

Research article

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A new species of mossy frog (Anura: Rhacophoridae) from Northeastern Vietnam

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Abstract. We describe a new species of *Theloderma* from northeastern Vietnam based on morphological differences and molecular divergence. *Theloderma khoii* sp. nov. is distinguishable from its congeners on the basis of a combination of the following characters: large size, SVL 52.1 mm in male, 59.4 mm

in female; head length and width equal; vomerine teeth present; snout pointed and truncated, eye large, ED 4.7 mm in male, 5.6 mm in female, spinules on upper eyelid; tibiotarsal articulation reaches to the posterior border of the eye or the tip of the snout; dorsal skin very rough with large irregular gland ridges and warts, ventral surface of body granular; tips of all digits dilated but all considerably smaller than tympanum; dorsal surface mossy green or olive mottled with dark magenta. The distribution of the new species is unknown but probably extends into adjacent high elevation forested areas in Ha Giang Province, Vietnam and in Yunnan Province, China with an extent of occurrence of only <1000 km² and continuing decline in the quality of its habitat due to deforestation. Thus, we suggest the species should be considered Endangered following IUCN's Red List categories.

Keywords. *Theloderma khoii* sp. nov., karst forest, molecular phylogeny, taxonomy, Ha Giang Province.

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Introduction

The genus *Theloderma* Tschudi, 1838 is a poorly known group of tree frogs due to their cryptic habits and because they are infrequently encountered (Nguyen *et al.* 2014). This genus is currently comprised of 26 species distributed throughout Southeast Asia, southern China and northeastern India (Hou *et al.* 2017; Poyarkov *et al.* 2018; Frost 2021). *Theloderma* is characterized by the combination of the following morphological characters: (1) distinct tympanum, (2) rounded canthus rostralis, (3) absence of bony ridges from canthus rostralis to occiput, (4) skin of head not co-ossified to the skull, (5) having some degree of tuberculate skin, sometimes with calcified warts on the dorsum, and (6) Y-shaped terminal phalanges (Liem 1970; McLeod & Norhayati 2007; Rowley *et al.* 2011; Nguyen *et al.* 2015, 2016; Poyarkov *et al.* 2015, 2018; Dever 2017; Hou *et al.* 2017). However, morphological synapomorphies are lacking for the genus, and the monophyly of *Theloderma* is not certain (Bain *et al.* 2009; Li *et al.* 2009; Rowley *et al.* 2011) according to the recent review by Dubois *et al.* (2021). Many species were described based on a single or a few specimens only, and are still imperfectly known (Stuart & Heatwole 2004; Orlov *et al.* 2006; Bain *et al.* 2009). To date, 16 species have been recorded from Vietnam: *T. albopunctatum* (Liu & Hu, 1962), until recently recorded as *T. asperum* (Boulenger, 1886); *T. annae* Nguyen, Pham, Nguyen, Ngo & Ziegler, 2016; *T. auratum* Poyarkov, Kropachev, Gogoleva & Orlov, 2018; *T. bicolor* (Bourret, 1937); *T. corticale* (Boulenger, 1903); *T. gordonii* Taylor, 1962; *T. laeve* (Smith, 1924); *T. lateriticum* Bain, Nguyen & Doan, 2009; *T. nebulosum* Rowley, Le, Hoang, Dau & Cao, 2011; *T. palliatum* Rowley, Le, Hoang, Dau & Cao, 2011; *T. petilum* (Stuart & Heatwole, 2004); *T. rhododiscus* (Liu & Hu, 1962); *T. ryabovi* Orlov, Dutta, Ghate & Kent, 2006; *T. truongsongense* (Orlov & Ho, 2005); *T. vietnamense* Poyarkov, Orlov, Moiseeva, Pawangkhanant, Ruangsuwan, Vassilieva, Galoyan, Nguyen & Gogoleva, 2015 (formerly recorded as *T. stellatum* Taylor, 1962) (Orlov & Ho 2005; Orlov *et al.* 2006, 2012; Orlov 2007; Bain *et al.* 2009; Nguyen *et al.* 2009, 2012, 2015, 2016; Rowley *et al.* 2011; Poyarkov *et al.* 2015, 2018).

During recent field work in Ha Giang Province, northeastern Vietnam, specimens of a tree frog species were collected in deep narrow valleys within limestone mountains, between elevations of 1320 to 1750 m a.s.l. The area was covered by primary and secondary broadleaved karst forests. Molecular comparisons revealed that this taxon was nested within the genus *Theloderma*. Closer morphological examination showed that the newly discovered population from Ha Giang is clearly distinguishable from other known members of *Theloderma* by a combination of morphological features. The phylogenetic analyses revealed this taxon to be sister to *T. bicolor* with a strong support value, but being distinctly separated from it. Due to morphological and molecular differences between the newly collected specimens and all other congeneric species, we herein describe the newly discovered *Theloderma* population from Ha Giang as a new species.

Material and methods

Sampling

Field surveys were conducted in June 2020 by H.Q. Nguyen and Q.K. Le in Tung Vai and Cao Ma Po Communes, Quan Ba District, Ha Giang Province, northeastern Vietnam. Specimens were collected between 19:00 and 23:30 h. 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 4–6 hours, and then later transferred to 70% ethanol for permanent storage. Tissue samples from liver were preserved separately in 95% ethanol. Preserved specimens were deposited in the collection of the Vietnam National Museum of Nature (VNMN), Hanoi, Vietnam. Other specimens referred to in this paper (see Appendix 1) are deposited at the American Museum of Natural History (AMNH), Graduate School of Human and Environmental Studies, Kyoto University (KUHE), and Institute of Ecology and Biological Resources (IEBR), Hanoi.

