First records of the fanged frogs *Limnonectes bannaensis* Ye, Fei & Jiang, 2007 and *L. utara* Matsui, Belabut & Ahmad, 2014 (Amphibia: Anura: Dicroglossidae) in Thailand

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Abstract

Background

The taxonomic status of the Thai populations belonging to the *Limnonectes kuhlii* species complex is controversial, due to phenotypic similarity in the cryptic species complex. Recently, some studies on this group in Thailand have discovered four new species: *L.
taylori, L. megastomias, L. jarujini and L. isanensis. Even so, the diversity of this group is still incomplete.

**New information**

Based on an integrative approach encompassing genetic and morphological analyses, we conclude that the *Limnonectes* populations from Nan Province (northern) and Yala Province (southern) of Thailand are conspecific with *L. bannaensis* Ye, Fei & Jiang, 2007 and *L. utara* Matsui, Belabut & Ahmad, 2014, respectively. These are the first records of these species in Thailand. Our study highlights the importance of using DNA sequence data in combination with morphological data to accurately document species identity and diversity. This is especially important for morphologically cryptic species complexes and sympatrically occurring congeners.

**Keywords**

Nan Province, Yala Province, 16S rRNA, Cryptic species, Species complex

**Introduction**

*Limnonectes* Fitzinger, 1843 is the most species rich genus of Asian frogs of the family Dicroglossidae presently comprising 78 species distributed throughout East and Southeast Asia (Frost 2021). This genus is generally characterised by a morphological crypsis and contains several potentially undescribed cryptic species, especially within widespread species complexes, such as the *L. kuhlii* (Tschudi 1838) complex (McLeod 2010, Suwannapoom et al. 2016). In terms of a systematic framework, the *L. kuhlii* species complex in Thailand had been previously explored and four species are currently recorded within the country (McLeod 2008Matsui et al. 2010b, McLeod et al. 2012). According to established records, *L. megastomias* McLeod, 2008 is only found in Nakhon Ratchasima and Sa Kaew Provinces of eastern Thailand. Two other species (*L. taylori* Matsui, Panha, Khonsue & Kuraishi, 2010 and *L. jarujini* Matsui, Panha, Khonsue & Kuraishi, 2010), occur in the mountain ranges of western Thailand, with *L. taylori* documented from northern and *L. jarujini* from southern parts of the country (Matsui et al. 2010a). Furthermore, *Limnonectes isanensis* McLeod, Kelly & Barley, 2012 is only known to occur in Phu Luang National Park, Loei Province, Thailand (McLeod et al. 2012).

During the course of our recent herpetological surveys conducted in the northern and southern parts of Thailand from 2017-2018, we collected several *Limnonectes* specimens from previously not examined populations in Nan and Yala Provinces. Based on detailed morphological comparisons and a phylogenetic analyses, we confirm here the presence of two *Limnonectes* species previously unreported for Thailand: *L. bannaensis* Ye, Fei, Xie & Jiang, 2007 and *L. utara* Matsui, Belabut & Ahmad, 2014, respectively. Therefore, we report *L. bannaensis* and *L. utara* as two new records of amphibian species for Thailand,
provide morphological and morphometric descriptions of the collected specimens and remark on the natural history of these species, based on our field observations.

**Materials and methods**

**Sampling**

Field surveys were conducted in Bo Kluea District, Nan Province in December 2017, in Bannang Sata District, Yala Province in August 2018 (Fig. 1). A total of 11 specimens of *Limnonectes bannaensis* and four specimens of *L. utara* were collected. Liver tissue samples of all specimens were taken and preserved in 95% ethanol for molecular analysis. The specimens were fixed with 10% formalin for 24 hours and subsequently transferred to 70% ethanol. Specimens and tissues were subsequently deposited at the herpetological collections of the School of Agriculture and Natural Resources, University of Phayao (AUP), Phayao, Thailand.

![Figure 1](image)

Map showing the type-localities of *Limnonectes kuhlii* species complex in Thailand. Circles = Type localities of *L. taylori* (red), *L. jarujini* (purple), *L. megestomias* (orange) and *L. isanensis* (brown). Blue square (*L. bannaensis*) and yellow square (*L. utara*) represent the two new distribution records in Thailand reported here.

