

DOI: 10.30906/1026-2296-2019-26-4-201-204

**FIRST RECORD OF *Megophrys gigantea* LIU, HU ET YANG, 1960
(ANURA: MEGOPHRYIDAE) FROM VIETNAM****Anh Mai Luong,¹ Anh Van Pham,² Tao Thien Nguyen,^{3,5} Truong Quang Nguyen^{4,5*}***Submitted June 11, 2018*

Based on our recent field research in Lao Cai and Son La Provinces, Vietnam, we record *Megophrys gigantea* for the first time from outside of China. The newly collected specimens from Vietnam slightly differs from the type series from China in having a smaller head width in males (32.4 vs. 34 – 42 mm) and a smaller size in females (SVL 97.3 vs. 110.4 – 115.4 mm). In terms of genetic divergence, the specimens from Vietnam are separated from those from Yunnan, China by 0.1% based on the 16S rRNA fragment. Our finding brings the species number of the genus *Megophrys* to 20 in Vietnam.

Keywords: *Megophrys gigantea*; distribution; new record; taxonomy; Lao Cai Province; Son La Province.

INTRODUCTION

The genus *Megophrys* contains 77 recognized species with a wide distribution in Southeast Asia from the Himalayan region southward to Borneo (Frost, 2018). In Vietnam, 19 species are recorded and four of them have been recently described since 2009 (Nguyen et al., 2009), namely *Megophrys elfina* Poyarkov, Duong, Orlov, Gogoleva, Vassilieva, Nguyen, Nguyen, Che et Mahony; *Megophrys kouli* Mahony, Foley, Biju et Teeling; *M. latidactyla* Orlov, Poyarkov et Nguyen; and *M. rubrimera* Tapley, Cutajar, Mahony, Chung, Dau, Nguyen, Luong et Rowley (Frost, 2018). In addition, two species were recently recorded for the first time from Vietnam, namely *Megophrys daweimontis* Rao et Yang and *Megophrys synoria* (Stuart, Sok et Neang) (Le et al., 2015; Vassilieva et al., 2016).

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As a result of our recent field work in Lao Cai and Son La provinces, we herein record *Megophrys gigantea* Liu, Hu et Yang for the first time from Vietnam based on morphological data and genetic divergence. The species was previously known only from Jingdong and Yongde counties in Yunnan Province, China (Frost, 2018).

MATERIAL AND METHODS

Sampling. Field surveys were conducted by N. V. Hoang and D. D. Nguyen in October 2015 in Bat Xat District, Lao Cai Province; by S. B. Nenh and T. V. Dau in October 2016 in Muong La District, Son La Province, Vietnam (Fig. 1).

Specimens were collected between 19:00 and 22:00. After taking photographs, specimens were euthanized in a closed vessel with a piece of cotton wool containing ethylacetate (Simmons, 2002), fixed in 80% ethanol for four hours, and then transferred to 70% ethanol for permanent storage. Voucher specimens were subsequently deposited in the collections of the Institute of Ecology and Biological Resources (IEBR), Hanoi and the Tay Bac University (TBU), Son La Province.

Morphological examination. Measurements were taken with a digital caliper to the nearest 0.1 mm. Abbreviations are as follows: SVL, snout-vent length; HL, head length from posterior corner of mandible to tip of snout; HW, maximum head width, at the angle of jaws; IN,

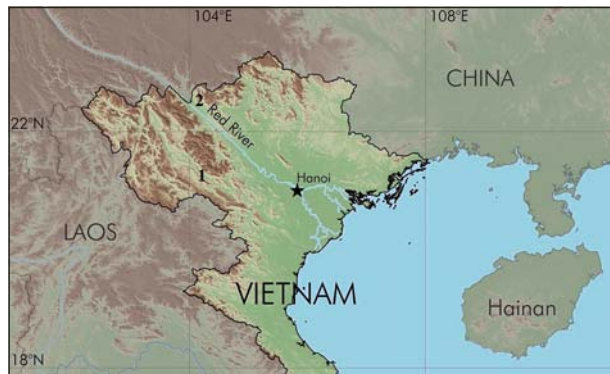


Fig. 1. Map showing the survey sites in northwestern Vietnam: 1, Muong La Nature Reserve in Son La Province; 2, Bat Xat District, Lao Cai Province.

