007). Sex was determined by gonadal inspection.

**Molecular analysis.** The tissue samples of *Limnonectes* were extracted using QIAamp DNA Mini kit (Qiagen, Germany) following protocols by the manufacturer. Total DNA was then amplified by HotStar Taq Mastermix (Qiagen, Germany). The standard PCR conditions were 95 °C for 15 min to active Taq; 35 cycles at 95 °C for the 30s, 50 °C for 45s, 72 °C for 1 min; and a final elongation at 72 °C for 10 min. The PCR volume contained 2 µL of each primer at 10 µmol/µL, 5 µL water, 10 µL of Mastermix and 2 µL DNA template. The primers used to amplify a fragment of the mitochondrial DNA 16S gene were AR (5'-CGCCTGTTTATCAAAA CAT-3') and BR (5'-CCGGTCTGAACTCA GATCACGT-3') (Palumbi et al., 1991). PCR products were visualized using electrophoresis through a 1% agarose gel, marker 1kb, in 1X TBE and stained with ethidium bromide and photographed under UV light. Successful amplifications were purified using GeneJet PCR Purification Kit (ThermoFisher Scientific, Lithuania). Cleaned PCR products were sent to 1<sup>st</sup> Base (Malaysia) for sequencing in both directions using the same primers. Sequences were validated with Sequencher v4.10 (Gene Codes, Ann Arbor, MI) with default setting and compared with data available on GenBank using BLAST Tool as implemented in the National Center for Biotechnology Information (NCBI, <https://blast.ncbi.nlm.nih.gov/>). The

sequences were uploaded on Genbank under accession numbers OM948736, OM948737, and OM948738.



Figure 2. Habitat of *Limnonectes fastigatus* in Chu Mom Ray National Park, Kon Tum province. a) Evergreen forest; b) Microhabitat of a stream

## RESULTS

### *Limnonectes fastigatus* Stuart, Schoen, Nelson, Maher, Neang, Rowley & McLeod, 2020

Specimens from Kon Tum province, Vietnam were identified as *Limnonectes fastigatus* based on morphological and molecular data in comparison with those of the type series from Cambodia.

*Specimens examined* (n = 7). Two adult males (IEBR A.4834, 4835) and one adult female (IEBR A.4836) collected by C. T. Pham on 1 April 2014, in the evergreen forest

near Ba Goc Ranger Station (14°26.24'N, 107°43.562'E, at an elevation of 978 m); two adult males (IEBR A.4837, 4838) and two adult females (IEBR A.4839, 4940) collected by C. T. Pham, T. D. Tran, and T. Q. Phan on 19 September 2019 in the evergreen forest near Rescue Center (14°27.237'N, 107°43.951'E, at an elevation of 1420 m), Chu Mom Ray National Park, Sa Thay district, Kon Tum province.

**Molecular analyses.** We successfully generated three sequences of 560 bp (16S gene) from specimens IEBR A.4837, IEBR 4839, and IEBR A.4840. They were identical and 97.9–98.0% similar to several sequences of *Limnonectes fastigatus* with the following GenBank accession numbers: MT459154, MT459155, MT459156, MT459157.

**Description.** Morphological characters of specimens from Kon Tum province agreed well with the original description of Stuart et al. (2020) (Figs. 3–5). Size large (SVL 69.1–77.8 mm, mean  $71.7 \pm 4.1$  mm,  $n = 4$  males; SVL 50.5–59.4 mm, mean  $56.4 \pm 5.1$  mm,  $n = 3$  females); habitus robust with moderately enlarged head (HL/SVL 0.45–0.46, HW/SVL

0.46–0.49 in males, HL/SVL 0.40–0.42, HW/SVL 0.41–0.44 in females); head slightly broader than long (HL 30.9–35.5 mm, HW 32.0–37.6 mm in males; HL 20.8–25.1 mm, HW 21.1–25.9 mm in females); snout round anteriorly in dorsal view, projecting beyond lower jaw; nostril lateral, about midway snout tip and eye (NS 4.8–5.2 mm, EN 5.2–5.4 mm in males; NS 3.7–4.2 mm, EN 3.7–4.3 mm in females); canthus rostralis indistinct; loreal region oblique and slightly concave; rostral length greater than eye diameter (RL 9.9–10.6 mm, ED 7.1–8.9 mm in males; RL 7.4–8.6 mm, ED 6.3–7.0 mm in females); interorbital distance wider than internarial distance and upper eyelid width (IOD 7.1–8.0 mm, IND 5.9–6.2 mm, UEW 3.7–4.7 mm in males; IOD 4.7–6.2 mm, IND 4.2–5.3 mm, UEW 3.0–4.0 mm in females); tympanum invisible; vomerine teeth in two oblique ridges; tongue cordiform, notched posteriorly; lower jaw with two tooth-like, odontoid processes robust thin and elongate with rounded tips (OH 4.8–5.8 mm in males, OH 2.4–3.0 mm in females); external vocal sac absent.

