Confirmation of *Leptobrachella ventripunctata* (Fei, Ye, and Li, 1990), based on molecular and morphological evidence in Thailand

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Abstract

Background

Thailand is considered a global biodiversity hotspot that is known to harbour a striking diversity of endemic species. However, several research studies have determined that the level of amphibian diversity in the country has been significantly underestimated. The megophryid genus *Leptobrachella* Smith, 1925 is currently known to include 89 species that are primarily distributed throughout southern China and Southeast Asia; however, only seven species have been found in Thailand.
New information

Based on an integrative approach encompassing genetic and morphological analyses, we have concluded that the population identified from Chiang Rai Province of Thailand is conspecific with *Leptobrachella ventripunctata* (Fei, Ye, and Li, 1990). Importantly, this is the first confirmation record of this species, based on molecular and morphological evidence in Thailand. The discovery of this species reaffirms that the diversity within the genus has been underestimated with many species yet to be discovered. In addition, the findings of our study further highlight the lack of existing knowledge on amphibian taxonomy and an underestimation of the biodiversity that exists along these national border areas.

Keywords

Megophryidae, national new record, Chiang Rai Province, 16S rRNA

Introduction

As a result of the increasing amounts of attention and effort devoted to herpetological research studies in Thailand over the past decade, the present knowledge of Thailand’s amphibian fauna has grown rapidly with newly-described taxa and the addition of new records from the country (e.g. Poyarkov et al. 2018, Suwannapoom et al. 2017, Suwannapoom et al. 2016, Wu et al. 2019, Yan et al. 2016). Currently, Thailand is known to be home to 194 amphibians, while at least 29 species are considered as being endemic (Poyarkov et al. 2021). However, many regions of the country, particularly along the northern borders of Thailand, have not yet been surveyed in full detail. Perhaps the most notable of these regions would be the tropical region along the Thailand-Myanmar border in Doi Tung, Chiang Rai Province.

The frog genus *Leptobrachella* Smith, 1925 is a key component of the Southeast Asian herpetofauna, which currently includes 89 species mainly being distributed throughout southern China, north-eastern India, Indochina, Malaya, Borneo and Natuna Island (Chen et al. 2018, Frost 2021). Frogs of this genus are small in size and inhabiting forest floors and rocky streams in hilly evergreen forests. Furthermore, frogs of this genus are often difficult to find and identify down to the species level due to their inconspicuous and morphological conservatism. However, recent increases in survey efforts, along with the use of updated molecular and acoustic data, have revealed an underestimation of the taxonomic diversity of the genus. These new efforts have established descriptions of many new species, while also yielding some newly-recorded species (e.g. Chen et al. 2018, Stuart and Rowley 2020, Yuan et al. 2017). According to Poyarkov et al. (2021), nine species of the genus *Leptobrachella* are distributed in Thailand, which include *L. fuliginosa*, *L. heteropus*, *L. melanoleuca*, *L. minima*, *L. pelodytoides*, *L. sola*, *L. bourreti*, *L. ventripunctata* and *L. zhangyapingi*. However, records of *L. bourreti* for Thailand seem to be based on misidentifications and need to be verified by further studies (Poyarkov et al. 2021).
There is no evidence of records for *L. ventripunctata* in Thailand (Poyarkov et al. 2021). Thus, only seven species of this genus are currently undisputed in Thailand.

The species *Leptobrachella ventripunctata* was originally described from Mengla County, Yunnan, China (Fei et al. 1990). Recently, relevant research studies have observed new distributions of this species. For example, Luong et al. (2019) reported distribution of this species in Dien Bien Province, Vietnam. In addition, Chen et al. (2018) recorded this species in Caiyanghe, Yunnan, China, as well as localities in Tuyen Quang, Thanh Hoa, Cao Bang, Lao Cai and Phu Tho Provinces in Vietnam. To date, this species is known to be distributed widely throughout southern Yunnan in China, Phongsaly in Laos and in the Son La, Vinh Phuc, Thanh Hoa, Tuyen Quang, Cao Bang and Dien Bien Provinces of northern Vietnam (Frost 2021).

During recent field surveys, conducted in Chiang Rai Province of northern Thailand in 2017, we collected one specimen that we have assigned to the genus *Leptobrachella*, based on morphological characteristics. Subsequent detailed morphological comparisons and phylogenetic analyses indicate that the newly-identified species in Chiang Rai Province should be assigned to *L. ventripunctata*. Therefore, we have further confirmed the presence of *L. ventripunctata* in Thailand.