internarial distance; SL, distance from anterior corner of eye to tip of snout; NS, distance from anterior edge of nostril to tip of snout; EN, distance from anterior corner of eye to posterior edge of nostril; EL, eye length, from anterior corner to posterior corner of eye; IOD, minimum distance between upper eyelids; UEW, maximum width of upper eyelid; FLL, forelimb length, from axilla to tip of third finger; HLL, hind limb length, from vent to tip of fourth toe; FL, thigh length, from vent to knee; TL shank length; FTL, inner toe length; IMT, length of inner metatarsal tubercle in foot; a.s.l., above sea level; NR, Nature Reserve. For webbing formula we followed Glaw and Vences (2007).

Molecular analysis. For the molecular analysis we used the protocols of Kuraishi et al. (2013), modified by Nguyen et al. (2014), for DNA extraction, amplification, and sequencing. Fragments of the mitochondrial DNA gene 16S rRNA, ~1100 bp was amplified using the primers following Kuraishi et al. (2013). Chromas Pro software (Technelysium Pty Ltd., Tewantin, Australia) was used to edit the sequences, which were aligned using MAFFT version 7 (Katoh and Standley, 2013) with default setting (FFT-NS-2 algorithm). We then checked the initial alignments by eye. We compared the sequences between the newly collected specimens from Vietnam and the Chinese specimens of *Megophrys gigantea* obtained from GenBank (see Chen et al., 2017). Pairwise comparisons of uncorrected sequence divergences (p -distance) were calculated for 16S rRNA fragments.

RESULTS

Genetic divergence

The comparisons of the resulting 1089 bp long fragment of the 16S rRNA between the specimens of *Megophrys gigantea* from Son La and Lao Cai Provinces,



Fig. 2. *Megophrys gigantea* from Son La Province, Vietnam (TBU ML.2016.28, adult male): A, dorsolateral view; B, ventral view. Photos by A. V. Pham.

northwestern Vietnam (TBU ML.2016.28 and IEBR 4383 (YT.130), GenBank accession number LC483950) and that from Ailao Shan, Yunnan, China (GenBank accession number KX811899) showed only a negligible single base pair difference (corresponding to 0.1%). Thus, we assigned the newly collected specimens from Vietnam to *Megophrys gigantea*.

Description of the Specimens of *Megophrys gigantea* from Vietnam (Fig. 2)

Specimens examined ($n = 2$): One adult male TBU ML.2016.28, collected on 8 October 2016, in the forest near Nam Nghiep Village (21°34.955' N 104°17.161' E, elevation 2220 m a.s.l.), Ngoc Chien Commune within Muong La Nature Reserve, Muong La District, Son La Province and one adult female IEBR 4383 (Field No. YT.130), collected on 15 October 2015, in the forest of Y Ty Commune, Bat Xat District, Lao Cai Province (22°39.140' N 103°36.590' E, elevation 2370 m a.s.l.).

Morphological characters of specimens from Lao Cai and Son La provinces matched the descriptions of Liu et al. (1960) and Fei et al. (2009, 2010). Body elongate (SVL 85.1 mm in the male and 97.3 mm in the female); head wider than long (HW 32.4 mm, HL 30.0 mm, HL/SVL 0.35, HW/SVL 0.38 in the male and HW 44.4 mm, HL 41.2 mm, HL/SVL 0.42, HW/SVL 0.46 in the female); snout pointed, barely projecting beyond upper jaw (SL 10.3 mm; SL/HL 0.34 in the male and SL 11.4 mm, SL/HL 0.28 in the female), longer than horizontal diameter of eye (EL 9.4 mm, SL/EL 1.1 in the male and EL 10.5 mm, SL/EL 1.09 in the female); canthus sharp; loreal region concave; nostril lateral, closer to eye than to tip of snout (NS 5.6 mm, EN 4.8 mm in the male and NS 5.5 mm, EN 6.0 in the female); interorbital space flat, broader than upper eyelid and internarial distance (IOD 9.4 mm, UEW 6.0 mm, IN 9.2 mm in the male and IOD 13.9 mm, UEW 7.6 mm, IN 10.4 mm in the female); tympanum indistinct; vomerine teeth absent; tongue heart-shaped, slightly notched posteriorly; vocal openings inner.