Morphological characters

Measurements of preserved specimens were taken with a digital caliper to the nearest 0.1 mm. Abbreviations are as follows:

DAE	=	Distance between anterior corners of eyes
DPE	=	Distance between posterior corners of eyes
ED	=	Eye diameter
EN	=	Distance from anterior corner of the eye to the nostril
fd1–4	=	Width of discs of fingers I–IV (maximal width of discs)
FeL	=	Femur length (from vent to knee)
FLL	=	Forelimb length (from axilla to elbow)
FoL	=	Foot length (from tarsus to the tip of fourth toe)
FTL	=	Fourth toe length
fw1–4	=	Width of fingers I–IV (measured at middle of the knuckles close to the disc)
HAL	=	Hand length (from elbow to the tip of third finger)
HL	=	Head length (from the back of the mandible to the tip of the snout)
HW	=	Maximum head width (across angle of jaws)
IMT	=	Inner metatarsal tubercle length
IN	=	Internarial distance
IOD	=	Interorbital distance
MAE	=	Distance between angle of jaw and anterior corner of the eye
MN	=	Distance from the back of the mandible to the nostril
MPE	=	Distance between angle of jaw and posterior corner of the eye
NS	=	Distance from nostril to the tip of the snout
OMT	=	Outer metatarsal tubercle length
OPT	=	Outer palmar tubercle length
SNL	=	Snout length (from anterior corner of eye to the tip of the snout)
SVL	=	Snout-vent length
TbL	=	Tibia length (from knee to tarsus)
TbW	=	Maximum tibia width
td1–4	=	Width of discs of toes I–IV (maximal width of discs)
TFL	=	Third finger length
tw1–4	=	Width of toes I–IV (measured at middle of the knuckles close to the disc)
TYD	=	Tympanum diameter
TYE	=	Distance from anterior margin of tympanum to posterior corner of the eye
UEW	=	Maximum width of upper eyelid

Table 1. Samples of *Theloderma* Tschudi, 1838 used and included in the molecular analyses.

No	Species	Voucher	Locality	GenBank No.	Reference
1	<i>T. bicolor</i>	VNMN 010811	Lai Chau, Viet Nam	LC641699	This study
2	<i>T. bicolor</i>	VNMN 010821	Lai Chau, Viet Nam	LC641700	This study
3	<i>T. bicolor</i>	VNMN 3536	Lao Cai, Viet Nam	KJ802915	Nguyen <i>et al.</i> 2014
4	<i>Theloderma</i> sp.	VNMN 012757	Ha Giang, Viet Nam	LC641701	This study
5	<i>Theloderma</i> sp.	VNMN 012758	Ha Giang, Viet Nam	LC641702	This study
6	<i>T. corticale</i>	DYS2	Guangxi, China	KY495636	Hou <i>et al.</i> 2017
7	<i>T. corticale</i>	VNMN 03556	Vinh Phuc, Viet Nam	LC012841	Nguyen <i>et al.</i> 2015
8	<i>T. corticale</i>	VNMN J2892	Tuyen Quang, Viet Nam	KJ802916	Nguyen <i>et al.</i> 2014
9	<i>T. corticale</i>	AMNH A161499	Vinh Phuc, Viet Nam	DQ283050	Frost <i>et al.</i> 2006
10	<i>T. leporosum</i>	LJT W46	Malaysia	KC465841	Li <i>et al.</i> 2016
11	<i>T. leporosum</i>	Tlep1	Selangor, Malaysia	KT461922	Poyarkov <i>et al.</i> 2015
12	<i>T. leporosum</i>	KUHE 52581	Kenaboi, Malaysia	AB847128	Matsui <i>et al.</i> 2014
13	<i>T. lateriticum</i>	AMNH 168757	Lao Cai, Viet Nam	LC012848	Nguyen <i>et al.</i> 2015
14	<i>T. lateriticum</i>	IEBR 3745	Lao Cai, Vietnam	LC641703	This study
15	<i>Nyctixalus pictus</i>	FMNH 231094	Sabah, Malaysia	AF458135	Wilkinson <i>et al.</i> 2002

Terminology for describing eye coloration in life and webbing formula followed those of Glaw & Vences (1997, 2007). Sex was determined by the examination of the presence of male nuptial pads and gonadal inspection.

Molecular data and phylogenetic analyses

We used the protocols of Kuraishi *et al.* (2013), modified by Nguyen *et al.* (2015), for DNA extraction, amplification and sequencing. Fragments of three mitochondrial DNA genes, 12S rRNA, tRNA^{val} and 16S rRNA, were amplified using the primers following Kuraishi *et al.* (2013). *Nyctixalus pictus* (Peters, 1871) was selected as outgroup according to Nguyen *et al.* (2015) (Table 1).