**Molecular analysis**

Genomic DNA was extracted from liver tissues preserved in 95% ethanol using the standard phenol-chloroform extraction protocol (Sambrook et al. 1989). Partial fragments of the mitochondrial 16S rRNA were amplified for all samples via the polymerase chain reaction (PCR) using the following primers: 16SAR (5'-CGCCTGTTTAYCAAAAACAT-3') and 16SBR (5'-CCGGTYTGAACTCAGATCAYGT-3'). PCR amplifications were performed in a 25 µl reaction volume with the following cycling conditions: initial denaturing step at 95°C for 4 min, 35 cycles of denaturing at 94°C for 40 s, annealing at 55°C for 30 s, extending at 72°C for 1 min and a final extension step at 72°C for 10 min. PCR products
were directly sequenced using an ABI 3730xl DNA automated sequencer with both forward and reverse primers.

Matrilineal genealogies were reconstructed to examine genealogical relationships amongst *Limnonectes*, based on the 16S rRNA gene fragment. Homologous sequences of the related species of *Limnonectes* and those of the outgroups (*Fejervarya limnocharis* (Gravenhorst) and *Fejervarya iskandari* Veith, Kosuch, Ohler & Dubois) were downloaded from GenBank (see Table 1).

Table 1.
Sequences and voucher specimens of *Limnonectes* and outgroup taxa used in molecular analyses for this study with sampling localities.

