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NHÀ XUẤT BẢN KHOA HỌC TỰ NHIÊN VÀ CÔNG NGHỆ

THE FIRST RECORDS OF *Limnonectes nguyenorum* McLeod, Kurlbaum & Hoang (Amphibia: Anura: Dicroglossidae) FROM LAO CAI PROVINCE, NORTHWEST VIETNAM

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Abstract: We report Nguyen's fanged frog (*Limnonectes nguyenorum*) for the first time from Lao Cai Province in northwest Vietnam. Specimens were collected in June 2018 from Mount Fansipan in Hoang Lien National Park and identified based on morphological and molecular data. Our record extends the known elevation range of the species (up to 1.300m vs. 1.030m in previous records), brings the number of *Limnonectes* species recorded from Lao Cai Province to three, and is the first record of *Limnonectes nguyenorum* from Mount Fansipan.

Keywords: Fansipan, fanged-frog, Hoang Lien National Park.

INTRODUCTION

The fanged frog genus *Limnonectes* Fitzinger 1843 is comprised of 73 species which are distributed widely in Asia from eastern and southern China, eastwards to Japan, throughout Indochina and southwards into Malaysia, Indonesia, the Philippines, and New Guinea (Frost 2019). To date, 19 species have been reported from Vietnam (Frost 2019).

Nguyen's fanged frog (*Limnonectes nguyenorum* McLeod, Kurlbaum & Hoang 2015) is endemic to northern Vietnam and has been recorded from three provinces (Ha Giang - type locality, Vinh Phuc, and Son La provinces) (McLeod et al. 2015; Ziegler et al. 2015; Pham et al. 2016). This species belongs to the *Limnonectes kuhlii* group, which is known to harbour cryptic species; several new species have been recently described from this group based on a combination of morphological and molecular data (Suwannapoom et al. 2016; Pham et al. 2017, 2018).

Mount Fansipan in the northwest Vietnam is known to support a high diversity of amphibians with more than 80 species recorded to date, including several species that are currently believed to be endemic to the mountain (Ohler et al. 2000; Nguyen et al. 2013; Rowley et al. 2013; Tapley et al. 2017a, 2018). However, only one species of *Limnonectes* has been reported from the mountain, *Limnonectes banaensis* Ye, Fei, Xie, & Jiang (previously known as *L. kuhlii* (Tschudi), Ohler et al. 2000), and only two have been reported from Lao Cai Province (Nguyen et al. 2009). During fieldwork on Mount Fansipan, Hoang Lien National Park (Hoang Lien NP) in June 2018, we encountered several *Limnocectes* in a small stream in Cat Cat Village, Sapa District, Lao Cai Province. Molecular and morphological data confirm the identity of the specimens as *L. nguyenorum* and we herein report the first record of the species on Mount Fansipan and for Lao Cai Province.

MATERIALS AND METHODS

Field work was conducted from 8 to 15 June 2018 in Mount Fansipan, Hoang Lien NP, Sapa District, Lao Cai Province. Specimens were collected at night (N22 19.561 E103 49.805, 1.300 m asl; in datum WGS 84) and photographed in life before being humanely euthanized via application of Orajel (20% benzocaine) to the ventral surface of the frog.

Tissue samples (liver) for molecular analyses were extracted from freshly euthanized specimens and stored in EDTA/DMSO prior to fixation and storage of specimens in 10% formalin and subsequent storage in 70% ethanol. Specimens were deposited at the Hoang Lien NP Museum (HLNP), Sapa, Vietnam.

Measurements were taken with a digital calliper to the nearest 0.1 mm. Morphological terminology followed McLeod (2008): SVL = snout-vent length; HW = head width; HL = head length; FOL = foot length; TBL = tibia length; FEL = femur length; UAL = upper arm length; PAL = palm length; O = outer metacarpal tubercle length; M = middle metacarpal tubercle length; T = thenar tubercle length; RL = rostrum length; EN = eye-nostril distance; ED = eye diameter; TD = tympanum diameter; MN = mandible-nostril distance; OH = odontoid height; IO = interorbital distance; IN = internarial distance; UEW = upper eyelid width.