Chromas Pro software (Technelysium Pty Ltd., Tewantin, Australia) was used to edit the sequences, which were aligned using MAFFT ver. 7 (Kato & Standley 2013) with default settings. We then checked the initial alignments by eye and adjusted slightly. Phylogenetic trees were constructed by using maximum likelihood (ML) and Bayesian inference (BI). Prior to ML and Bayesian analyses, we chose the optimum substitution models for entire sequences using Kakusan 4 (Tanabe 2011) based on the Akaike information criterion (AIC). The best model selected for ML was the general time reversible model (GTR: Tavaré 1986) with a gamma shape parameter (G: 0.220 in ML and 0.258 in BI). The BI summarized two independent runs of four Markov Chains for 10 000 000 generations. A tree was sampled every 100 generations and a consensus topology was calculated for 70 000 trees after discarding the

first 30 000 trees (burn-in = 3 000 000). We checked parameter estimates and convergence using Tracer ver. 1.5 (Rambaut & Drummond 2009). The strength of nodal support in the ML tree was analyzed using non-parametric bootstrapping (MLBS) with 1000 replicates. We regarded tree nodes in the ML tree with bootstrap values of 70% or greater as sufficiently resolved (Huelsenbeck & Hillis 1993), and nodes with a BPP of 95% or greater as significant in the BI analysis (Leaché & Reeder 2002). Pairwise comparisons of uncorrected sequence divergences (p-distance) were calculated for 16S rRNA fragments only between species of the genus *Theloderma*.

In the preliminary analyses combining all 26 known *Theloderma* species and *Nyctixalus pictus* as outgroup, the newly discovered population from Ha Giang Province, *Theloderma bicolor* and *T. corticale* formed a clade with strong support (Bayesian posterior probability [BPP] = 95, bootstrap support [MLBS] = 89%). These results clearly suggest placement of the rhacophorid species from Ha Giang Province in the genus *Theloderma*.

Aligned, combined sequences of 16S rRNA yielded a total of 920 bp. Of 920 nucleotide sites, 219 were variable and 207 were parsimony informative within the ingroup. The ML and Bayesian analyses produced topologies with $-\ln L = 2967.76$ and 2993.59, respectively. Phylogenetic analyses employing ML and BI methods yielded slightly different topologies only among referenced species, and only the BI tree is presented in Fig. 1. The newly discovered population of *Theloderma* from Ha Giang was

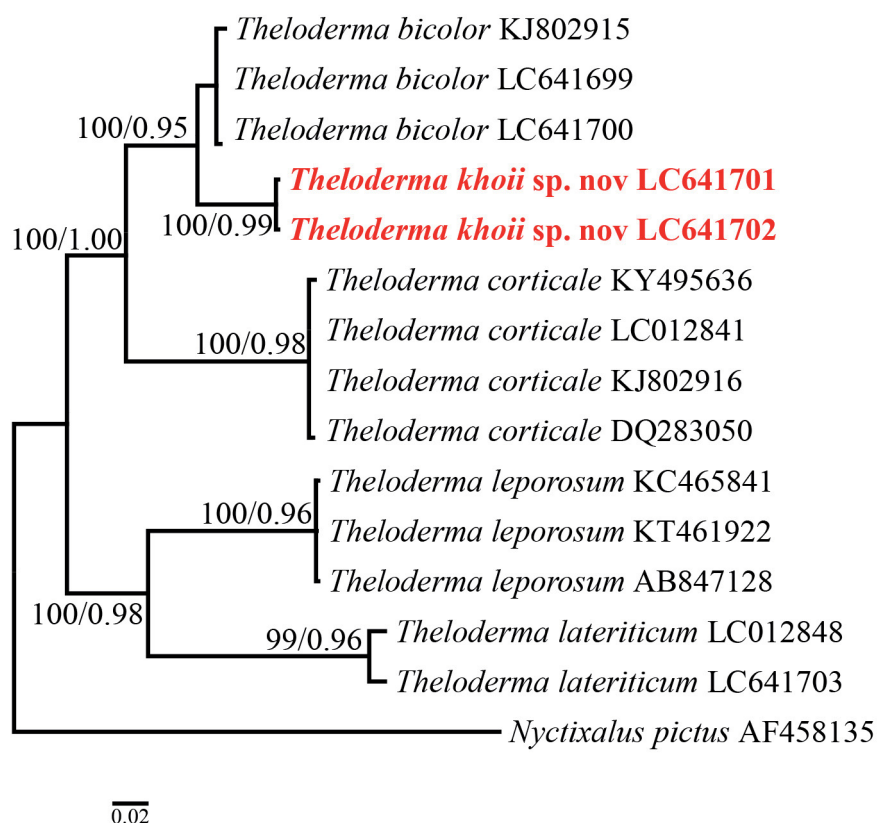


Fig. 1. BI tree from a 920 bp sequence of mitochondrial 16S rRNA gene of *Theloderma* Tschudi, 1838 and outgroup species. Numbers above and below branches are Bayesian posterior probabilities (values ≥ 0.95 shown) and ML bootstrap values (values ≥ 70 shown), respectively; for GenBank accession numbers, see Table 1.

Table 2. Uncorrected p-distances (%) between species of *Theloderma* Tschudi, 1838 estimated from 16S rRNA sequences.