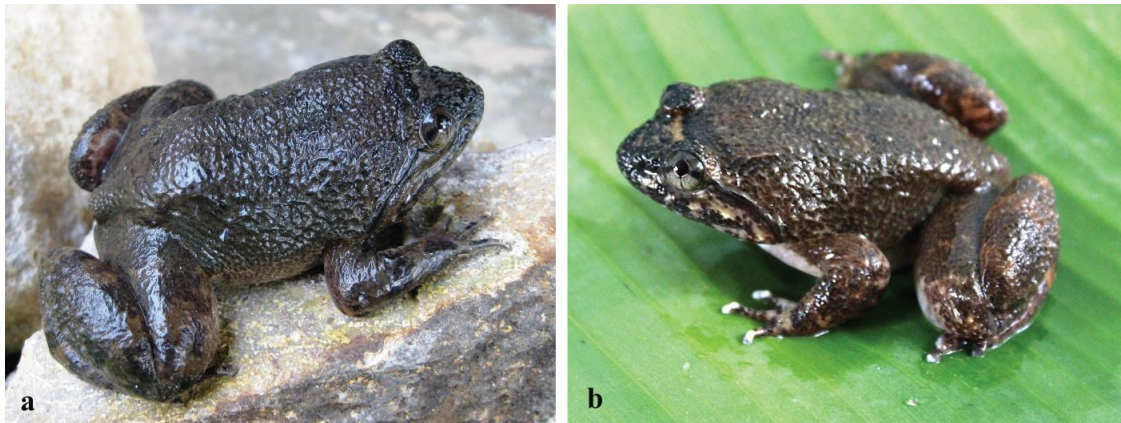


Figure 3. Dorsolateral view of *Limnonectes fastigatus* in life: a) IEBR.A 4836 (female); b) IEBR.A 4834 (male)

Forelimbs. Arms short; upper arm length (UAL 10.6–12.7 mm in males, 8.2–11.2 mm in females), forearm length (FAL 27.1–32.7 mm in males, 20.5–23.7 mm in females); relative finger lengths: I < II < IV < III;

fingers free of webbing; dermal ridge on sides of fingers II and III; tips of fingers blunt, not expanded; subarticular tubercles prominent, round, formula 1, 1, 2, 2; inner metatarsal tubercle large, oval; outer

metatarsal tubercle small, elongate; finger I of males with nuptial pad, composed of minute spines on the dorsal surface and medial edge.

Hind limbs. Tibia length shorter than thigh length (FeL 32.5–36.9 mm, Tbl 28.5–32.3 mm in males; FeL 26.6–28.5 mm, Tbl 22.5–26.8 mm in females), approximately 2.2–2.4 times longer than wide; tips of toes blunt, slightly round; relative length of toes: I < II < V < III < IV; toes webbed to the middle of terminal phalanx at base of toe pad, webbing formula  $I0^+-0^+II0^+-0^+III0^+-0^+IV0^+-0^+V0^+$ ; dermal ridge on outer sides of toes I and V; subarticular tubercles prominent,

elongate, formula 1, 1, 2, 3, 2; inner metatarsal tubercle elongate; outer metatarsal tubercle absent; tibio-tarsal articulation reaching behind the eye.

Skin texture in life. Dorsal surface of head and body crenulate; small tubercles on the upper eyelid; flanks, around cloaca, and dorsal surface of limbs and thighs with small tubercles; supratympanic fold distinct, extending from eye to angle of the jaw; dorsolateral fold absent; dorsal surface of tibia and foot distinctly tuberculate, covered with moderately dense, small, tubercles; throat, chest, belly and ventral surface of thighs smooth.

