

First record of *Theloderma lateriticum* Bain, Nguyen et Doan, 2009 (Anura Rhacophoridae) from China with redescribed morphology

Weicai Chen^{1,2*}, Xiaowen Liao³, Shichu Zhou³ & Yunming Mo³

¹Key Laboratory of Beibu Gulf Environment Change and Resources Utilization of Ministry of Education, Nanning Normal University, Nanning 530001, China

²Guangxi Key Laboratory of Earth Surface Processes and Intelligent Simulation, Nanning Normal University, Nanning 530001, China

³Natural History Museum of Guangxi, Nanning 530012, China

*Corresponding author, e-mail: chenweicai2003@126.com

ABSTRACT

Theloderma lateriticum Bain, Nguyen et Doan, 2009 (Anura Rhacophoridae) is recorded for the first time outside of Vietnam. The new locality record is from Shiwandashan National Nature Reserve, southern Guangxi, China, adjoining to Vietnam. We complemented and improved the morphological characters, including tadpole's morphology and advertisement calls.

KEY WORDS

Theloderma lateriticum; new national record; distribution; southern China.

Received 26.01.2019; accepted 05.03.2019; published online 28.03.2019

INTRODUCTION

Theloderma lateriticum Bain, Nguyen et Doan, 2009 (Anura Rhacophoridae) was described based on a single specimen (Voucher no. AMNH 168757/IEBR A. 0860, adult male). The type locality is the Hoang Lien Mountains, Lao Cai Province, northwestern Vietnam, between 1,300-1,400 meters elevation (Bain et al., 2009). Then, two new distribution records for Vietnam were reported, Yen Tu, Bac Giang (Voucher no. VNMN 1215, 1216, two adult males) and Ta Sua, Son La (Voucher no. TBUPAE 226, male; TBUPAE 227; female), respectively (Hecht et al., 2013; Nguyen et al., 2015; Pham & Nguyen, 2018). However, Nguyen et al. (2015) pointed out that Yen Tu and Ta Sua specimens present a vocal slit, but holotype lacks the vocal slit. Otherwise,

these specimens displayed high genetic variation, ranging from 0.5 to 4.9 based on combined sequences of 12S rRNA, tRNAval, and 16S rRNA yielded a total of 2412 bp positions (Nguyen et al., 2015).

In 2017, we carried out the monitoring of amphibians at Shiwandashan National Nature Reserve, Guangxi, China (21.844043° - N, 107.891647° E, 532 m asl). We found *Theloderma lateriticum* breeding in PVC buckets (diameter = 25 cm, height = 20 cm) that were used to monitor amphibians. Bain et al. (2009) have suggested that this species may also occur in neighboring southeastern Yunnan Province, China, and northeastern Laos. Herein, we reported the first record of *T. lateriticum* from China, and redescribed its morphological characters and constructed its phylogeny based on mitochondrial DNA genes fragments.

MATERIAL AND METHODS

Morphological data

All specimens were fixed in 10% formalin then stored in 75% ethanol. Before fixing in formalin, muscle tissue was collected and then stored in 100% ethanol for DNA extraction. Specimens were deposited at the Natural History Museum of Guangxi (NHMG).

Morphological measurements were taken with digital calipers to the nearest 0.1 mm. Measurements include snout-vent length (SVL); head length from tip of snout to rear of jaw (HL); head width at the commissure of the jaws (HW); snout length from tip of snout to the anterior corner of eye (SNT); diameter of the exposed portion of the eyeball (ED); interorbital distance (IOD); horizontal diameter of tympanum (TD); distance from anterior edge of tympanum to posterior corner of eye (TED); internarial space (IN); eye-nostril distance from anterior of eye to nostril (EN); tibia length with the hindlimb flexed (TIB); forelimb length from elbow to tip of the third finger (FLL); thigh length from vent to knee (THL); pes length from tip of the fourth toe to base of the inner metatarsal tubercles (PL); manus length from tip of the third digit to base of tubercle on prepollex (ML); diameter of the third finger disc (FTD3), and diameter of the fourth toe disc (HTD4). The webbing formula followed Myers & Duellman (1982). Tadpole labial tooth row formula (LTRF) followed Altig & McDiarmid (1999).