	1.	2.	3.	4.
1. <i>Theloderma khoii</i> sp. nov. VNMN 012757	–			
2. <i>T. bicolor</i> VNMN 010821	4.6			
3. <i>T. corticale</i> LC012841	11.7	10.8		
4. <i>T. lateriticum</i> IEBR 3745	17.1	14.8	17.50	
5. <i>T. leporosum</i> AB847128	13.6	12.9	15.3	14.1

sister to *Theloderma bicolor* from Lao Cai and Lai Chau provinces, Vietnam (KJ802915, LC641699, LC641700), with their monophyly being fully supported (BPP = 1.00, MLBS = 100%).

The interspecific uncorrected genetic p-distances of the fragment of 16S rRNA gene examined between the newly discovered population of *Theloderma* from Ha Giang and all congeners analyzed varied from approximately 4.6% (compared with *T. bicolor*) to 17.1% (compared with *T. lateriticum*).

This degree of pairwise divergence in the 16S rRNA gene in frogs has been interpreted previously as indicative of differentiation at the species level (Vences *et al.* 2005). Furthermore, the new population is also clearly separated morphologically from all nominal species of *Theloderma*. Thus, we conclude that the rhacophorid species from Ha Giang, Vietnam is a distinct species in the genus *Theloderma*.

Results

Class Amphibia Gray, 1825
Order Anura Fischer von Waldheim, 1813
Family Rhacophoridae Hoffman, 1932
Genus *Theloderma* Tschudi, 1838

***Theloderma khoii* sp. nov.**

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Figs 2–3

Diagnosis

Theloderma khoii sp. nov. shows the diagnostic characters of the genus *Theloderma*, for instance a distinct tympanum, round canthus rostralis, bony ridges from canthus rostralis to occiput absent, and skin of head not co-ossified to the skull, and also molecular analyses revealed it to be nested within *Theloderma*. The new species is distinguished from its congeners and other small rhacophorid species by a combination of the following characters: 1) large-sized frog of the genus *Theloderma* (SVL 52.2 mm in a single male, 59.4 mm in a single female); 2) head length and width equal; 3) vomerine teeth present; 4) snout pointed and truncated (SNL/SVL 16.3% in male; 16.0% in female); 5) eye large, eye diameter about a half of snout length (ED/SNL 54.60% in male; 58.9% in female), spinules on upper eyelid; 6) tibiotarsal articulation reaching to the posterior border of the eye or the tip of the snout; 7) dorsal skin very rough, with large irregular gland ridges and warts, ventral surface granular; 8) fingers rudimentary webbed, toes almost four-fifths webbed, tips of all digits dilated but all considerably smaller than

tympanum; 9) dorsal surface mossy green or olive mottled with dark magenta; 10) lateral and ventral surfaces black with irregular lemon yellow pattern and some white granules; 11) male with large nuptial pads and an inner vocal sac.

Etymology

The specific epithet is in honor of Professor Dr Le Vu Khoi of the VNU Hanoi University of Science, Ha Noi, Vietnam, in recognition of his great support of teaching, mentoring, research and conservation work in Vietnam. As common names we suggest Khoi's Mossy Frog (English) andẾch cây sần khôì (Vietnamese).

Type material

Holotype

VIETNAM • adult ♂; northern Vietnam, Ha Giang Province, Quan Ba District, in the karst forest near Tung Vai Commune; 1641 m a.s.l; 8 Jun. 2020; H.Q. Nguyen leg.; VNMN 012757.

Paratype

VIETNAM • adult ♀; same collection data as for holotype; VNMN 012758.

Description

Holotype

SIZE. Large, body distinctly flattened (SVL 52.2 mm).

HEAD. Very strongly depressed, length and width equal (HL 19.7 mm, HW 19.7 mm), snout pointed and truncated, snout length double the diameter of eye (SNL 8.5 mm, ED 4.7 mm).

CANTHUS ROSTRALIS. Oval, loreal region very oblique, slightly concave; interorbital distance wider than internarial distance and upper eyelid width (IOD 6.0 mm, IN 3.7 mm, UEW 4.5 mm); distance between anterior corners of eyes (DAE 10.1 mm) about 59.8% distance between posterior corners of eyes (DPE 16.3 mm).

NOSTRIL. Oval, inside rather enlarged tubercle on tip of snout, nostrils nearer to tip of snout than to eyes (NS 3.1 mm, EN 6.1 mm).

PUPIL. Circular.

TYMPANUM. Distinct, diameter less than eye diameter and slightly larger than width of third finger disc (TYD 3.7 mm), tympanum separated from eye by distance (TYE) 65.0% of tympanum length (TYD).

PINEAL OCELLUS. Absent; conical tubercles on eyelid, smaller than those in the back.

SUPRATYMPANIC FOLD. Distinct, interrupted, extending from behind eye to beyond level of axilla, composed of large irregular glandular ridges.

VOMERINE TEETH. Arranged in two small oblique groups between choanae and widely separated from each other.

TONGUE. Large, accounting for almost half of mouth, heart-shaped, dorsal surface smooth, notched and free posterior.

FORELIMBS. Moderately strong and long (FLL 13.2 mm, HAL 15.1 mm, 54.3% of SVL), much shorter than hindlimbs (FLL 87.5% of HAL); relative length of fingers: I < II < IV < III; tips of fingers dilated into

large, rounded and flattened discs, with grooves separating dorsum of discs from venter; disc of finger III approximately two times width of finger III (fd3/fw3 219.2%) but smaller than tympanum diameter (fd3/TYD 83.1%); subarticular tubercles large and distinct, with indistinct supernumerary tubercles; formula of subarticular tubercles: 1, 1, 2, 2; well-developed nuptial pad on lateral side of thumb; outer side of fourth finger fringed, outer edge of fourth finger distinctly serrated; webbing between fingers slightly developed, about 50% between base of finger up to proximal subarticular tubercles (Fig. 3).

