

FIRST RECORD OF *Leptobrachella shiwandashanensis* CHEN, PENG,
PAN, LIAO, LIU & HUANG, 2021 (ANURA: MEGOPHRYIDAE)
FROM VIETNAM

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SUMMARY

Leptobrachella shiwandashanensis was originally described from the evergreen forest of Mount Shiwandashan, Guangxi province, China. We herein record this species for the first time from Vietnam based on a new amphibian collection from Quang Son commune, Hai Ha district, Quang Ninh province. Morphological characteristics of the specimens from Quang Ninh province, Vietnam resemble those of the type series from China in the following characteristics: small size; head wider than long; pale brown dorsal surfaces, with distinct dark brown inverse-triangle-shaped marking between eyes; flanks with irregular black spots; ventral surface creamy white with brown spots on lateral margin; throat and chest nearly immaculate creamy white; fingers and toes free of webbing; dermal fringes on sides of fingers and toes absent; tibio-tarsal articulation reaching to posterior of the eye; iris bicolored, upper half brownish-red, transitioning to silver in the lower half. However, the specimens from Vietnam slightly differ from the type series from China by having a smaller size in males and a somewhat different dorsal colour pattern in the female. Genetic divergences between sequences of the Vietnamese specimens and those of *L. shiwandashanensis* from China are approximate 1.7–1.9% (16S gene). In addition to our morphological and molecular comparisons, we provide first natural history notes of *L. shiwandashanensis* from Vietnam.

Keywords: *Leptobrachella shiwandashanensis*, molecular divergence, morphology, Quang Ninh province.

1. INTRODUCTION

The genus *Leptobrachella* Smith, 1925 currently contains 93 species that inhabit hilly evergreen forests throughout Southern China, Northeastern India, and Myanmar through Thailand and Vietnam to Malaya and Borneo and Natuna Island (Frost, 2022). In Vietnam, 31 species of *Leptobrachella* are currently known and 17 of which have been described in the last ten years (Frost 2022). *Leptobrachella shiwandashanensis* was originally described by Chen et al. (2021) from the evergreen forest of Mount Shiwandashan at elevations between 300 and 600 m above sea level, Guangxi province, China.

During our recent field survey in Northern Vietnam, specimens of a species of *Leptobrachella* were collected in Hai Ha district, Quang Ninh province. Morphological and molecular analyses showed that these specimens are representatives of *Leptobrachella*

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shiwandashanensis thus representing the first country record of this species from Vietnam.

2. RESEARCH METHODOLOGY

The field survey in Quang Ninh province was conducted in May 2016. Specimens were collected between 19:00 and 23:00. After taking photographs in life, specimens were anaesthetized and 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), Hanoi, Vietnam.

Morphological characters: Measurements followed Rowley et al. (2016) and were obtained with a digital caliper to the nearest 0.1 mm. These measurements included snout-vent length (SVL); head length from the tip of

snout to rear of jaws (HL); head width at commissure of jaws (HW); rostral length from the tip of snout to the anterior eye corner (RL); diameter of the exposed portion of eyeball (ED); interorbital distance (IOD), the shortest distance between the anterior corners of the orbits; horizontal diameter of tympanum (TD); distance from anterior edge of the tympanum to posterior eye corner (TED); internarial space distance (IN); nostril-snout length (NS); distance from the eye to nostril (EN), measured from the anterior corner of the eye to the posterior margin of the nostril; tibia length with flexed hindlimb (TIB); forelimb length from elbow to the tip of third finger (FLL); thigh length from vent to knee (THL); manus length from the tip of third digit to proximal edge of the inner palmar tubercle (ML); pes length from the tip of fourth toe to the proximal edge of inner metatarsal tubercle (PL); and maximum diameter of femoral gland (FEM). Sex was determined by direct observation of calling in life or the presence of internal vocal sac openings.

Molecular analysis. Two samples of *Leptobranchella shiwandashanensis* were used for molecular analysis. Tissue samples were extracted using PureLink™ RNA Micro Scale Kit (Thermo Fisher Scientific company), following the manufacturers' instructions. DNA was amplified using PCR Applied Biosystems. PCR volume consisted of 25 µl, including 12 µl of Mastermix, 6 µl of water, 1 µl of each primer at concentration of 10 pmol/µl, and 5 µl of DNA. A fragment of the mitochondrial gene (16S) with ~570 base pairs length was amplified using the primer pair LR-N-13398 (5'-CGCCTGTTTACCAAAAACAT-3'; forward) and LR-J 12887 (5'-CCGGTCTGAACTCAGATCACGT-3'; reverse) (Simon et al., 1994). PCR conditions: 94 °C for 5 minutes of initial denaturation; with 35 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 30 s, and extension at 72 °C for 45 s; and the final extension at 72 °C for 7 minutes (Hoang et al. 2019). PCR products were sent to Apical Scientific company for sequencing (<https://apicalscientific.com>). 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 ON974311 and ON974312.