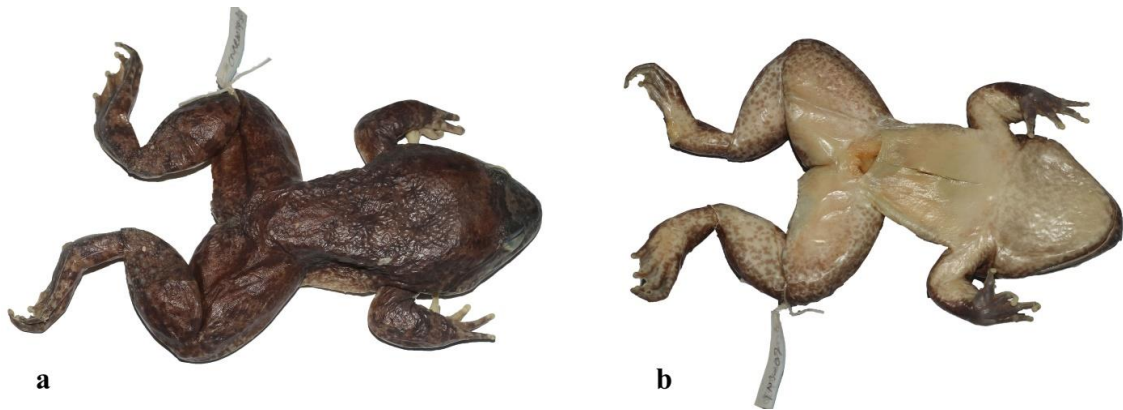


Figure 4. *Limnonectes fastigatus* (IEBR.A 4834, male) in preservative: a) Dorsolateral view; b) Ventral view

Coloration in life. Iris light brass with dark gray-brown lines bisecting eye vertically and horizontally through pupil; head with a pale yellow bar in the anterior interorbital region and a narrow dark brown bar in the posterior interorbital region; dorsum and flanks with dark gray-brown marking; lips with dark bars; supratympanic fold black; dorsal surface of limbs irregular, yellowish-brown with dark crossbars; ventral surface of limbs, throat and chest white with brown markings; belly immaculate white; toe webbing brown; the tip of fingers and toes white.

*Ecological notes.* The specimens were found between 19:00 and 23:00 in the water or on the ground in rocky streams (Fig. 2b).

The surrounding habitat was a mixed secondary evergreen forest of the large, medium, and small hardwoods and shrubs (Fig. 2a).

*Distribution.* The species was previously known only from Virachey National Park in Ratanakiri and Stung provinces, northeastern Cambodia (Stuart et al., 2020). The new record of this species in the Central Highlands of Vietnam is approximately 40 to 70 km distant from the locality in Cambodia (Fig. 1).

*Remarks.* The specimens from Vietnam have a slightly larger size in males (SVL 69.1–77.8 mm, mean  $71.7 \pm 4.1$  mm,  $n = 4$  males vs. 50.3–70.3 mm, mean  $59.7 \pm 7.8$  mm,  $n = 7$  in specimens from Cambodia) and different color on dorsum (dark gray-brown

marking vs. golden brown with dark gray-brown marking in specimens from Cambodia). Specimens from Cambodia were collected at an elevation from 160 to 650 m (Stuart et al., 2020), while specimens from Vietnam were collected at a higher elevation from 978 to 1420 m elevation (Stuart et al., 2020; this study).



Figure 5. *Limnonectes fastigatus* (IEBR.A 4834, male): a) Shape of odontoid processes; b) Right hand; c) Right foot

## DISCUSSION

Our findings bring the total species number of the genus *Limnonectes* to 10 in Vietnam, namely *Limnonectes bannaensis* Ye, Fei, Xie & Jiang; *L. dabanus* (Smith); *L. fastigatus*; *L. gyldenstolpei* (Andersson); *L. khammonensis* (Smith); *L. kiziriani*; *L. nguyenorum*; *L. phuyenensis*; *L. poilani* (Bourret); and *L. quangninhensis* (Nguyen et al., 2009; Pham et al., 2017, 2018, 2020; Frost, 2021). Our preliminary data showed

that Hai Van Mountain Pass, situated between Thua Thien Hue province and Da Nang City, is the southernmost record of *L. bannaensis* in Vietnam (Pham et al., 2018). Other populations to the South of Hai Van Mountain Pass should be assigned to the recently described species, *L. kiziriani* and *L. phuyenensis* (Pham et al., 2018, 2021), or represent new species. The previous record of *Limnonectes* cf. *bannaensis* from Chu Mom Ray National Park, Kon Tum province by Jestrzowski et al. (2013) should be re-identified as *L. fastigatus*.