**Materials and methods**

**Sampling**

Field work was conducted in the environs of Doi Tung, Chiang Rai Province, Thailand (20°19'36.1"N, 99°49'35.0"E, 650 m a.s.l.) and one specimen was collected by Chatmongkon Suwannapoom on 16 July 2017 (Fig. 1). The specimen was photographed in situ. The specimen was euthanised using benzocaine, then liver tissue was extracted, which was stored in 95% ethanol. The voucher specimen was fixed with 10% formalin and later stored in 70% ethanol. The voucher specimen and tissue sample were then deposited in the herpetological collections of the School of Agriculture and Natural Resources, University of Phayao (AUP), Phayao, Thailand.

**Molecular analysis**

Genomic DNA was extracted from the liver tissue sample using standard phenol-chloroform protocols (Sambrook et al. 1989). A partial fragment of the mitochondrial gene 16S rRNA (16S) was amplified and sequenced using the following primers: 16SAR (5’- CGCCTGTATTAYCAAAAACAT-3’; Kocher et al. 1989) and 16SBR (5’- CCGGTGTAACTCAGATCAYGT-3’; Kocher et al. 1989). Amplification was performed in a 25 µl volume reaction according to the following procedure: initial denaturation at 95°C for 5 min, 35 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min and a final extension at 72°C for 10 min. PCR products were purified using a Gel Extraction Mini Kit (A T G C, Bangkok, Thailand). All sequencing was
conducted on an ABI PRISM 3730 automated sequencer (Applied Biosystems, Foster City, CA, USA). The new sequence was first assembled and edited using AutoSeqMan (Sun 2018).

To study the existing phylogenetic relationships amongst *Leptobrachella*, phylogenetic trees were reconstructed, based on the partial mitochondrial 16S rRNA gene. Homologous sequences of the related species in the genus *Leptobrachella* and those of the outgroups *Megophrys glandulosa* (KIZ048439) and *Leptobrachium huashen* (KIZ049025) (Chen et al. 2018) were downloaded from GenBank (Table 1). All sequences were aligned using MUSCLE 3.6 (Edgar 2004), visually checked for accuracy and then trimmed to minimise missing characters in MEGA v.6.0.6 (Tamura et al. 2013).

![Map showing the new record in Thailand (red circle) and the type locality of *L. ventripunctata* (red star) in China.](image)