HINDLIMBS. Strong and long, tibia length about five times greater than tibia width (TbL 27.3 mm, TbW 5.65 mm, TbL/TbW 4.6), tibia longer than thigh length and foot length (TbL/FeL 107%; TbL/FoL 108%), tibio-tarsal articulation reaching posterior edge of snout when hindlimbs pressed forward as opposed to eye; relative length of toes $I < II < V < III < IV$; toes with rounded discs, tips of toes dilated into flattened discs with grooves that separate dorsum from venter, smaller than discs on fingers; webbing formula $I(1/3) - (1)II(0) - (1/2)III(0) - (1)IV(1) - (1/3)V$ (Fig. 3); large subarticular tubercles roundish: 1, 1, 2, 3, 2; large inner metatarsal tubercle oval and raised (IMT 2.7 mm), outer metatarsal tubercle absent; with small warts on inner aspect of metatarsal area; outer side of fifth toe fringed, outer edge of fringe with five distinct serrations.

SKIN TEXTURE IN LIFE. Dorsal surface of head, dorsum, arms and legs above very rough, with large irregular glandular ridges ordered symmetrically in middle vertebral region; a large, distinct horn gland behind head in X-shape; on top of each supraorbital 3–4 enlarged glands, distinctly conical; lateral parts of body become granular; large conical tubercles on back of thighs near vent; throat and chest with some small blurred pattern; belly and ventral surface and underside of thigh with thickened flat granules or



Fig. 2. *Theloderma khoii* sp. nov., holotype, ♂ (VNMN 012757). Dorsolateral view, in life.

warts on groin; axillary region and underside of tibia smooth; surroundings of tympanum with small tubercles, with 4–5 enlarged tubercles posteriorly.

COLORATION IN LIFE. Dorsal surface mossy-green or light-olive mixed with mossy-green, resembling moss; tips of skin ridges and tubercles reddish-brown; flank with clearly shaped dark brown pattern, ventrally joining a yellowish-green band; dorsal surfaces of forelimbs and hindlimbs with army-green bands, and some irregular patches on webbing; both finger and toe discs green, in center deep olive; nuptial pad ivory; throat and chest dark-brown with yellowish-green warts; belly has black shape of irregular size with yellowish-green pattern and some cream warts; pupil black, iris yellowish-green with irregular black reticulation; tympanum dark-olive (Fig. 2).

COLORATION IN PRESERVATIVE. In ethanol, dorsal surfaces of head, dorsum, and upper part of flanks dark indigo with brown markings and blotches; tympanum light brown; forelimb, dorsal surface of thigh, tibia and foot black with lights bands, posterior part of thigh below vent dark-grey with large ivory bands; chin and throat light-grey with small ivory spots; chest and belly dark to black, with cream areas on chest (Fig. 3).

Variation of paratype

Male smaller than female, with single vocal sac and nuptial pads. For measurements of the type series see Table 3.

Comparison with other species

We compared *Theloderma khoii* sp. nov. with other members of the genus *Theloderma* based on morphological specimen examination (see Appendix 1) and data obtained from the literature (e.g., Tschudi 1838; Boulenger 1903; Smith 1924; Ahl 1927, 1931; Bourret 1937, 1942; Liu & Hu 1962; Taylor 1962; Chanda 1994; Inger *et al.* 1999; Chanard 2003; Bain & Nguyen 2004; Stuart & Heatwole



Fig. 3. *Theloderma khoii* sp. nov., holotype, ♂ (VNMN 012757). Dorsal (left) and ventral view (right) during fixation.

Table 3. Measurements (in mm) and proportions of the type series of *Theloderma khoii* sp. nov. (for abbreviations see Material and methods).

	VNMN 012757	VNMM 012758		VNMN 012757	VNMM 012758
	♂	♀		♂	♀
	holotype	paratype		holotype	paratype
SVL	52.2	59.4	TYD	3.7	4.0
HW	19.7	20.7	TYE	2.4	2.6
HL	19.7	21.5	FLL	13.2	14.7
MN	18.5	19.4	HAL	15.1	19.6
MFE	13.1	14.0	TFL	3.54	5.54
MBE	8.8	8.7	fd3	3.2	4.2
SNL	8.5	9.5	FeL	25.6	27.1
ED	4.6	5.6	TbL	27.3	29.6
UEW	4.5	5.7	TbW	6.0	7.0
IN	3.7	3.6	FoL	25.3	27.2
IOD	6.1	5.5	FTL	25.1	26.9
DAE	10.1	11.8	SNL/SVL	16.3%	16.0%
DPE	16.3	17.7	ED/SNL	54.6%	58.9%
NS	3.0	2.9	TYE/TYD	65.1%	63.6%
EN	6.1	6.2	fd3/TYD	83.1%	76.5%

2004; Orlov & Ho 2005; Orlov *et al.* 2006, 2012; McLeod & Norhayati 2007; Bain *et al.* 2009; Chan & Norhayati 2009; Fei *et al.* 2009, 2012; Jiang *et al.* 2009; Kunz *et al.* 2010; Rowley *et al.* 2011; Nguyen *et al.* 2014).