Total genomic DNA was extracted from the newly collected tissue samples with DNeasy tissue extraction kits (Qiagen), and we used the primers 16SAR and 16SBR of (Palumbi et al. 1991) to amplify 550 base pairs (bp) of the 16S rRNA gene. Standard PC protocols were used and PCR products were purified with ExoSap-IT (USB Corporation, OH, USA). Purified templates were sequenced directly by Macrogen (Seoul, Korea). Sequences were validated with Sequencher 4.10 (Gene Codes, Ann Arbor, MI), aligned with the Clustal option in MEGA 7 (Kumar et al. 2016), and refined by eye. The newly obtained sequence was uploaded on GenBank under accession number MK951684. Mean uncorrected genetic distances (*p*-distances) between sequences and species were calculated using MEGA 7.0.

RESULTS

Molecular confirmation. The male specimen from Mount Fansipan (HLNP 2018.003) was identical to that from an adult holotype *Limnonectes nguyenorum* (VNMN A.2015; GenBank: HM067250). The uncorrected *p*-distance from the two sequences (MK951684 and HM067250) was 0.17 % (528 bp).

Specimens examined: HLNP 2018.003 (adult male) and HLNP 2018.004 (adult female).

Morphological description. Small sized frog, (SVL 41.6 mm in male, 40.5 mm in the) (Figure 1); slightly enlarged head (HL 38% of SVL in male, 40% SLV in female); head shorter than wide in male (HL 83% HW in male, 98% HW in female); canthus rostralis indistinct and rounded, lores flat; distinct supratympanic fold; tympanum not visible; odontoid processes in male robust with rounded tips, angled posteriorly (OH 17% HL in male), odontoid processes present but very short in female (OH 8% HL). Fingertips rounded, relative length of fingers decreasing III-IV-II-I; no webbing on fingers, dermal ridges on sides of fingers present; on male specimen, nuptial pad on finger I present with minute spines on inner edge, nuptial pad absent in female. Toe tips rounded, relative length of toes decreasing IV-III-V-II-I. Tibia short (TBL 49% SVL in male, 46% in female), longer than femur length in male (TBL 104% FEL) but shorter in female (TBL 92% FEL). Full webbing between toes, inner metatarsal tubercle oval, a skin ridge from inter metatarsal to tarsus present. Skin on top of the head and venter smooth, skin on dorsum and flanks very feebly crenulate with tubercle numbers increasing on the lower part of dorsum; precloacal area, and dorsal surfaces of shank and foot covered with heterogeneous tubercles. Colour in life: head yellow-brown with black bar on posterior interorbital region, dorsum and dorsal surfaces of limbs yellow-brown, supratympanic fold dark brown, tips of tubercles white; lateral sides yellowish, ventral surface of throat and chest white with black marbling, ventral surface of limbs and abdomen white, dorsal surface of thighs and tibiae with dark brown bars; iris golden brown in upper half and greyish white in lower half. Colour in preservative: head, dorsum and dorsal part of limbs brown; ventral surface of throat and chest white with black marbling, ventral survey of abdomen whitish; iris white.