**Table 1.**
Localities, voucher ID and GenBank numbers for all samples used in this study.

| ID | Species                    | Voucher ID       | Locality         | 16S rRNA     | Reference          |
|----|---------------------------|------------------|------------------|--------------|--------------------|
| 1  | *Leptobrachella bourreti* | KIZ01599         | Jinxiu, Guangxi, China | MH055870    | Chen et al. 2018   |
| 2  | *Leptobrachella bourreti* | KIZ048947        | Dawei Shan, Yunnan, China | MH055871    | Chen et al. 2018   |
| 3  | *Leptobrachella eos*      | ZMMU-NAP-02278   | Vientiane, Laos  | MH055880    | Chen et al. 2018   |
| 4  | *Leptobrachella tengchongensis* | SYS a004598 | Gaoligong Shan, Yunnan, China | KU589209 | Yang et al. 2016   |
| 5  | *Leptobrachella tengchongensis* | SYS a003766 | Gaoligong Shan, Yunnan, China | MH055897 | Chen et al. 2018   |
| ID | Species | Voucher ID | Locality | 16S rRNA  | Reference |
|----|---------|------------|----------|-----------|-----------|
| 6  | *Leptobrachella puhoatensis* | IEBR ADPH049 | Pu Hu Nature Reserve, Thanh Hoa, Vietnam | MH055898 | Chen et al. 2018 |
| 7  | *Leptobrachella liui* | SYS a004035 | Wugong Shan, Jiangxi, China | MH055916 | Chen et al. 2018 |
| 8  | *Leptobrachella laui* | SYS a002450 | Shenzhen, Guangdong, China | MH055904 | Chen et al. 2018 |
| 9  | *Leptobrachella maershanensis* | KIZ07614 | Mao'er Shan, Guangxi, China | MH055927 | Chen et al. 2018 |
| 10 | *Leptobrachella minima* | NCSM 79224 | Louangphrabang, Luang Prabang, Laos | MH055845 | Chen et al. 2018 |
| 11 | *Leptobrachella aereus* | NCSM 76038 | Vilabuly, Savannakhet, Laos | MH055809 | Chen et al. 2018 |
| 12 | *Leptobrachella ventripunctata* | ROM 35750 | Pu'er, Yunnan, China | MH055828 | Chen et al. 2018 |
| 13 | *Leptobrachella ventripunctata* | ROM 28378 | Sapa, Lao Cai, Vietnam | MH055829 | Chen et al. 2018 |
| 14 | *Leptobrachella ventripunctata* | KIZ020421 | Jinuo Shan, Yunnan, China | MH055825 | Chen et al. 2018 |
| 15 | *Leptobrachella ventripunctata* | KIZ014379 | Caiyanghe, Yunnan, China | MH055826 | Chen et al. 2018 |
| 16 | *Leptobrachella ventripunctata* | KIZ020428 | Maandi, Yunnan, China | MH055827 | Chen et al. 2018 |
| 17 | *Leptobrachella ventripunctata* | IEBR ADPH050 | Pu Hu Nature Reserve, Thanh Hoa, Vietnam | MH055819 | Chen et al. 2018 |
| 18 | *Leptobrachella ventripunctata* | NCSM 77715 | Viengthong, Houaphanh, Laos | MH055820 | Chen et al. 2018 |
| 19 | *Leptobrachella ventripunctata* | NCSM 80550 | Boun Tay, Phongsaly, Laos | MH055821 | Chen et al. 2018 |
| 20 | *Leptobrachella ventripunctata* | NCSM 80783 | Kham, Xiangkhouang, Laos | MH055822 | Chen et al. 2018 |
| 21 | *Leptobrachella ventripunctata* | Tissue ID: YPX32065 | Huanglianshan National Nature Reserve, Yunnan, China | MH055823 | Chen et al. 2018 |
| 22 | *Leptobrachella ventripunctata* | KIZ013621 | Wenlong, Yunnan, China | MH055824 | Chen et al. 2018 |
| 23 | *Leptobrachella ventripunctata* | ZMMU-NAP-05008 | Muong Nhe Nature Reserve, Dien Bien, Vietnam | MH055830 | Chen et al. 2018 |
| 24 | *Leptobrachella ventripunctata* | ZMMU-A-5225 | Xuan Son National Park, Phu Tho, Vietnam | MH055835 | Chen et al. 2018 |
| 25 | *Leptobrachella ventripunctata* | ZMMU-NAP-02272 | Hoa Binh, Vietnam | MH055836 | Chen et al. 2018 |
| 26 | *Leptobrachella ventripunctata* | ZMMU-NAP-05061 | Kim Son, Nghe An, Vietnam | MH055837 | Chen et al. 2018 |
| 27 | *Leptobrachella ventripunctata* | ZMMU-NAP-02277 | Thuong Xuan, Thanh Hoa, Vietnam | MH055838 | Chen et al. 2018 |
| ID | Species                | Voucher ID   | Locality                                                                 | 16S rRNA    | Reference          |
|----|------------------------|--------------|---------------------------------------------------------------------------|-------------|--------------------|
| 28 | *Leptobrachella* ventripunctata | ROM 27051    | Quang Thanh Village, Cao Bang, Vietnam                                   | MH055832    | Chen et al. 2018   |
| 29 | *Leptobrachella* ventripunctata | ZMMU-NAP-02270 | Bac Giang, Vietnam                                                        | MH055833    | Chen et al. 2018   |
| 30 | *Leptobrachella* ventripunctata | ZMMU-A-5604-06331 | Vinh Phuc, Vietnam                                                       | MH055834    | Chen et al. 2018   |
| 31 | *Leptobrachella* ventripunctata | SYS a004536   | Zhushihe, Yunnan, China                                                  | MH055831    | Chen et al. 2018   |
| 32 | *Leptobrachella* ventripunctata | ROM 6945      | Na Hang Nature Reserve, Tuyen Quang, Vietnam                             | MH055839    | Chen et al. 2018   |
| 33 | *Leptobrachella* ventripunctata | AUP-00326     | Doi Tung, Chiang Rai, Thailand                                            | OK430887    | This study         |
| 34 | *Leptobrachella* nyx    | AMNH A163810  | Mount Tay Conn Linh, Ha Giang, Vietnam                                   | DQ283381    | Frost 2021         |
| 35 | *Leptobrachella* nyx    | ROM 35606     | Malipo, Yunnan, China                                                    | MH055814    | Chen et al. 2018   |
| 36 | *Leptobrachella* pluvialis | ROM 30685     | Fansipan, Lao Cai, Vietnam                                               | MH055843    | Chen et al. 2018   |
| 37 | *Leptobrachella* nahangensis | ROM 7035     | Na Hang Nature Reserve, Tuyen Quang, Vietnam                             | MH055853    | Chen et al. 2018   |
| 38 | *Leptobrachella* melanoleuca | KIZ018031    | Kapoe, Ranong, Thailand                                                  | MH055967    | Chen et al. 2018   |
| 39 | *Leptobrachella* dringi | KUHE:55610    | Gunung Mulu, Malaysia                                                     | AB847553    | Matsui et al. 2014 |
| 40 | *Leptobrachella* heteropus | KUHE 15487   | Larut, Perak, Malaysia                                                   | AB530453    | Matsui et al. 2014 |
| 41 | *Leptobrachella* kajangensis | LSUHC:4431   | Tioman, Malaysia                                                          | LC202001    | Matsui et al. 2017 |