*Limnonectes fastigatus* is morphologically similar to *L. kiziriani* and *L. phuyenensis*, but it differs from *L. kiziriani* by having significantly larger mean total body sizes of both sexes, with SVL of adult males 50.3–70.3 mm, mean  $59.7 \pm 7.8$ ,  $n = 7$  (vs. 42.7–53.9 mm, mean  $48.6 \pm 4.5$ ,  $n = 7$  in *L. kiziriani*) and adult females 48.9–67.4 mm, mean  $59.7 \pm 6.0$ ,  $n = 6$  (vs. 40.2–55.0 mm, mean  $49.1 \pm 4.6$ ,  $n = 12$  in *L. kiziriani*); by having significantly larger mean body lengths of both sexes, with BL of adult males 29.6–38.0 mm, mean  $34.3 \pm 3.4$ ,  $n = 7$  (vs. 23.9–29.4 mm, mean  $26.6 \pm 2.4$ ,  $n = 7$  in *L. kiziriani*), adult females 27.9–41.3 mm, mean  $36.1 \pm 4.6$ ,  $n = 6$  (vs. 23.2–32.9 mm, mean  $29.1 \pm 3.1$ ,  $n = 12$  in *L. kiziriani*), and by having a significantly different male head shape; from *L. phuyenensis* by having a different odontoid processes shape (thin and elongated vs. robust with very sharp tips in *L. phuyenensis*), nostril midway between snout tip and eye (vs. nostril closer to tip of snout than to eye in *L. phuyenensis*), and belly creamy white in preservative (vs. belly with brown flecking or vermiform marking in *L. phuyenensis*) (Pham et al., 2018, 2020; Stuart et al., 2020; this study).

Genetically, the new population from Vietnam is less than 2.5% divergent from the type series from Cambodia, while the distance between the species pair of *L. fastigatus* and *L. kiziriani* is  $> 4.0\%$  based on the same fragment of the mitochondrial 16S gene

(Pham et al., 2018). We consider the molecular divergence between Vietnamese and Cambodian populations within the intraspecific level.

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## REFERENCES

- Frost D. R., 2021. Amphibian Species of the World: an on-line reference. Version 6.0. American Museum of Natural History, New York, USA. Available from <https://amphibiansoftheworld.amnh.org/> (accessed on 10 March 2021).
- Glaw F., Vences M., 2007. A field guide to the amphibians and reptiles of Madagascar. Third Edition, Frosch Verlag, Cologne, pp. 496.
- Jestrzemski D., Schütz S., Nguyen T. Q., Ziegler T., 2013. A survey of amphibians and reptiles in Chu Mom Ray National Park, Vietnam, with implications for herpetofaunal conservation. *Asian Journal of Conservation Biology*, 2: 88–110
- Matsui M., Panha S., Khonsue W., Kuraishi N., 2010. Two new species of the “*kuhlii*” complex of the genus *Limnonectes* from Thailand (Anura: Dicroglossidae). *Zootaxa*, 2615: 1–22.
- McLeod D. S., 2008. A new species of big-headed, fanged dicroglossine frog (Genus *Limnonectes*) from Thailand. *Zootaxa*, 1807: 26–46.
- McLeod D. S., Kurlbaum S., Hoang N. V., 2015. More of the same: a diminutive new species of the *Limnonectes kuhlii* complex from northern Vietnam (Anura: Dicroglossidae). *Zootaxa*, 3947: 201–214. <https://doi.org/10.11646/zootaxa.3947.2.4>
- Palumbi S. R., Martin A., Romano S., McMillan W. O., Stice L., Grabowski G., 1991. The simple fool’s guide to PCR. Department of Zoology and Kewalo Marine Laboratory, Hawaii, pp. 94.
- Pham C. T., Do D. T., Le M. D., Ngo H. N., Nguyen L. T., Ziegler T., Nguyen T. Q., 2020. A new species of *Limnonectes* (Amphibia: Anura: Dicroglossidae) from Vietnam. *Zootaxa*, 4894(3): 387–402. <https://doi.org/10.11646/zootaxa.4894.3.5>
- Pham C. T., Le M. D., Ngo H. T., Ziegler T., Nguyen T. Q., 2018. A new species of *Limnonectes* (Amphibia: Anura: Dicroglossidae) from Vietnam. *Zootaxa*, 4508: 115–130. <https://doi.org/10.11646/zootaxa.4508.1.7>
- Pham C. T., Le M. D., Nguyen T. T., Ziegler T., Wu Z. J., Nguyen T. Q., 2017. A new species of *Limnonectes* (Amphibia: Anura: Dicroglossidae) from Vietnam. *Zootaxa*, 4269: 545–558. <https://doi.org/10.11646/zootaxa.4269.4.8>
- Simmons J. E., 2002. Herpetological collecting and collections management. Revised edition. Society for the Study of Amphibians and Reptiles. *Herpetological Circular*, 31: 1–153.
- Stuart B. L., Schoen S. N., Nelson E. E. M., Maher H., Neang T., Rowley J. J. L., McLeod D. S., 2020. A new fanged frog in the *Limnonectes kuhlii* complex (Anura: Dicroglossidae) from northeastern Cambodia. *Zootaxa*, 4894: 451–473. <https://doi.org/10.11646/zootaxa.4894.3.1>