Species of *Theloderma* inhabiting Indochina, Sumatra, southern China and eastern India can be assigned to three different size groups (Dever 2017): small species (SVL < 35 mm), such as *T. albopunctatum* (Liu & Hu, 1962); *T. annae* Nguyen, Pham, Nguyen, Ngo & Ziegler, 2016; *T. asperum* (Boulenger, 1886); *T. auratum* Poyarkov, Kropachev, Gogoleva & Orlov, 2018; *T. baibungense* Jiang, Fei & Huang, 2009; *T. lacustrinum* Sivongxay, Davankham, Phimmachak, Phoumixay & Stuart, 2016; *T. laeve* (Smith, 1924); *T. lateriticum* Bain, Nguyen & Doan, 2009; *T. licin* McLeod & Ahmad, 2007; *T. nebulosum* and *T. palliatum* Rowley, Le, Hoang, Dau & Cao, 2011; *T. petilum* (Stuart & Heatwole, 2004); *T. pyaukkya* Dever, 2017; *T. rhododiscus* (Liu & Hu, 1926); *T. (Stelladerma in Poyarkov et al. 2015) stellatum* Taylor, 1926; *T. (Stelladerma in Poyarkov et al. 2015) vietnamense* (Poyarkov *et al.*, 2015); and *T. truongsongense* (Orlov & Ho, 2005); medium-sized species with SVL of 40–45 mm, *T. horridum* (Boulenger, 1903); *T. moloch* (Annandale, 1912); *T. phrynoderma* (Ahl, 1927); *T. nagalandense* and *T. ryabovi* Orlov, Dutta, Ghate & Kent, 2006; and *T. schmardana* (Kelaart, 1853); large-sized species with SVL 48–75 mm (Orlov 1997), including *T. bicolor* (Bourret, 1937); *T. corticale* (Boulenger, 1903); *T. gordonii* Taylor, 1962; *T. leporosum* Tschudi, 1838; and *Theloderma khoii* sp. nov.

Compared to *T. bicolor*, another large-sized group member in Vietnam, *Theloderma khoii* sp. nov. can be distinguished by its coloration pattern: the lateral part from the axilla to groin is black with an irregular yellowish-green pattern in *T. bicolor* vs flank pattern with clear brown-dark speckles, ventrally joining the yellowish-green band in *Theloderma khoii* sp. nov.; a comparatively lighter belly pattern, consisting

of irregular blackish brown speckles with whitish pattern in *T. bicolor* vs belly irregular black with yellowish-green pattern and some cream warts, and the presence of few smaller bluish spots on the throat, in *Theloderma khoii* sp. nov. (Fig. 4); by the comparatively dark-colored posterior part of the thighs, consisting of black blotches and irregular light-green or olive reticulations (vs black with yellow-greenish bands and a few red-brown warts in *Theloderma khoii* sp. nov.; Fig. 4); and foot webbing well-developed: I(0) – (1)II(0) – (1/2)III(0) – (1)IV(1) – (0)V (vs I(1/3) – (1)II(0) – (1/2)III(0) – (1)IV(1) – (1/3)V in *Theloderma khoii* sp. nov.; Fig. 4). *Theloderma khoii* sp. nov. (Bourret 1937; Hou *et al.* 2017) differs from *T. corticale* by its shorter body size: SVL 52.2 mm in male and 59.4 mm in female