**Outgroups**

| ID | Species                  | Voucher ID   | Locality     | 16S rRNA    | Reference          |
|----|--------------------------|--------------|--------------|-------------|--------------------|
| 42 | *Megophrys glandulosa*   | KIZ048439    | Yunnan, China | KX811762    | Chen et al. 2016   |
| 43 | *Leptobrachium* huashen  | KIZ049025    | Yunnan, China | KX811931    | Chen et al. 2016   |

Phylogenetic reconstructions using Bayesian Inference (BI) and Maximum Likelihood (ML) were executed in the CIPRES web server (Miller et al. 2010). Data were tested in JMODELTEST 2.1.7 (Darriba et al. 2012) using Bayesian Information Criteria to provide the best-fitting nucleotide substitution models (BIC; Posada 2008). For BI analyses, two separate runs were performed with four Markov chains using the GTR+I+G model. Each run was conducted for 10 million generations, while every 100 generations were sampled with a burn-in value of 25%. Convergence was assessed by the average standard deviation of split frequencies (below 0.01) and ESS values (over 200) in TRACER 1.5 (Rambaut and Drummond 2009). ML analysis was performed using RAxML with 1,000 bootstrap replications using the rapid bootstrap feature under the GTR+G model (random
seed value of 12,345) (Stamatakis 2014). Apart from the phylogenetic tree-based methods, we also calculated the degree of row pairwise sequence divergence using uncorrected p-distances and complete deletion implemented in MEGA v.6.0.6 (Tamura et al. 2013).

Morphology

Measurements were taken using a digital caliper to the nearest 0.1 mm. Abbreviations are presented following the method employed by Matsui (1984) for 25 morphological characteristics: (1) Snout-vent length (SVL); (2) Head length (HL); (3) Head width (HW); (4) Snout length (SL); (5) Distance from the centre of the nostril to the tip of the snout (SN); (6) Nostril-eye distance (N-EL); (7) Eye diameter (ED); (8) Tympanum diameter (TD); (9) Internarial distance (IND); (10) Interorbital distance (IOD); (11) Upper eyelid width (UEW); (12) Forelimb length (FLL); (13) Lower arm length (LAL); (14) Hand length (HAL); (15) First finger length (1FL); (16) Third finger disc diameter (3FDD); (17) Outer palmar tubercle length (OPTL); (18) Inner palmar tubercle length (IPTL); (19) Tibia length (TL); (20) Foot length (FL); (21) Hind-limb length (HLL); (22) Fourth toe disc diameter (4TDD); (23) Inner metatarsal tubercle length (IMTL); (24) Outer metatarsal tubercle length (OMTL) and (25) First toe length (1TOEL).

Figure 2. Phylogram of *Leptobrachella ventripunctata* (Fei, Ye, and Li, 1990), ...
**Data resources**

**Molecular Phylogeny**

The final aligned dataset of 16S nucleotide sequences contained 41 individuals with 510 bp. Amongst the 510 sites, 324 were established as conserved sites and 184 were considered variable sites, of which 127 were found to be potentially parsimony-informative sites (excluding outgroups). The Bayesian Inference (BI) and Maximum Likelihood (ML) phylogenetic trees yielded essentially identical topologies. This was true, except for the poorly-supported nodes, which have been integrated in Fig. 2. The phylogenetic analysis suggested that the newly-collected singular specimen from Chiang Rai was nested in the genus *Leptobrachella* and formed a monophyletic clade with *L. ventripunctata* obtained from China, Laos and Vietnam with strong support (Bayesian posterior probabilities (PP) = 1.00; ML bootstrap support (BS) = 100%).