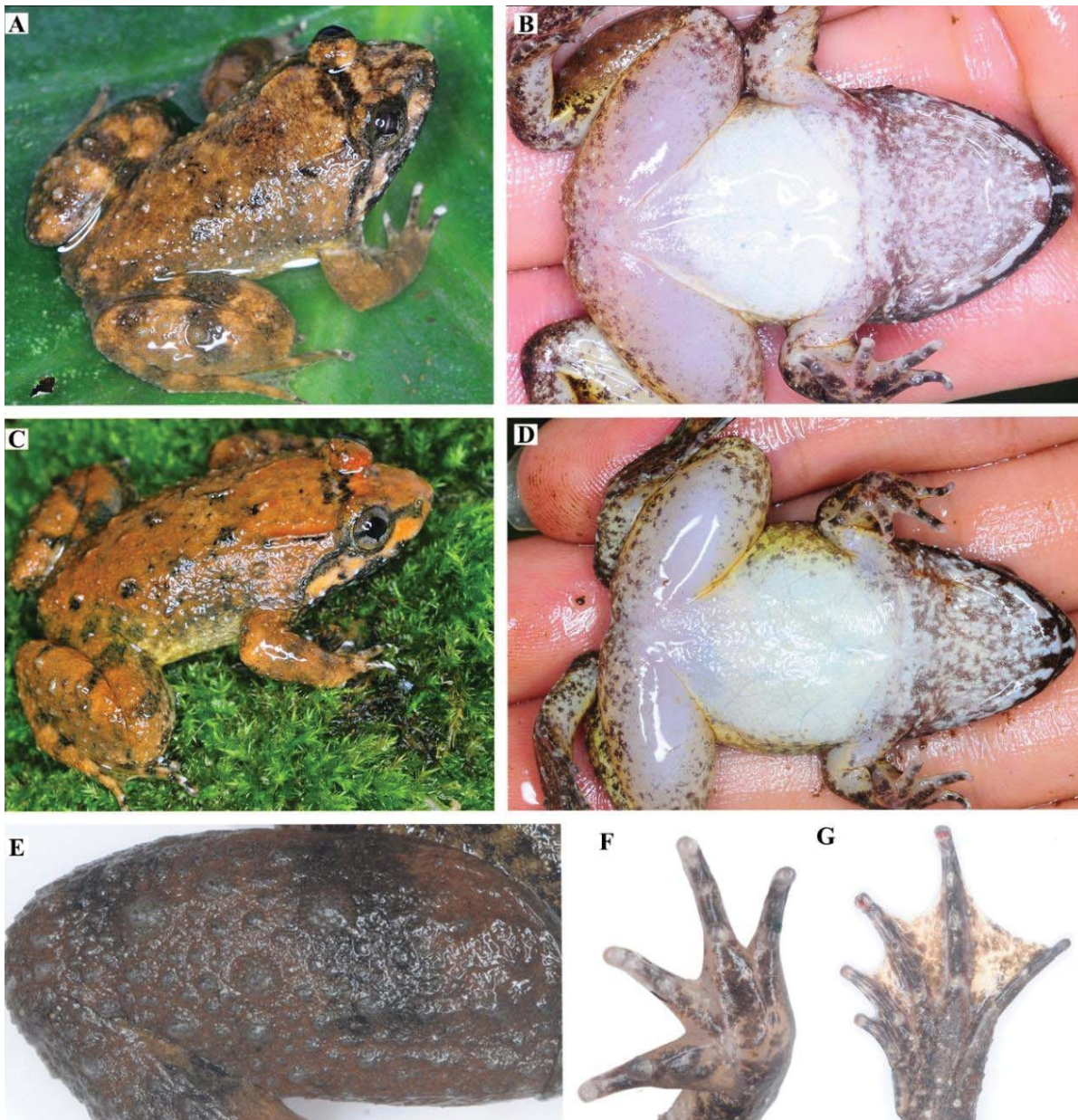


Figure 1. *Limnonectes nguyenorum* from Mount Fansipan in life. A, B: lateral and ventral views of male HLNP 2018.0003; C, D: lateral and ventral views of gravid female HLNP 2018.0004; E-G: leg, planar, and palmar view of male HLNP 2018.0003.