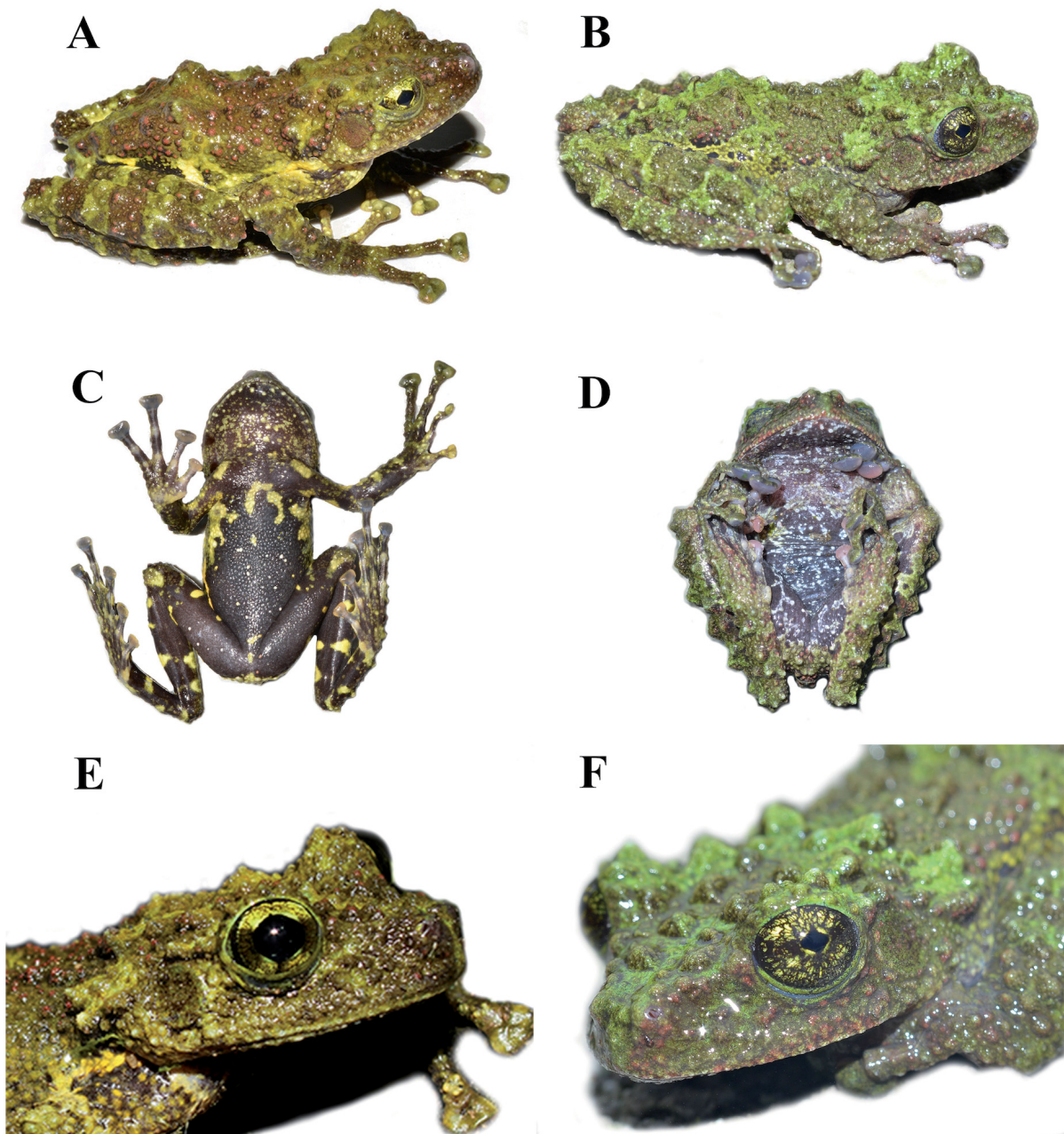


Fig. 4. A, C, E. *Theloderma khoii* sp. nov., holotype, ♂ (VNMN 012757). B, D, F. *Theloderma bicolor* (Bourret, 1937) (VNMN 010811). Dorsal (A–B) and ventral views (C–D) as well as lateral head (E–F).

in *Theloderma khoii* sp. nov. vs SVL up to 67 mm in *T. corticale*; by colour pattern: a much lighter belly pattern, yellow-lemon with irregular small black patterns and creamy warts (vs dark belly pattern, blackish ground color with thin yellowish-green pattern and some cream warts in *Theloderma khoii* sp. nov.); by skin texture: the absence of enlarged warts in the scapular area (vs large irregular prominent warts on dorsum, forming large groups at the base of the head and scapular area in *Theloderma khoii* sp. nov.); by the head being slightly wider than long (vs head length and width equal in *Theloderma khoii* sp. nov.); and foot webbing well developed: webbing formula: I(0) – (1 + 12)II(0) – (1)III(0) – (1)IV(1) – (0)V (vs I(1/3) – (1)II(0) – (1/2)III(0) – (1)IV(1) – (1/3)V in *Theloderma khoii* sp. nov.).

Theloderma khoii sp. nov. differs from *T. gordonii* by its coloration pattern: dorsal surface mossy-green or light-olive mixed with mossy-green, resembling moss; ventral yellowish-green band in *Theloderma khoii* sp. nov. vs dorsum dark brown or coffee with some clusters light brown or orange on enlarged gland ridges; ventral surface dark blue with numerous irregular grayish white patterns and speckles in *T. gordonii*. Tympanum and tympanic fold distinct in *Theloderma khoii* sp. nov. vs tympanum and tympanic fold invisible in *T. gordonii*; head width equal to head length in *Theloderma khoii* sp. nov. vs head width greater than head length in *T. gordonii* (Qui *et al.* 2018).

Ecological notes

Theloderma khoii sp. nov. appears to be closely associated with karstic environments. Specimens were found at night between 19:00 and 23:30 h near cave entrances and in valleys surrounded by limestone cliffs, about 5–6 m from water sources (Fig. 5). Advertisement calls, eggs and tadpoles of the species have not been recorded during our field surveys. The main habitat at the type locality was secondary



Fig. 5. Habitat of *Theloderma khoii* sp. nov. at the type locality in Quan Ba District, Ha Giang Province, Northern Vietnam.

karst forest of medium and small hardwoods mixed with shrubs and vines. One specimen was found on a leaf, about 0.5–1.2 m above the ground, the other specimen was collected on a limestone cliff, about 0.5–1.2 m above the ground. The air temperature at the time of collection ranged from 18.3 to 23.2°C and relative humidity from 91 to 100%. In Tung Vai, several species of *Theloderma* have been recorded, viz. *T. albopunctatum* (Liu & Hu, 1962), *T. gordonii* (Taylor, 1962), *T. lateriticum* (Bain, Nguyen & Doan, 2009), and *T. rhododiscus* (Liu & Hu, 1962), but all were found in tree holes filled with water or on tree leaves near streams in the valleys. Other tree frogs that were found at the same site were *Polypedates* sp. (of the *P. megacephalus* Hallowell, 1861 species complex), *Zhangixalus duboisi* (Ohler, Marquis, Swan & Grosjean, 2000), *Z. jodiae* Nguyen, Ninh, Orlov, Nguyen & Ziegler, 2020, *Z. franki* Ninh, Nguyen, Orlov, Nguyen & Ziegler, 2020, *Z. pachyproctus* Yu, Hui, Hou, Wu, Rao & Yang, 2019 and *Kurixalus* sp.