| #  | Species        | Voucher | Locality                   | GenBank    | Reference             |
|----|----------------|---------|----------------------------|------------|-----------------------|
| 1  | *L. bannaensis* | CIB 200901116 | China, Yunnan, Jinghong | AB526312   | Matsui et al. 2010    |
| 2  | *L. bannaensis* | FMNH 255140 | Laos, Huaphahn, Vieng Tong | HM067133   | McLeod 2010           |
| 3  | *L. bannaensis* | FMNH 258519 | Laos, Phongsaly, Phongsaly | HM067158   | McLeod 2010           |
| 4  | *L. bannaensis* | VNMN A.2015.41 | Vietnam, Ha Giang, Vi Xuyen | HM067246   | McLeod 2010           |
| 5  | *L. bannaensis* | AMNH 106430 | Vietnam, Vinh Phuc, Tam Dao | HM067272   | McLeod 2010           |
| 6  | *L. bannaensis* | KIZ 024971 | China, Yunnan, Xishuangbanna, Mengla, Yiwu | KU599847   | Suwannapoom et al. 2016 |
| 7  | *L. bannaensis* | KIZ 024970 | China, Yunnan, Xishuangbanna, Mengla, Yiwu | KU599848   | Suwannapoom et al. 2016 |
| 8  | *L. bannaensis* | KIZ 011793 | China, Yunnan, Xishuangbanna, Mengla, Bubang | KU599849   | Suwannapoom et al. 2016 |
| 9  | *L. bannaensis* | KIZ 011726 | China, Yunnan, Xishuangbanna, Mengyang | KU599850   | Suwannapoom et al. 2016 |
| 10 | *L. bannaensis* | KIZ 011727 | China, Yunnan, Xishuangbanna, Mengyang | KU599851   | Suwannapoom et al. 2016 |
| 11 | *L. bannaensis* | KIZ 022207 | China, Guangxi, Hulong, Pinglongshan | KU599856   | Suwannapoom et al. 2016 |
| 12 | *L. bannaensis* | KIZ 011608 | Vietnam, Thanh Hoa, Quan Hoa | KU599857   | Suwannapoom et al. 2016 |
| 13 | *L. bannaensis* | KIZ YPX18365 | Vietnam, Quang Tri, Bac Huong Hoa | KU599861   | Suwannapoom et al. 2016 |
| #  | Species         | Voucher  | Locality                   | GenBank  | Reference     |
|----|----------------|----------|----------------------------|----------|---------------|
| 14 | L. bannaensis  | AUP-00481| Thailand, Nan, Bo Kluea    | MZ493348| This study    |
| 15 | L. bannaensis  | AUP-00484| Thailand, Nan, Bo Kluea    | MZ493349| This study    |
| 16 | L. bannaensis  | AUP-00485| Thailand, Nan, Bo Kluea    | MZ493350| This study    |
| 17 | L. bannaensis  | AUP-00488| Thailand, Nan, Bo Kluea    | MZ493351| This study    |
| 18 | L. cintalubang | KUHE 47859| Malaysia, Borneo, Sarawak, Serian | AB981409|Matsui et al. 2010a |
| 19 | L. fragilis     | CIB 20081089| China, Hainan, Wuzhi Shan | AB526315| Matsui et al. 2010 |
| 20 | L. fujianensis  | CIB ZJ 200806223| China, Jiangxi, Zixi | AB526311| Matsui et al. 2010 |
| 21 | L. isanensis    | KUHE 19284| Thailand, Loei, Phu Luang | AB526314| Matsui et al. 2010 |
| 22 | L. isanensis    | KUHE 19320| Thailand, Loei, Phu Luang | AB558955| Matsui et al. 2010a |
| 23 | L. jarujini     | KUHE 19514| Thailand, Kanchanaburi, Sangkhla Buri | AB558940|Matsui et al. 2010a |
| 24 | L. jarujini     | KUHE 19690| Thailand, Surat Thani, Khlong Saeng | AB558950|Matsui et al. 2010a |
| 25 | L. longchuanensis| KIZ048424| China, Yunnan, Dehong, Longchuan | KU599867| Suwannapoom et al. 2016 |
| 26 | L. longchuanensis| KIZ048527| China, Yunnan, Yingjiang, Tongbiguan | KU599869| Suwannapoom et al. 2016 |
| 27 | L. kuhlii       | GMU unnumbered| Indonesia, Java, Purwerojo | AB526316|Matsui et al. 2010 |
| 28 | L. megastomias  | FMNH 266221| Thailand, Sa Kaew, Pang Si Da | HM067184| McLeod 2010 |
| 29 | L. megastomias  | KU 307760| Thailand, Nakon Ratchasima | HM067201| McLeod 2010 |
| 30 | L. namiyei      | KUHE L0809191| Japan, Okinawa, Okinawajima | AB526309|Matsui et al. 2010 |
| 31 | L. quangninhensis| IEBR 3969| Vietnam, Quang Ninh, Hai Ha | KY595927| Pham et al. 2017 |
| 32 | L. quangninhensis| IEBR 3970| Vietnam, Quang Ninh, Hai Ha | KY595928| Pham et al. 2017 |
| 33 | L. selatan      | KUHE54079| Malaysia, Genting, Pahang | AB981384|Matsui et al. 2010 |
| 34 | L. selatan      | KUHE54080| Malaysia, Genting, Pahang | AB981385|Matsui et al. 2010 |
| # | Species | Voucher | Locality | GenBank   | Reference       |
|---|---------|---------|----------|-----------|-----------------|
| 35 | *L. taylori* | KUHE 19101 | Thailand, Chiang Mai, Doi Inthanon | AB558929 | Matsui et al. 2010a |
| 36 | *L. taylori* | KUHE 19868 | Thailand, Chiang Mai, Tha Ton | AB981390 | Matsui et al. 2010a |
| 37 | *L. utara* | KUHE54064 | Malaysia, Larut, Perak | AB981377 | Matsui et al. 2010 |
| 38 | *L. utara* | KUHE54065 | Malaysia, Larut, Perak | AB981378 | Matsui et al. 2010 |
| 39 | *L. utara* | AUP 01705 | Thailand, Yala, Bannang Sata | MZ493344 | This study |
| 40 | *L. utara* | AUP 01706 | Thailand, Yala, Bannang Sata | MZ493345 | This study |
| 41 | *L. utara* | AUP 01707 | Thailand, Yala, Bannang Sata | MZ493346 | This study |
| 42 | *L. utara* | AUP 01708 | Thailand, Yala, Bannang Sata | MZ493347 | This study |

**Outgroup**

| # | Species | Voucher | Locality | GenBank   | Reference       |
|---|---------|---------|----------|-----------|-----------------|
| 43 | *F. limnocharis* | AMNH A-161230 | Vietnam, Nghe An, Con Cuong, Pu Mat | AY843588 | Faivovich et al. 2005 |
| 44 | *F. iskandari* | UI unnumbered | Indonesia, Java, Banyuwangi | AB526324 | Matsui et al. 2010 |