Molecular data

Genomic DNA was extracted from muscle using QIAgen DNeasy tissue extraction kits. The primers 16SAR and 16SBR of Palumbi et al. (1991) were used to amplify around 540 base pair fragment of the 16S rRNA gene, with standard PCR protocols. PCR products were directly sequenced using ABI 3730 DNA analyzer (Applied Biosystems, USA). Newly determined sequences were submitted for BLAST searching to ensure that the target fragment had been amplified (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) (Altschul et al., 1997) and deposited in GenBank (MH521262–3), then were aligned using Clustalx in MEGA 7 (Kumar et al., 2016) with the default settings. Given the close relationship be-

tween *Theloderma* and *Nyctixalus*, we included homologous DNA of two genera *Theloderma* and *Nyctixalus* downloaded from GenBank for phylogenetic analyses (Table 1). Uncorrected pairwise genetic variation was calculated in MEGA 7 using a ~530 bp mtDNA 16S fragment. The Akaike Information Criterion (AIC) implemented in MrModeltest 2.3 (Nylander, 2004) was used to identify the best-fitting models of DNA substitution for our data. Bayesian inference (BI) method was used to reconstruct phylogenetic relationships and carried out using MrBayes 3.12 (Ronquist & Huelsenbeck, 2003). Four independent Markov Chain Monte Carlo searches were run for 20 million generations, sampled every 1000 generations, each with four chains and default priors. A 50% majority-rule consensus tree was constructed to calculate the Bayesian posterior probabilities (BPP) of the tree nodes.

Bioacoustics analysis

The advertisement calls were recorded with an ICD recorder (Sony ICD-TX50) at a distance of approximately 0.2–0.3 m. Ambient temperature was taken with a TP-2200 (A-volt). Calls were analyzed with Raven Pro 1.5 software (<http://www.birds.cornell.edu/brp/raven/RavenOverview.html>) with default setting.

RESULTS

Systematics

Classis AMPHIBIA Linnaeus, 1758

Ordo ANURA Hogg, 1839

Familia RHACOPHORIDAE Hoffman, 1932

Genus *Theloderma* Tschudi, 1838

Theloderma lateriticum Bain, Nguyen et Doan, 2009

EXAMINED MATERIAL. NHMG1704001, NHMG1706010–11, adult males, and NHMG1706012, adult female from Shiwandashan National Nature Reserve, Guangxi, China (21.844123° N, 107.891561° E, 510 m asl), collected by Weicai Chen, Yunming Mo, and Shichu Zhou on 21 April and 24 June, 2017.

DESCRIPTION. Habitus slender. Head wider than long ($HL/HW = 0.83$). Snout slightly subacuminate in dorsal view, rounded in lateral view, and projecting beyond lower jaw; nostril oval, oblique, much closer to tip of snout than to eye, internarial shorter than interorbital distance ($IN/IOD = 0.83$); canthus rostralis distinct, rounded; lores oblique, concave; interorbital region slightly concave, interorbital distance longer than upper eyelid width ($IOD/UEW = 1.43$); pupil diamond-shaped, horizontal; eye diameter shorter than snout length ($ED/SNT = 0.86$); tympanum distinct, rounded, 58% of eye diameter, tympanic rim elevated relative to skin of temporal region; dorsolateral folds absent; pineal ocellus absent; vomerine teeth absent; choanae oval, at margins of roof of mouth; tongue elongated-cordiform, attached anteriorly, deeply notched posteriorly; supratympanic fold from posterior margin of eye to level slightly posterior to axilla; vocal sac absent (Figs. 1–4, Table 2).

Forelimb slender. Finger tips with well-expanded discs having distinctly circummarginal grooves, finger III disc width 62% tympanum diameter; relative finger lengths $I < II < IV < III$; fingers without webbing; subarticular tubercles distinct, surfaces rounded, formula 1, 1, 2, 2; accessory palmar tubercles indistinct; nuptial pad present, elongated, covering prepollex area (Figs. 5, 6).

Hindlimb slender. Toe tips with distinctly expanded discs with circummarginal grooves, diameter of discs slightly shorter than those of fingers; toes slender; relative toe lengths $I < II < III \leq V < IV$; toes moderately webbed, webbing formula: $I 1 - 1 - II 1 + - 1 - III 1 + - 1 + IV 1 + - 2 - V$; subarticular tubercles rounded, distinct, formula 1, 1, 2, 3, 2; inner metatarsal tubercle oval, elongated; outer metatarsal tubercle and supernumerary tubercle absent (Figs. 5, 6).