Distribution

Theloderma khoii sp. nov. is currently known only from the type locality in Ha Giang Province, northeastern Vietnam (Fig. 6). The species was recorded at elevations between 1320 and 1750 m a.s.l.

Conservation status

The range of the new species is not expected to extend outside of Mount Tung Vai and it is expected to be found in evergreen forests of Yunnan Province, southern China. However, the actual distributional range should be the focus of further studies. Currently, we assume that the new species is restricted geographically, likely having an Extent Of Occurrence (EOO) of only < 1000 km². The new species likely occurs in one threat-defined location, which has been characterized as an area with a continuing decline in the quality of its habitat due to deforestation (Meyfroidt *et al.* 2013). Therefore, *Theloderma khoii* sp. nov. likely qualifies as Endangered (EN) B1ab(iii) in accordance with the categories and criteria of the IUCN Red List of Threatened Species (IUCN 2012).

Discussion

In our phylogenetic analysis, *Theloderma khoii* sp. nov. is sister to *T. bicolor*, from which it differed by a 4.6% genetic divergence in fragments of the 16S gene (Table 2). The distribution range of *T. bicolor* encompasses the west of the Red River including northwestern Vietnam (Lai Chau and Lao Cai provinces), and also Jingdong and Luechun counties, central and southeastern Yunnan, China (Frost 2021), whereas *Theloderma khoii* sp. nov. is currently only known from Ha Giang Province, north of the Red River (Fig. 6). The discovery of *Theloderma khoii* sp. nov. supports the hypothesis that the Red River is a barrier to gene flow and dispersal (Zhi *et al.* 2016).

Morphologically, *Theloderma khoii* sp. nov. can clearly be distinguished from other members of *Theloderma* in having the dorsal surface being green or olive with mossy markings. The moss-green coloration on the dorsal surface of the new species, which can blend remarkably well into the background of stones covered with lichens or tree leaves, seems to be an adaptation to the life mode associated with the karst environment (Nguyen *et al.* 2016). The rich biodiversity of karst forests in Tung Vai as well as elsewhere in northeastern Vietnam is currently under threat due to the effects of border road construction, expanding agriculture, and illegal timber logging (Bain & Nguyen 2004; Ziegler *et al.* 2014; Nguyen *et al.* 2018).

Vietnam has more *Theloderma* species than any other country; a total of 16 species have been recorded to date (Frost 2021). The discovery of an additional species of *Theloderma* in Vietnam suggests that the current species richness of the genus remains underestimated. More studies using an integrative approach, i.e., combining morphological and molecular data, will help to reveal the extent of species richness of *Theloderma* in the poorly studied regions of northern Vietnam.



Fig. 6. Map showing distribution of *Theloderma bicolor* (Bourret, 1937) (blue circles) in Lao Cai and Lai Chau provinces, Vietnam and Jingdong and Luechun counties, Yunnan Province, China (west of the Red River) and the type locality (red circle) of *Theloderma khoii* sp. nov. in Ha Giang Province, Vietnam (north of the Red River).

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Appendix 1. Examined specimens.

Theloderma bicolor (Bourret, 1937)

VIETNAM • 2 ♂♂; Lao Cai Province, Sa Pa District; IEBR 3740, 3741 • 1 ♂; same collection data as for preceding; VNMN 3536 • 1 ♂, 1 ♀; Lai Chau Province, Sin Ho District; VNMN 010811, 010821.

Theloderma corticale (Boulenger, 1903)

VIETNAM • 1 ♂, 1 ♀; Bac Giang Province, Son Dong District; IEBR A.2013.84, 2013.85 • 2 ♂♂; Hoa Binh Province, Lac Son District; IEBR 3758, 3759 • 1 ♂, 1 ♀; Vinh Phuc Province, Tam Dao District; VNMN 03556, J2892.

Theloderma gordonii Taylor, 1962

VIETNAM • 1 ♂; Hoa Binh Province, Lac Son District; IEBR 3737 • 1 ♂, 1 ♀; Vinh Phuc Province, Tam Dao NP; IEBR 3738, 3739.

Theloderma lateriticum Bain, Nguyen & Doan, 2009

VIETNAM • 3 ♂♂; Lao Cai Province, Sa Pa District; IEBR 3745 to 3747; • 4 ♂♂, 2 ♀♀; Hoa Binh Province, Lac Son District; IEBR 3748 to 3753.

Theloderma truongsongense (Orlov & Ho, 2005)

VIETNAM • 3 ♂♂, 1 ♀; Quang Binh Province, Le Thuy District; IEBR 3754 to 3757.

Theloderma vietnamense Poyarkov, Orlov, Moiseeva, Pawangkhanant, Ruangsawan, Vassilieva, Galoyan, Nguyen & Gogoleva, 2015

VIETNAM • 3 ♂♂; Kien Giang Province, Phu Quoc District; IEBR 3742 to 3744.

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