Trees were reconstructed using Bayesian Inference (BI) and Maximum Likelihood (ML). JMODELTEST v.2.1.7 (Darriba et al. 2012) was used to select an appropriate nucleotide substitution model for BI. The GTR+G model was chosen as the best-fit model following the Bayesian Information Criterion (BIC, Posada 2008). The CIPRES web server (Miller et al. 2010) was selected to implement BI. The Monte Carlo Markov chain length was run for 10,000,000 generations and sampled every 1,000 generations. A burn-in value of 25% was used. Convergence was assessed by the average standard deviation of split frequencies (below 0.01) and the ESS values (over 200) in TRACER v.1.5 (Rambaut A and Drummond A 2007). ML was performed using RAxML with 1,000 bootstrap replicates (Stamatakis et al. 2008).

**Morphometric analysis and morphological comparisons**

Morphometric measurements were taken using digital callipers to the nearest 0.1 mm, following Matsui (1984) and McLeod 2008) abbreviations of the morphometric traits are as follows: snout-vent length (SVL), horizontal eye diameter (ED), eye nostril distance (END), rostrum length distance (RLD), thigh (femur) length (FEL), foot length (FOL), head length (HL), head width (HW), internarial distance (IN), interorbital width (IO), lower arm length (LAL), mandible-nostril distance (MN), palm length (PAL), relative finger length (RFL), relative toe length (RTL), shank (tibia) length (TBL), tympanum diameter (TD) and upper eyelid width (UEW). The digital-webbing formulae followed Savage (1975). Morphological
comparisons were made with specimens of morphologically related congeners, deposited at the University of Phayao (AUP).

Data resources

Molecular phylogeny

Sequencing generated a total of 492 base pairs (bp) of 16S rRNA for *Limonectes bannaensis* and *L. utara*. All newly-generated sequences were submitted to GenBank (Accession numbers MZ493344-MZ493351, see Table 1). Interspecific uncorrected p-distances between the newly-discovered population of *L. bannaensis* collected from Nan Province in Thailand and the other known species of *Limonectes* varied from 6.3% (in relation to *L. quangninhensis*) to 12.1% (in relation to *L. cintalubang*) (Suppl. material 1). The uncorrected p-distance between the newly-found populations of *L. bannaensis* from Nan Province and the topotypic *L. bannaensis* (Mengyang, Yunnan, China) is 2.1%. Both ML and BI analyses recovered the Nan population nested within a strongly supported clade, together with topotypic *L. bannaensis* (see Fig. 2). The newly-discovered population of *L. utara* from Yala Province, Thailand and the congeners varied from 5.8% (in relation to *L. selatan*) to 13.5% (in relation to *L. cintalubang*) (see Suppl. material 1). The uncorrected p-distance between the newly-discovered populations of *L. utara* from Yala Province and the topotypic *L. utara* (Larut, Perak, Malaysia) is 0.2%. Both ML and BI analyses recovered the Yala population within a strongly supported clade, together with topotypic *L. utara* (Fig. 2).
Taxon treatments

*Limnonectes bannaensis* Ye, Fei, Xie & Jiang, 2007

**Materials**

a. scientificName: *Limnonectes bannaensis*; class: *Amphibia*; order: *Anura*; family: *Dicroglossidae*; genus: *Limnonectes*; specificEpithet: *bannaensis*; scientificNameAuthorship: Ye, Fei, Xie & Jiang, 2007; country: Thailand; countryCode: TL; stateProvince: Nan; locality: Doi Phu Kha; verbatimElevation: 750; verbatimLatitude: 19°03'21.3"N; verbatimLongitude: 101°10'47.8"E; eventDate: 17 December, 2017; fieldNotes: collected by C. Suwannapoom, P. Pawangkhanant; individualCount: 1; sex: male; lifeStage: adult; catalogNumber: AUP-00481; language: en; collectionCode: Amphibians; basisOfRecord: Preserved Specimen

b. scientificName: *Limnonectes bannaensis*; individualCount: 1; sex: adult male; catalogNumber: AUP-00482; basisOfRecord: Preserved Specimen; dynamicProperties: collection date, collector and Location as the AUP-00481

c. scientificName: *Limnonectes bannaensis*; individualCount: 1; sex: adult male; catalogNumber: AUP-00483; basisOfRecord: Preserved Specimen; dynamicProperties: collection date, collector and Location as the AUP-00481