Interspecific genetic divergence (uncorrected p-distance) between the new sample obtained from Chiang Rai, Thailand and the other species of *Leptobrachella* varied from 4.9% (versus *L. bourreti*) to 17.9% (versus *L. heteropus*) (Fig. 3, Suppl. material 1, Suppl. material 2). The genetic divergence between the individual specimen of *Leptobrachella*, collected from Chiang Rai and the *L. ventripunctata* specimens collected from Vietnam, Laos and China, was found to be very small (0–0.4%, Suppl. material 2).

Morphologically, the specimen from Chiang Rai shows a similar appearance with original description of *L. ventripunctata*: pupil vertical; iris distinctly bicoloured; fingers with lateral dermal fringes, absent for toes; belly creamy white with many scattered brown spots. Therefore, we determined that AUP-00326 belonged to *L. ventripunctata*.

![Heatmap of pairwise uncorrected p-distances of the 16S rRNA gene of Leptobrachella species included in phylogenetic analyses. The colours red to blue indicate high to low divergences.](image-url)
Taxon treatment

*Leptobrachella ventripunctata* (Fei, Ye & Li, 1990)

**Material**

- **scientificName:** *Leptobrachella ventripunctata*; **class:** Amphibia; **order:** Anura; **family:** Megophryidae; **genus:** *Leptobrachella*; **specificEpithet:** ventripunctata; **country:** Thailand; **countryCode:** TL; **stateProvince:** Chiang Rai; **locality:** Doi Tung; **verbatimElevation:** 650 m; **verbatimLatitude:** 20°19'36.1"N; **verbatimLongitude:** 99°49'35.0"E; **individualCount:** 1; **sex:** male; **lifeStage:** adult; **catalogNumber:** AUP-00326; **basisOfRecord:** preserved specimen

**Description**

Morphological descriptions of the specimen obtained from Thailand (measurements shown in Suppl. material 3) are as follows: adult male with SVL 28.9 mm; head length (HL 13.1 mm, 45.3% of SVL) longer than width (HW 11.6 mm, 40.1% of SVL); snout slightly pointed, projecting beyond lower jaw; nostrils slightly closer to eyes than tip of snout (N-EL 2.5 mm, 8.7% of SVL; SN 2.7 mm, 9.3% of SVL); canthus rostralis distinct; loreal region concave; snout (SL 5.3 mm, 18.3% of SVL) longer than eye diameter (EL 4.6 mm, 15.9% of SVL); width of upper eyelid (UEW 4.5 mm) larger than interorbital distance (IOD 3.5 mm) and internasal distance (IND 3.1 mm); tympanum distinct, rounded, length (TD 2.4 mm, 8.3% of SVL) nearly half the diameter of the eye; vomerine teeth absent; pupil vertical; tongue pyriform, deeply notched posteriorly; supratympanic fold distinct, running from posterior corner of eye towards axilla (Fig. 4).

![Figure 4](https://example.com/figure4.png)

**Figure 4.** Male of *L. ventripunctata* (AUP-00326) in life. A. Dorsolateral view B. ventral view C. lateral view of the head. Photo by P. Pawangkhanant.

Forelimbs slender; relative finger lengths: I4).

Hind-limbs long, foot slightly shorter than tibia, tibia length (TL) 56.1% of SVL, foot length (FL) 51.6% of SVL; tibial-tarsal articulation beyond the tip of the eye when the hind-limb is adpressed along the side of the body; heels overlapping when the flexed legs are held at right angles to the body axis; relative toe length: I4).

Dorsal surfaces of head, body, thigh, tibia and flank of body appear relatively rough with numerous granular spots; ventral surfaces smooth; pectoral gland and femoral
gland distinct, oval; pectoral glands larger than femoral glands; supra-axillary gland raised. Ventrolateral gland distinctly visible, forming discontinuous cream-white lines on flanks (Fig. 4).

**Color in life:** Dorsal surface appears reddish brown with small reddish tubercles; a dark inverted triangular marking in the interorbital region, a "W"-shaped marking appears between axillae; flanks scattered with some distinct moderate black blotches; upper lips with three black vertical bars; loreal and tympanic region with distinct black markings; supratympanic ridge appears reddish and lower margin of supratympanic fold appears black; elbow to upper arm and tibio-tarsal articulation distinct and reddish-brown in colour on the dorsum; dorsal surface of lower arms, legs, fingers and toes with dark brown crossbars; throat pale with some dusting; belly creamy white with many scattered brown spots; ventrolateral glands, pectoral glands and femoral glands appear white or yellowish-white; ventral surface of limbs appear grey brown with dark brown and white speckling or dots; iris distinctly bicoloured, typically bright orange-red on upper half and silvery-white on lower half (Fig. 4).