3. RESULTS AND DISCUSSION

Leptobranchella shiwandashanensis Chen, Peng, Pan, Liao, Liu & Huang, 2021 (Figure 1)

Specimens examined (n = 9). Eight adult males IEBR A.5120–A.5127 and one adult female IEBR A.5128 collected by C.T. Pham & T.V. Nguyen, on 9 May 2016, in a rocky stream along the border between Vietnam and China (21°35.865'N, 107°40.159'E, at elevations from 768 to 950 m), Tai Chi village, Quang Son commune, Hai Ha district, Quang Ninh province.

Specimens from Quang Ninh province were identified as *L. shiwandashanensis* based on morphological and molecular data in comparison with those of the type series from China.

Molecular analyses: We successfully generated two sequences of 560 bp (16S gene) from voucher specimens IEBR A.5120 and A.5128. They were 98.1–98.3% similar to the sequences of the type series of *L. shiwandashanensis* from China (GenBank accession numbers: MZ326691–326695).

Description: Morphological characters of the specimens from Quang Ninh province agreed well with the original description of Chen et al. (2021). Size small (SVL 24.3–28.5 mm, n = 8 males; SVL 32.3 mm, n = 1 female); head wider than long (HW 8.9–9.7 mm, HL 8.4–9.4 mm in males; HL 9.6 mm, HW 9.5 mm in female); snout protruding, projecting slightly over the lower jaw; nostril oval-shaped, closer to the tip of snout than eye (NS 1.2–1.8 mm, EN 1.7–2.9 mm in males; NS 2.1 mm, EN 2.4 mm in female); canthus rostralis round; loreal region sloping and slightly concave; rostral length greater than eye diameter (RL 3.6–5.2 mm, ED 3.1–3.6 mm in males; RL 4.4 mm, ED 3.7 mm in female); interorbital space flat, interorbital distance wider than internasal distance (IOD 2.8–3.8

mm, IN 2.0–2.7 mm in males; IOD 3.5 mm, IN 2.8 mm in female); pupil vertical; tympanum distinct and round, diameter smaller than eye diameter (TD 1.4–2.0 mm in males; TD 2.1 mm in female); supratympanic fold distinct, extending from the corner of the eye to supra-axillary gland; vomerine teeth absent; vocal sac openings located laterally on the floor of mouth; tongue with a shallow notch at the posterior tip.

Forelimb long and slender; fingers long and slender (ML 5.5–6.7 mm in males, ML 8.0 mm in female); relative length of fingers I<II<IV<III; dermal fringes on sides of fingers absent; fingers free of webbing; tips of fingers slightly swollen; subarticular tubercles absent; inner palmar tubercle prominent, separated from the small outer palmar tubercle; nuptial pad absent in males.

Hindlimb relatively long; toes long and slender (PL 11.4–12.7 mm in males, PL 13.8 mm in female); relative toe length I<II<V<III<IV; dermal fringes on sides of toes absent; tips of toes round, slightly swollen; subarticular tubercles absent; inner metatarsal tubercle pronounced large, oval; outer metatarsal tubercle absent; toes free of webbing; tibio-tarsal articulation reaching to posterior of the eye; heels not meeting when

thighs oppressed at right angles to the body.

Dorsal surface shagreened with small tubercles and ridges, more prominent on the shoulder and dorsal surfaces of limbs; pectoral gland small, oval; femoral gland small, oval, located on the posteroventral surface of thighs, closer to the knee than to the vent; supra-axillary glands distinct and rounded and interrupted ventrolateral glandular line distinct; ventral surface of throat, chest, and belly smooth; ventral surface of thighs with small tubercles.

Coloration in life: Dorsum pale brown with distinct dark brown inverse triangle-shaped marking between eyes; tympanum pale brown; a brown supratympanic line from posterior corner of the eye to supra-axillary glands; posterior corner of eye creamy white; upper lip with wide brown bars; flanks with irregular black spots; dorsal surface of limbs yellowish brown with dark-brown crossbars; ventral surface creamy white with brown spots on lateral margin; throat and chest near immaculate creamy white; pectoral and femoral glands creamy white, supra-axillary glands pale orange; pupil black; iris bicolored, upper half brownish-red, transitioning to silver in the lower half.