d. scientificName: *Limnonectes bannaensis*; individualCount: 1; sex: adult male; catalogNumber: AUP-00484; basisOfRecord: Preserved Specimen; dynamicProperties: collection date, collector and Location as the AUP-00481

e. scientificName: *Limnonectes bannaensis*; individualCount: 1; sex: adult male; catalogNumber: AUP-00485; basisOfRecord: Preserved Specimen; dynamicProperties: collection date, collector and Location as the AUP-00481

f. scientificName: *Limnonectes bannaensis*; individualCount: 1; sex: adult male; catalogNumber: AUP-00486; basisOfRecord: Preserved Specimen; dynamicProperties: collection date, collector and Location as the AUP-00481

g. scientificName: *Limnonectes bannaensis*; individualCount: 1; sex: adult male; catalogNumber: AUP-00487; basisOfRecord: Preserved Specimen; dynamicProperties: collection date, collector and Location as the AUP-00481

h. scientificName: *Limnonectes bannaensis*; individualCount: 1; sex: adult male; catalogNumber: AUP-00488; basisOfRecord: Preserved Specimen; dynamicProperties: collection date, collector and Location as the AUP-00481

i. scientificName: *Limnonectes bannaensis*; individualCount: 1; sex: adult female; catalogNumber: AUP-00489; basisOfRecord: Preserved Specimen; dynamicProperties: collection date, collector and Location as the AUP-00481

j. scientificName: *Limnonectes bannaensis*; individualCount: 1; sex: adult male; catalogNumber: AUP-00490; basisOfRecord: Preserved Specimen; dynamicProperties: collection date, collector and Location as the AUP-00481

k. scientificName: *Limnonectes bannaensis*; individualCount: 1; sex: adult female; catalogNumber: AUP-00491; basisOfRecord: Preserved Specimen; dynamicProperties: collection date, collector and Location as the AUP-00481
Description

Morphological characters of specimens from Nan Province agreed with the descriptions by Ye et al. (2007). Large body size, with males SVL of 80.7 mm (n = 9) and females SVL of 75.4 mm (n = 2). The complete morphometric description of each specimen is presented in Suppl. material 2. They are morphologically distinct in comparison between sexes. Males can be distinguished from females by the dorsal skin texture of the male appearing to be smoother, with less tubercles, supratympanic fold dark brown, indistinct, throat heavily pigmented. Head longer than wide (males HL of 36.8 mm, HW 34.9 mm, n = 9 and females HL of 34.9 mm, HW 32.9 mm, n = 2). Fore limbs robust, relatively short, fingers moderately slender, finger length formula: II < I < IV < III (Fig. 3D), toe length formula: I < II < V < III < IV (Fig. 3E), tips of toes expanded into round elevated pads lacking grooves, toe webbing well-developed, complete, webbing formula: I 0 – 0 II 0 – 0 III 0 – 0 IV 0 – 0 V. Skin on dorsum weakly granulated with few fine folds on the back and a few small rounded tubercles scattered on the rear of the dorsum, ventrally smooth. Colouration in life: black stripes present on areas around the folds (Fig. 3A and C), dorsum light red brown with confluent dark brown markings (Fig. 3A and B and Fig. 4), dark transverse bars on upper surface of hind limbs, side of head and lateral surfaces of body lighter brown, lower lip white marbled with brown, belly white with brown vermiform markings, dark brown bar between eyes edged with thin yellowish-brown bars, lower half of iris golden, upper half brown, separated by a dark brown horizontal band, nuptial pad white. Colouration in preservative: after three years in preservative, the colouration pattern did not change, dorsal and lateral body colouration faded to brown, dark brown bars on upper lip turned less distinct, lower lip turned dark with light mottling, ventre immaculate, ventral portions of limbs mottled around margins, palmar and plantar surfaces turned dark brown.

Figure 3. Male of *Limnonectes bannaensis* (AUP-00485) in life. A. Dorsal view; B. Ventral views; C. Dorsal view of leg (notice the tubercles); D. Palmar view of hand; E. Ventral view of foot.
Distribution

This species is known from southern China, northern and central Vietnam and northern Laos (Frost 2020). This is the first record for Thailand, ca. 266 km southwest from the type locality in Jinghong City, Mengla County, Yunnan Province, China (Ye et al. 2007).