![Figure 5. Habitat at collection site of *L. ventripunctata* in Chiang Rai Province, Thailand.](doi)

**Distribution**

This species was previously only known to be indigenous to extreme southern Yunnan, China; Phongsaly, Xiangkhouang and Houaphanh Provinces in Laos; as well as Son La, Vinh Phuc, Thanh Hoa, Tuyen Quang, Cao Bang, Nghe An, Bac Giang, Phu Tho, Hoa Binh, Lao Cai and Dien Bien Provinces in Vietnam (Chen et al. 2018, Frost 2021). Importantly, this first specific record of *L. ventripunctata* in Thailand substantially expands the known area of distribution of this species.
Ecology

This species lives mainly in the litter on both sides of streams. An individual specimen was observed at night sitting on the branch of a shrub that was about 1-2 m off the ground in an evergreen forest surrounded by trees near a stream with nearby herbaceous plants (Fig. 5). This species has been associated with the sympatric distribution of Micryletta inornata and Limnonectes taylori.

Discussion

Poyarkov et al. (2021) mentioned the distribution of L. ventripunctata in the extreme north of Thailand, but did not provide any evidence, such as the specific locality, voucher specimens or DNA sequences. This is the first record of the species with morphological data and molecular evidence.

Thailand represents an important component of the Indo-Burma biodiversity hot-spot. Its northern region lies at a biogeographic crossroads where the fauna of China, Indochina, India and Himalaya converge (Myers et al. 2000). Recently, along with an increasing intensity of field investigations and the application of new techniques, biodiversity studies in this region have expanded. Consequently, many new species and records have been described and reported (e.g. Lorphengsy et al. 2021, Wu et al. 2019). The confirmation of L. ventripunctata in this study increases the total number of known amphibian species in Thailand from 125 (Khonsue and Thirakhupt 2001) to 194, along with the known number of Leptobrachella species from seven to eight. The following species of Leptobrachella are known to be from Thailand: L. ventripunctata, L. pelodytoides, L. fuliginosa, L. heteropus, L. melanoleuca, L. minima, L. sola and L. zhangyaping. The discovery of this species reaffirms that, to date, the diversity within the genus has been underestimated with many species yet to be discovered in this country.

Furthermore, our study further highlights a lack of existing knowledge on amphibian taxonomy and identifies an underestimation of the potential biodiversity along these national border areas. In recent years, many new recorded species have been found in border areas, such as Nidirana chapaensis (Yuan et al. 2019), Gracixalus quangi (Lorphengsy et al. 2021) and Theloderma pyaukkya (Du et al. 2020), while a number of studies have called for enhanced surveys of species diversity in these border areas (Chen et al. 2020, Chen et al. 2018, Wu et al. 2020, Yuan et al. 2019). The findings of our research further support this view. The newly-recorded species we reported was found along the border of Thailand and Myanmar, which is very close to Laos. Currently, distributions of this species have been recorded in Laos and Thailand, whereas they have not been definitively reported in Myanmar (Chen et al. 2018, Frost 2021). Considering that it is located in the same zoogeographic region comprised of continuous forests habitats, we suspect that this species is also present in Myanmar. In the future, it will be important to strengthen levels of international cooperation in order to further clarify the full range of this species.
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References