Smooth dorsal skin without distinct skin ridge, but dorsal surfaces of head, back, limbs and outer



Figures 1–4. Dorsolateral (Fig. 1), dorsal (Fig. 2) and ventral view (Fig. 3) of NHMG1704001 (adult male) 241 in life, and (Fig. 4) dorsolateral view of NHMG1706012 (female) in life.

margin of foot are interspersed with some asperities; coarsely granular venter; absent dermal fringe and velvety and ovoid nuptial pad on prepollex area.

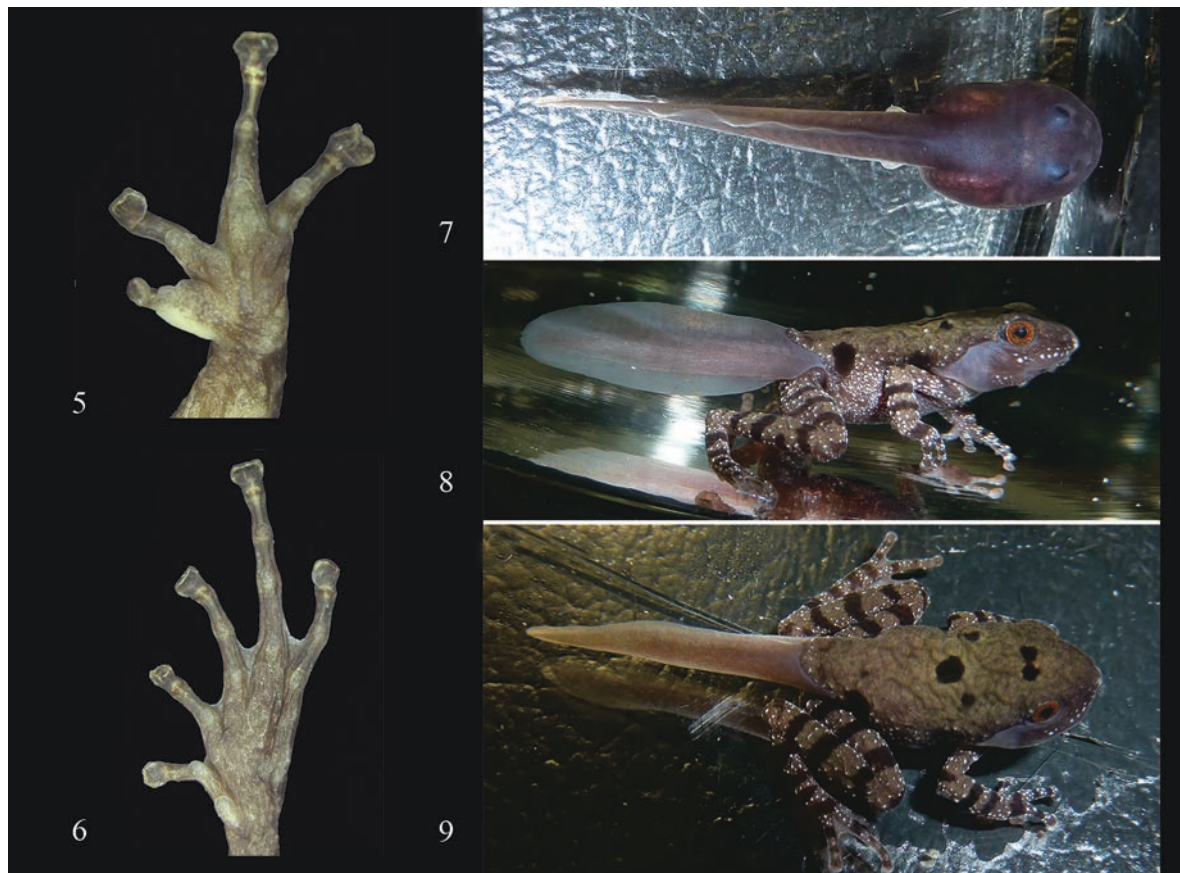
Color in life. Tip of snout, loreal region, upper eyelids, supratympanic fold and shoulder are brick-red; dorsum has several irregular brown markings; brown upper lip contains several white spots; flanks are brown with black blotches, and the lower portion of flanks exhibits white spots; grey-brown forelimbs with two dark brown transverse bands; brown hindlimbs with three dark brown transverse bands; grey digit tips with white spots; grey-brown venter with white spots; dark brown pupils and brick-red iris with deeply red ring along the margin (Figs. 1–4).

Color in preservative. Brown body with dark brown markings. Brown ventral surface with grey spots. Brick-red faded on the tip of snout, loreal region, upper eyelids, supratympanic fold and shoulder.