Ecology

Specimens were found between 19:00 to 21:00 h in small rocky streams (Fig. 5). Most specimens were found in the water. The surrounding habitat was secondary evergreen forest of medium growth. Other anuran species found in sympatry include: *Limnonectes taylori*, *Kurixalus bisacculus* (Taylor), *Leptobrachium* cf. *huashen* Fei & Ye, *Leptobrachella* cf. *minima* (Taylor) and *Amolops cremnobatus* Inger & Kottelat.

Figure 4. doi
Colour variations of *Limnonectes bannaensis* A. Dorsal view of male (AUP-00481); B. Dorsal view of female (AUP-00491).

Figure 5. doi
Habitat of *Limnonectes bannaensis* in Bo Kluea District, Nan Province, northern Thailand.
**Limnonectes utara** Matsui, Belabut & Ahmad, 2014

**Materials**

a. **scientificName:** *Limnonectes utara*; **class:** Amphibia; **order:** Anura; **family:** Dicroglossidae; **genus:** *Limnonectes*; **specificEpithet:** utara; **scientificNameAuthorship:** Matsui, Belabut & Ahmad, 2014; **country:** Thailand; **countryCode:** TL; **stateProvince:** Yala; **locality:** Bannang Sata; **verbatimElevation:** 680; **verbatimLatitude:** 6°11’39.5”N; **verbatimLongitude:** 101°18’28.2”E; **eventDate:** 21 August, 2018; **fieldNotes:** P. Pawangkhanant, C. Suwannapoom; **individualCount:** 1; **sex:** female; **lifeStage:** adult; **catalogNumber:** AUP-01706; **language:** en; **collectionCode:** Amphibians; **basisOfRecord:** Preserved Specimen

b. **scientificName:** *Limnonectes utara*; **individualCount:** 1; **sex:** female; **lifeStage:** adult; **catalogNumber:** AUP-01706; **basisOfRecord:** Preserved Specimen; **dynamicProperties:** collection date, collector and Location as the AUP-01705

c. **scientificName:** *Limnonectes utara*; **individualCount:** 1; **sex:** male; **lifeStage:** adult; **catalogNumber:** AUP-01707; **basisOfRecord:** Preserved Specimen; **dynamicProperties:** collection date, collector and Location as the AUP-01705

d. **scientificName:** *Limnonectes utara*; **individualCount:** 1; **sex:** male; **lifeStage:** adult; **catalogNumber:** AUP-01708; **basisOfRecord:** Preserved Specimen; **dynamicProperties:** collection date, collector and Location as the AUP-01705

**Description**

Morphological characters of specimens from Yala Province agreed with the description by Matsui et al. (2014): Body size moderate, with males SVL of 70.7 mm (n = 2) and females SVL of 46.1 mm (n = 2). The complete morphometric description of each specimen is presented in Suppl. material 2. Head slightly longer than wide (males HL of 32.8 mm, HW 29.8 mm, n = 2 and females HL of 20.0 mm, HW 19.6 mm, n = 2). Snout obtusely pointed in dorsal view, obtuse in profile, projecting beyond the lower jaw. Eye diameter shorter than snout length, canthus rostralis rounded, loreal region sloping and concave, nostril dorsolaterally orientated, placed closer to tip of snout than to eye, internarial distance equal to upper eyelid width. Fore limb robust, relatively short and moderately slender fingers, finger length formula: II < I < IV < III (Fig. 6F), toe length formula, I < II < V < III < IV (Fig. 6E), tips of toes expanded into round, elevated pads lacking grooves, toe webbing complete, webbing formula, I 0 – 0 II 0 – 0 III 0 – 0 IV 0 – 0 V.

Skin on dorsal surfaces of head, fore limbs and body feebly crenulate, skin of body flanks rough with moderately, roundish and non-pearl tipped tubercles, skin around vent, knees and shanks distinctly tuberculate, covered with moderately, small, low tubercles with translucent spinules, ventral surfaces smooth, pair of faint, but broken dorsolateral folds extending from posterior of eye to vent.

Colouration in life: dorsum light brown with confluent dark brown markings (Fig. 6A), head with narrow light bands placed anteriorly to the dark interorbital bar, blackish-brown stripe on canthus rostralis, sides of head pale brown with dark markings. Ventral surfaces of hand and foot dark brown (Fig. 6E and F). Colouration in preservative: after
two years in preservative, dorsal colouration slightly faded, but other than that, no obvious change in colour pattern has occurred.