Figure 1. Dorsolateral view (A) and ventral view (B) of *L. shiwandashanensis* (male IEBR A.5120) in life; dorsolateral view (C) and ventral view (D) of *L. shiwandashanensis* (female IEBR A.5128) in life (Photos: Pham The Cuong)

Ecological notes: The specimens were found between 19:00 and 23:00 on leaves, in a rocky stream along the border between Vietnam and China. The surrounding habitat on both sides consisted of secondary forests with little impact, mossy rocks, and many herbaceous plants in the stream bed. Air temperature was 25.4–29.1°C and relative humidity was 70–82%.

Distribution: The species was previously

known from the evergreen forest of Mount Shiwandashan at elevations between 300 and 600 m above sea level, Golden Camellia National Nature Reserve and Shiwandashan National Nature Reserve, Guangxi province, China (Frost, 2022). The new record of this species in Quang Ninh province, Vietnam is approximately about 20 km distant from the known locality in China (Figure 2).



Figure 2. Map showing the distribution of *Leptobranchella shiwandashanensis*
 Red circle: The type locality in China, Red rectangle: New record in Vietnam

4. CONCLUSION

The male specimens from Vietnam have a slightly smaller size than those of the type series (SVL 24.3–28.5 mm, mean 27.4 ± 1.2 mm, $n = 8$ males vs. 26.8–29.7 mm, mean 28.0 ± 0.9 mm, $n = 11$ males from China) and slightly different dorsal coloration in the female (dark brown vs. pale brown in specimens from China) (Chen et al. 2021). The type specimens from China were collected at elevations from 300 to 600 m above sea level (Chen et al., 2021), while the Vietnamese specimens were found at

elevations between 768 and 950 m above sea level.

Our findings bring the total species number of the genus *Leptobranchella* to 32 in Vietnam. In the past 10 years, 17 new species of the genus *Leptobranchella* have been discovered from Vietnam (Frost 2022, this study). However, most of field studies have been conducted in Central Vietnam (Rowley et al. 2016, 2017; Hoang et al. 2019). Further field work in northern Vietnam, particularly along the border between Vietnam and China, will

help to uncover the actual diversity of this amphibian group.

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GHI NHẬN MỚI LOÀI CỐC MÀY *Leptobrachella shiwandashanensis* CHEN, PENG, PAN, LIAO, LIU & HUANG, 2021 (ANURA: MEGOPHRYIDAE) Ở VIỆT NAM

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TÓM TẮT

Loài Cóc máy *Leptobrachella shiwandashanensis* được phát hiện và mô tả ở rừng thường xanh, núi Shiwandashan, tỉnh Quảng Tây, Trung Quốc. Kết quả phân tích mẫu vật các loài lưỡng cư thu được từ xã Quan Sơn, huyện Hải Hà, tỉnh Quảng Ninh, chúng tôi lần đầu tiên ghi nhận loài này cho khu hệ lưỡng cư Việt Nam. Đặc điểm hình thái của các mẫu vật thu được ở tỉnh Quảng Ninh, Việt Nam giống với các mẫu vật ghi nhận ở Trung Quốc ở các đặc điểm sau: Kích thước nhỏ; đầu rộng hơn dài; lưng có màu nâu nhạt, giữa hai mắt có một hình tam giác ngược màu nâu đậm; hai bên sườn có các đốm màu đen; bụng màu kem có các đốm màu nâu ở hai bên; họng và ngực màu trắng; giữa các ngón tay và ngón chân không có màng bơi; không có diềm da ở các ngón tay và ngón chân; khi gấp dọc thân khớp cổ-chày đặt phía sau mắt; mống mắt có hai màu, nửa trên màu nâu đỏ, nửa dưới màu bạc. Tuy nhiên, các mẫu vật thu ở Việt Nam có kích thước nhỏ hơn ở con đực và con cái có màu nâu đậm hơn so với các mẫu vật ở Trung Quốc. Sai khác di truyền giữa các mẫu vật thu ở Việt Nam so với các mẫu vật ở Trung Quốc khoảng 1,7–1,9% (gen 16S). Bên cạnh đó, chúng tôi cũng cung cấp đặc điểm hình thái và môi trường sống của loài này ở Việt Nam.

Từ khóa: Hình thái, *Leptobrachella shiwandashanensis*, Quảng Ninh, sai khác di truyền.

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