Tadpole. Tadpoles were assigned to the new

species because the color pattern resembled that of adults (Figs. 7–9). Tadpoles exhibit a rounded and depressed body shape; dorsal eyes and nares; nares are nearer to the snout than eyes; medial vent tube; sinistral spiracle; broadly rounded tail tip. The oral apparatus is anteroventral. The labial tooth row formula (LTRF) is $3(2-3)/3$ ($n = 4$, stage 32–38). The marginal papillae have a large dorsal gap and lack a medial gap on the lower labium. The body is dark brown, and the tail fin is pale brown. Measurements (in mm) of four tadpoles at developmental stages 32–38 (Gosner, 1960) are as follows: total length, 26.0–28.7 mm; body length, 10.1–10.7 mm; maximum tail height, 5.6–6.8 mm; tail muscle height, 3.1–3.8 mm; interorbital distance, 2.8–3.2 mm; internarial distance, 1.6–2.1 mm; oral disk width, 2.2–2.5 mm and oral disk height, 0.8–0.9 mm.

MOLECULAR ANALYSES. Our preliminary phylogenetic trees were similar to Poyarkov et al. (2018),



Figures 5, 6. Ventral view of the hand (Fig. 5), and of the foot (Fig. 6) of NHMG1704001. Figures 7–9. Tadpole of *Theloderma lateriticum*. Dorsal view of tadpole (Fig. 7), lateral view (Fig. 8), and dorsal view of metamorphs (Fig. 9).