![Image](image1.png)

**Figure 6.** doi
Male of *Limnonectes utara* (AUP-01708) in life. **A.** Dorsal view; **B.** Lateral view of head; **C.** Dorsolateral view of head; **D.** Dorsal view of leg (notice the tubercles); **E.** Ventral view of foot; **F.** Ventral view of hand.

**Distribution**

Prior to these records, this species was considered endemic to Peninsular Malaysia. This is the first country record for Thailand, ca. 158 km northeast from the type locality [Bukit Larut (= Larut Hill), Perak State, Peninsular Malaysia] (Matsui et al. 2014).

![Image](image2.png)

**Figure 7.** doi
Habitat of *Limnonectes utara* in Bannang Sata District, Yala Province, southern Thailand.
Ecology

Specimens were found after 20:00 h in small rocky streams. Most specimens were found in the water. All specimens were collected in evergreen forests along hillside streams and small tributaries varying in width from 1 m to 2 m (Fig. 7). Other syntopic anuran species include: *Limnonectes plicatellus* (Stoliczka), *Nyctixalus pictus* (Peters) and *Rhacophorus rhodopus* Liu & Hu.

Discussion

In this study, we examined newly-collected samples of *Limnonectes* species related to the *L. kuhlii* species complex, from previously not surveyed areas in northern and southern Thailand. From a biogeographic perspective, according to Matsui et al. (2010a), *L. taylori* was thought to be the unique representative of the *L. kuhlii* species complex in northern Thailand, whereas *L. jarujini* was believed to occur in the southern part of the country, the biogeographic distribution between these species being located between Thong Pha Phum and Khao Laem National Parks in Kanchanaburi Province. With the exception of *L. bannaensis* and *L. utara*, it was already known which other species of the *L. kuhlii* complex occur in northern and southern Thailand; therefore, it is not a result that can be obtained from phylogenetic analysis. Actually, phylogeny corroborates the identification of the collected specimens and, thus, demonstrates that *L. bannaensis* and *L. utara* occur in northern and southern Thailand, respectively. Our new records of *L. bannaensis* and *L. utara* from Thailand increase to 20 the number of *Limnonectes* species occurring in the country.

Our study and others like this (e.g. Suwannapoom et al. 2016) further highlight the importance of using molecular data in combination with traditional morphological characteristics. This is especially important for species complexes whose members have sympatric distribution, which is the case with the *L. kuhlii* complex. We recorded sympatric occurrence of *L. taylori* and *L. bannaensis*, which were observed sharing the same habitats at Bo Kluea, Nan Province, northern Thailand. Consistent with the findings of previous studies involving the *Limnonectes* species complex (e.g. Suwannapoom et al. 2016), our results demonstrate that species living in sympathy are not necessarily close relatives (i.e. sister taxa).

These two sympatric members of the *Limnonectes* species complex in Bo Kluea, Nan Province, are difficult to distinguish from each other, based only on morphological evidence. The application of molecular methods is crucial for reliable identification and can guide morphological re-examinations, further elucidating fine-scale differences in morphological characteristics that represent species-specific variations. Identification of tadpoles, juveniles and adult females still remains challenging in the field. Our study underscores that the herpetofaunal diversity of Thailand still remains underestimated and also illustrates the special role of evergreen forests with regard to biodiversity conservation in the country.
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Supplementary materials

**Suppl. material 1: Genetic distance between species of *Limnonectes*.** [doi](https://doi.org/10.1080/10635150802429642)

**Authors:** Chatmongkon Suwannapoom

**Data type:** Table

**Brief description:** The pairwise uncorrected p-distance (%) of 16S rRNA gene between species of *Limnonectes*.

**Download file** (16.92 kb)
Suppl. material 2: Measurement (in mm) and proportions of the series of *Limnonectes bannaensis* and *L. utara* [doi](#)

**Authors:** Chatmongkon Suwannapoom  
**Data type:** Table  
**Brief description:** Measurements (in mm) and proportions of the series of *Limnonectes bannaensis* from Nan Province and *L. utara* from Yala Province. (M = Male, F = Female; N/a = Not applicable; for other abbreviations, see Materials and Methods).

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