• Chen JM, Zhou WW, Poyarkov NA, Stuart BL, Brown RM, Lathrop A, Wang YY, Yuan ZY, Jiang K, Hou M, Chen HM, Suwannapoom C, Nguyen SN, Duong TV, Papenfuss TJ, Murphy RW, Zhang YP, Che J (2016) A novel multilocus phylogenetic estimation reveals unrecognized diversity in Asian horned toads, genus Megophrys sensu lato (Anura: Megophryidae). Molecular Phylogenetics and Evolution 106: 28-43. https://doi.org/10.1016/j.ympev.2016.09.004
• Chen JM, Poyarkov NA, Suwannapoom C, Lathrop A, Wu YH, Zhou WW, Yuan ZY, Jin JQ, Chen HM, Liu HQ, Nguyen TQ, S.N. N, Duong TV, Eto K, Nishikawa K, Matsui M, Orlov NL, Stuart BL, Brown RM, Rowley JJ, Murphy RW, Wang YY, Che J (2018) Large-scale phylogenetic analyses provide insights into unrecognized diversity and historical biogeography of Asian leaf-litter frogs, genus Leptolalax (Anura: Megophryidae). Molecular Phylogenetics and Evolution 124: 162-171. https://doi.org/10.1016/j.ympev.2018.02.020
• Chen JM, Xu K, Poyarkov NA, Wang K, Yuan ZY, Hou M, Suwannapoom C, Che J (2020) How little is known about the little brown frogs: description of three new species of the genus Leptobrachella (Anura: Megophryidae) from Yunnan Province, China. Zoological Research 41: 292-313. https://doi.org/10.24272/j.issn.2095-8137.2020.036
• Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9: 772. https://doi.org/10.1038/nmeth.2109
• Du LN, Liu S, Hou M, Yu GH (2020) First record of Theloderma pyaukkya Dever, 2017 (Anura: Rhacophoridae) in China, with range extension of Theloderma moloch (Annandale, 1912) to Yunnan. Zoological Research 41: 576-58. https://doi.org/10.24272/j.issn.2095-8137.2020.083
• Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32: 1792-1797. https://doi.org/10.1093/nar/gkh340
• Fei L, Ye CY, Huang YZ (1990) Key to Chinese amphibians. Chongqing Branch, Science and Technology Literature Press, Chongqing, 364 pp. [In Chinese].
• Frost D (2021) Amphibian species of the World. http://research.amnh.org/herpetology/amphibia/. Accessed on: 2021-9-30.

• Khonsue W, Thirakhupt K (2001) A checklist of the amphibians in Thailand. The Natural History Journal of Chulalongkorn University 1: 69-82.

• Kocher TD, Thomas WK, Meyer A, Edwards SV, Pbo S, Villablancna FX, Wilson AC (1989) Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proceedings of the National Academy of Sciences 86: 6196-620. https://doi.org/10.1073/pnas.86.16.6196

• Lorphengsy S, Nguyen VT, Poyarkov NA, Wu YH, Pawangkhanant P, Passorn S, Che J, Suwannapoom C (2021) First national record of Gracixalus quangi Rowley, Dau, Nguyen, Cao & Nguyen, 2011 and G. yunnanensis Yu, Li, Wang, Rao, Wu &Yang, 2019 (Amphibia: Anura: Rhacophoridae) from Thailand. Biodiversity Data Journal 9: e67667. https://doi.org/10.3897/BDJ.9.e67667

• Matsui M (1984) Morphometric variation analyses and revision of the Japanese toads (genus Bufo, Bufonidae). Contributions from the Biological Laboratory, Kyoto University 26: 209-428.

• Matsui M, Nishikawa K, Yambun P (2014) A new Leptolalax from the mountains of Sabah, Borneo (Amphibia, Anura, Megophryidae). Zootaxa 9: 440-452. https://doi.org/10.11646/zootaxa.3753.5.3

• Matsui M, Elo K, Nishikawa K, Hamidy A, Belabut D, Ahmad N, Panha S, Khonsue W, Grismer LL (2017) Mitochondrial phylogeny of Leptolalax from Malay Peninsula and Leptobrachella (Anura, Megophryidae). Current Herpetology 36: 11-21. https://doi.org/10.5358/hsj.36.11

• Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In Gateway Computing Environments Workshop (GCE), 2010. ieee

• Myers N, Mittermeier RA, Mittermeier CG, Da Fonseca GA, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403: 853-858. https://doi.org/10.1038/35002501

• Posada D (2008) jModelTest: phylogenetic model averaging. Molecular Biology and Evolution 25: 1253-1256. https://doi.org/10.1093/molbev/msn083

• Poyarkov NA, Suwannapoom C, Pawangkhanant P, Aksornneam A, Duong TV, Korost DV, Che J (2018) A new genus and three new species of miniaturized microhylid frogs from Indochina (Amphibia: Anura: Microhylidae: Asterophryinae. Zoological Research 39: 130-157.