Forelimb long (FLL 48.8 mm, FLL/SVL 0.57 in the male and FLL 63.8 mm, FLL/SVL 0.66 in the female); relative finger lengths $I < II < IV < III$; fingers dermal fringe present, free of webbing; tips of fingers rounded, not swollen; subarticular tubercle distinct, formula 1:1:2:2; palmar tubercles two, oval; nuptial pads absent in male.

Hindlimb slender, long (HLL 133.8 mm, HLL/SVL 1.57 in the male and HLL 171.2 mm, HLL/SVL 1.76 in the female); tibia longer than thigh (TL 43.1 mm, FL 38.5 mm in the male and TL 50.6 mm, FL 48.1 mm in the female); relative toe lengths $I < II < V < III < IV$; tips of toes slightly swollen; toes dermal fringe present; webbing formula $II - IIII - 2III_{1/2} - 2_{3/4}IV_{2/3} - 1V$; inner metatarsal tubercle distinct, shorter than length of toe I (IMT 4.5 mm; FTL 6.7 mm in the male and IMT 5.1 mm, FTL 7.6 mm in the female); subarticular tubercles indistinct; tibiotarsal articulation reaching to posterior margin of eye when limb adpressed along body.

Skin. Dorsal surface smooth; supratympanic fold present, from posterior edge of eye to axilla; flanks smooth; ventral surface smooth; outer edge of the eyelid without a horn-like tubercle.

Coloration in life. Dorsal surface yellowish brown, without dark marking; loreal and supratympanic fold edged in black below; upper lip yellow; flanks yellowish brown; ventral surface brown with pattern yellow.

Ecological notes. Specimens were found on the bank of small streams between 20:00 and 22:00. The surrounding habitats were evergreen forest of larger hardwood and



Fig. 3. Habitat of *Megophrys gigantea*: a, Muong La Nature Reserve in Son La Province; b, Bat Xat District, Lao Cai Province.

shrub (Fig. 3). The female contained yellow eggs with black spots, 4.5 – 4.7 mm in diameter.

Remarks. The specimens of *M. gigantea* from Vietnam slightly differ from those in the descriptions of Fei et al. (2009, 2010) in having a smaller head width in males (HW 32.4 mm vs. 34 – 42 mm) and a smaller size in females (SVL 97.3 mm vs. 110.4 – 115.4 mm).

DISCUSSION

Discovery of *Megophrys gigantea* from Vietnam is not surprising, since the new recorded localities are approximately 120 km distant from the type locality of the species in Chingtung, Yunnan Province, southern China (Liu et al., 1960). The recorded elevations of the specimens from Vietnam (2220 – 2370 m) are within the altitudinal range of the species known in China (1400 – 2400 m) (Frost, 2018). *M. gigantea* was listed as Vulner-

abale in the IUCN Red List due to its extent of occurrence of less than 20,000 km² and area of occupancy of less than 2000 km², with all individuals in fewer than ten locations, and a continuing decline in the extent and quality of its habitat in Yunnan, China (Wu et Yang, 2004). The same threat to the species was found in Lao Cai and Son La provinces of Vietnam. Our new finding of *M. gigantea* bring the total species number of the genus *Megophrys* to 20 in Vietnam (after Nguyen et al., 2009; Frost, 2018).

Acknowledgments. We are grateful to the directorates of Muong La Nature Reserve (Son La Province) and Bat Xat Nature Reserve (Lao Cai Province) for support of our field work and issuing relevant permits. We thank N. V. Hoang and D. D. Nguyen (Thai Nguyen University), S. B. Nenh and T. V. Dau, (Tay Bac University), S. V. Cao, D. B. Giang, and C. V. Cao (Son La), C. T. Pham (Hanoi) for their assistance in the field. This research was partially funded by the Vietnam Academy of Science and Technology (the Project BSTMV. 08/16-19) to T. T. Nguyen and by the Ministry of Education and Training (Grant No. B2019-TTB-562-13) to A. V. Pham. Field equipment was donated by Idea Wild to A. V. Pham.

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