Measurements in detail. Male HLNP 2018.003: SVL 41.6, HW 19.3; HL 16.1; FOL 27.4; TBL 20.3; FEL 19.4; UAL 8.1; PAL 11.2; O 1.0; M 2.0; T 4.7; RL 7.0; EN 3.1; ED 5.0; MN 12.7; OH 2.7; IO 5.2; IN 4.6; UEW 3.0. Female HLNP 2018.004: SVL 40.5; HW 16.5; HL 16.2; FOL 26.7; TBL 18.6; FEL 20.2; UAL 8.2; PAL 9.3; O 0.9; M 1.8; T 2.0; RL 6.0; EN 3.0; ED 5.2; MN 12.9; OH 1.3; IO 4.0; IN 4.6; UEW 2.5.

Natural history. Specimens were collected at 20:00 h in the middle of a small, shallow stream that feeds into Cat Cat Stream (Figure 2). The stream was bordered by secondary forest above and rice fields below. The collection site is in close proximity to the outskirts of Cat Cat Village. The air temperature was 23.3°C and ambient humidity was 88.8%. A further five individuals were observed in the stream but were not collected.



Figure 2. Habitat in Cat Ca Village (left) and microhabitat where *L. nguyenorum* has been found (right).

DISCUSSION

The single male specimen from Mount Fansipan is consistent in morphology with type specimens except that it is slightly smaller in size (SVL 41.6 mm vs. >43 mm in the two type specimens, McLeod et al. 2015) and has tubercles present on the dorsum that were absent in the type specimens (McLeod et al. 2015). The coloration in life and the presence of minute spines on the inner edge of the nuptial pads on finger I have not been reported previously in this species. The single female specimen from Mount Fansipan is consistent in morphology with type specimens except that it has tubercles present on the dorsum that were absent in the type specimens (McLeod et al. 2015).

Our finding increases the number of *Limnonectes* species known from Lao Cai Province to three and Mount Fansipan to two (Nguyen et al. 2009; Ohler et al. 2000; this study). We expect that Mount Fansipan contains additional diversity within the genus *Limnonectes*.

Limnonectes nguyenorum was discovered at an elevation of 900 m (McLeod et al. 2015) and the highest elevation record for this species previously was 1030 m (Son La Province, Pham et al. 2016). Our record extends the known elevational distribution of the species to 1300 m.

Several new species of amphibian have been recently described from Mount Fansipan and the surrounding area of Hoang Lien NP (Nguyen et al. 2013; Rowley et al. 2013; Matsui et al. 2017; Tapley et al. 2017b; Tapley et al. 2018) indicating that the diversity of amphibians in this area is underestimated. Further study is necessary to document the true amphibian diversity of Mount Fansipan.

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**GHI NHẬN LẦN ĐẦU TIÊN LOÀI *Limnonectes nguyenorum* McLeod, Kurlbaum & Hoang
(Amphibia: Anura: Dicroglossidae) Ở TỈNH LÀO CAI, TÂY BẮC VIỆT NAM**

**Nguyễn Thành Luân, Tapley Benjamin, Cutajar Timothy, Nguyễn Thành Chung,
Portway Christopher, Harding Luke, Lương Văn Hào, Rowley L. J Jodi**

Tóm tắt: Chúng tôi ghi nhận phân bố mới loài ếch nhẽo nguyên (*Limnonectes nguyenorum*) ở tỉnh Lào Cai, Tây Bắc Việt Nam. Các mẫu vật được thu thập vào tháng 6 năm 2018 từ núi Phan Xi Păng tại Vườn Quốc gia Hoàng Liên và được định danh dựa trên dữ liệu hình thái và phân tử. Ghi nhận này mở rộng độ cao phân bố hiện biết của loài (lên 1.300 m so với 1.030 m trước đó) đồng thời nâng số loài ếch nhẽo (*Limnonectes*) ở tỉnh Lào Cai lên 3 loài. Đây cũng là ghi nhận đầu tiên của loài này ở núi Phan Xi Păng.

Từ khóa: Ếch nhẽo, Phan Xi Păng, Vườn Quốc gia Hoàng Liên.

Accepted by: Dr. Ngo Van Binh, Dr. Le Trung Dung