• Poyarkov NA, Van Nguyen T, Popov ES, Geissler P, Pawangkhanant P, Neang T, Suwannapoom C, Orlov NL (2021) Recent Progress in Taxonomic Studies, Biogeographic Analysis, and Revised Checklist of Amphibians in Indochina. Russian Journal of Herpetology 28: 1-110. https://doi.org/10.30906/1026-2296-2021-28-3A-1-110

• Rambaut A, Drummond AJ (2009) Tracer v1.5. Computer Program Available from:. http://tree.bio.ed.ac.uk/software/tracer/8

• Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. Second Edition. Cold Spring Harbor Laboratory Press, 1626 pp. [ISBN 0-87969-309-6]

• Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30: 1312-1313. https://doi.org/10.1093/bioinformatics/btu033
• Stuart BL, Rowley JJ (2020) A new *Leptobrachella* (Anura: Megophryidae) from the Cardamom Mountains of Cambodia. *Zootaxa* 4834: 556-572. https://doi.org/10.11646/zootaxa.4834.4.4

• Sun YB (2018) FasParser2: a graphical platform for batch manipulation of tremendous amount of sequence data. *Bioinformatics* 34: 2493-2495. https://doi.org/10.1093/bioinformatics/bty126

• Suwannapoom C, Yuan ZY, NA P, Yan F, Kamtaeja S, Murphy RW, Che J (2016) A new species of genus *Fejervarya* (Anura: Dicroglossidae) from northern Thailand. *Zoological Research* 37: 327-337.

• Suwannapoom C, Yuan ZY, Jiang K, Yan F, Gao W, Che J (2017) A new species of rain-pool frog (*Dicroglossidae: Fejervarya*) from western Thailand. *Zoological Research* 38: 243-250.

• Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 30: 2725-2729. https://doi.org/10.1093/molbev/mst197

• Wu YH, Suwannapoom C, Poyarkov NA, Pawangkhanant P, Xu K, Jin JQ, Murphy RW, Che J (2019) A new species of the genus *Xenophrys* (Anura: Megophryidae) from northern Thailand. *Zoological Research* 40: 564-574. https://doi.org/10.24272/j.issn.2095-8137.2019.032

• Wu YH, Yan F, Stuart BL, Prendini E, Suwannapoom C, Dahn HA, Zhang BL, Cai HX, Xu YB, Jiang K, Chen HM, Lemmon AR, Lemmon EM, Raxworthy CJ, Orlov NL, Murphy RW, Che J (2020) A combined approach of mitochondrial DNA and anchored nuclear phylogenomics sheds light on unrecognized diversity, phylogeny, and historical biogeography of the torrent frogs, genus *Amolops* (Anura: Ranidae). *Molecular Phylogenetics and Evolution* 148: 106789. https://doi.org/10.1016/j.ympev.2020.106789

• Yan F, Jiang K, Wang K, Jin JQ, Suwannapoom C, Li C, Vindum JV, Brown RM, Che J (2016) The Australasian frog family Ceratobatrachidae in China, Myanmar and Thailand: discovery of a new Himalayan forest frog clade. *Zoological Research* 37: 7-1.

• Yang JY, Wang YY, Chen GL, Rao DQ (2016) A new species of the genus *Leptolalax* (Anura: Megophryidae) from Mt. Gaoligongshan of western Yunnan Province, China. *Zootaxa* 4088: 379-394. https://doi.org/10.11646/zootaxa.4088.3.4

• Yuan Z, Sun R, Chen J, Rowley JJ, Wu Z, Hou S, Che J (2017) A new species of the genus *Leptolalax* (Anura: Megophryidae) from Guangxi, China. *Zootaxa* 4300: 551-57. https://doi.org/10.11646/zootaxa.4300.4.5

• Yuan ZY, Liu XL, Wang K, Wang J, Chen JM, Jin JQ, Wei PF, Zhou JJ, Che J (2019) *Nidirana chapaensis* (Bourret, 1937), one additional anuran species for the amphibian fauna of China. *Zootaxa* 4571: 580-588. https://doi.org/10.11646/zootaxa.4571.4.10
Supplementary materials

Suppl. material 1: Mean pairwise uncorrected p-distance  
doi
Authors: Wu et al.
Data type: Table
Brief description: The mean pairwise uncorrected p-distance (%) of 16S rRNA gene amongst
the species of *Leptobrachella*. Sample ID corresponds to those in Table 1
Download file (23.91 kb)

Suppl. material 2: Average uncorrected p-distances  
doi
Authors: Wu et al.
Data type: Table
Brief description: Average uncorrected p-distances amongst those calculated from 16S rRNA
gene sequences
Download file (28.48 kb)

Suppl. material 3: Measurement and proportions  
doi
Authors: Wu et al.
Data type: Table
Brief description: Measurement (in mm) of and proportions of *Leptobrachella ventripunctata.*
Download file (18.96 kb)