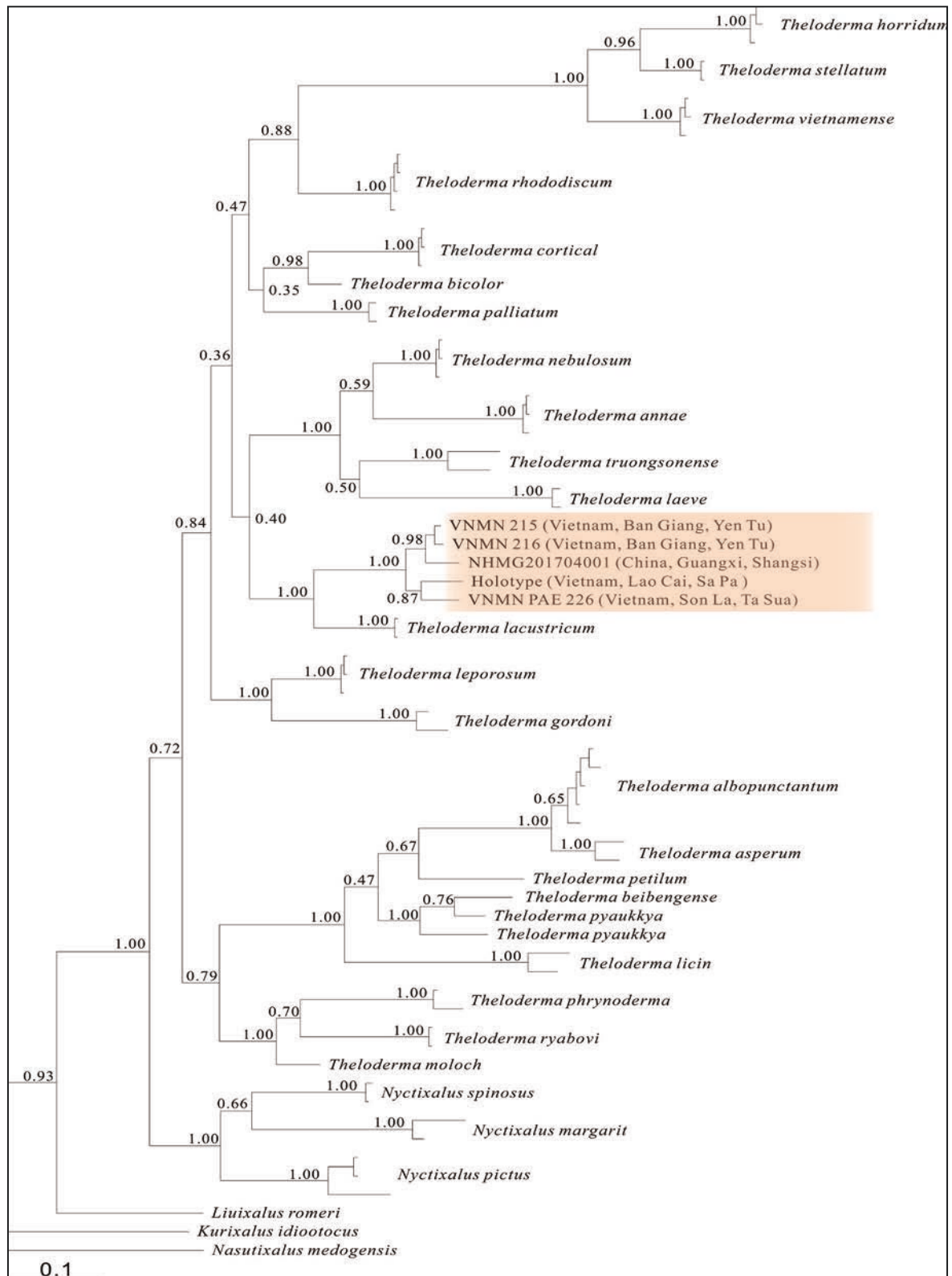


Figure 10. Bayesian inference tree reconstructed from 16S rRNA mitochondrial gene with *Kurixalus idiotocus*, *Liuixalus romeri*, and *Nasutixalus medogensis* as outgroups. Numbers above branches represent bootstrap supports for Bayesian posterior probabilities (BPP).

ID	Species	Voucher no.	Locality	GenBank no.	Reference
1	<i>Nyctixalus pictus</i>	FMNH 231095	Malaysia,Sabah, Lahad Datu	DQ283133	Frost et al., 2006
2	<i>N. pictus</i>	FMNH 231094	Malaysia	AF458135	Wilkinson et al., 2002
3	<i>N. pictus</i>	AH07001	Malaysia,Sarawak, Gunung Mulu	GU154888	Das and Haas, 2010
4	<i>N. spinosus</i>	ACD 1043	Philippine Islands, Mindanao	DQ283114	Frost et al., 2006
5	<i>N. spinosus</i>	pet trade	Philippine Islands, Mindanao	KT461916	Poyarkov et al., 2015
6	<i>N. margaritifer</i>	TNHCJAM 3030	Indonesia, Java	EU178087	Biju et al., 2008
7	<i>N. margaritifer</i>	KUHE 26135	Indonesia, Java	LC012864	Dever, 2017
8	<i>T. albopunctatum</i>	KIZ 060821217	China, Guangxi, Jinxiu	EF564522	Yu et al., 2008
9	<i>T. albopunctatum</i>	HN0806100	China, Hainan	GQ285678	Dever, 2017
10	<i>T. albopunctatum</i>	VNMN J2916	Vietnam, Vinh Phuc	KJ802913	Dever, 2017
11	<i>T. albopunctatum</i>	NHMG20160632	China, Guangxi, Shangsi	MH521263	This study
12	<i>T. annae</i>	IEBR3732	Vietnam, Hoa Binh	LC168170	Nguyen et al., 2016
13	<i>T. annae</i>	IEBR3733	Vietnam, Hoa Binh	LC168171	Nguyen et al., 2016
14	<i>T. annae</i>	IEBR3734	Vietnam, Hoa Binh	LC168172	Nguyen et al., 2016
15	<i>T. asperum</i>	VNMN J 2888	Vietnam, Tam Dao, Vin Phu	LC012853	Li et al., 2016
16	<i>T. asperum</i>	VNMN 4404	Vietnam, Ngoc Linh, Kon Tum	LC012854	Li et al., 2016
17	<i>T. asperum</i>	VNMN 4405	Vietnam, Kon Ka Kinh, Gia Lai	LC012855	Li et al., 2016
18	<i>T. baibungense</i>	YPX31940	China, Medog, Tibet	KU981089	Li et al., 2016
19	<i>T. bicolor</i>	IEBR A. 2011.4	Vietnam, Lao Cai, Sa Pa	JX046474	Gawor et al., 2012
20	<i>T. corticale</i>	AMNH A 161499	Vietnam, Dao, Vin Phu	DQ283050	Li et al., 2016
21	<i>T. corticale</i>	VNMN 3556	Vietnam, Tam Dao,	LC012841	Li et al., 2016
22	<i>T. corticale</i>	NHMG20161003	China, Guangxi, Shangsi	MG322125	Chen et al., 2018
23	<i>T. gordonii</i>	VNMN 03013	Vietnam,Nghe An	JN688167	Rowley et al., 2011
24	<i>T. gordonii</i>	VNMN PAE217	Vietnam,Son La	KJ802918	Nguyen et al., 2014
25	<i>T. horridum</i>	LJT W44	Malaysia	KC465843	Li et al., 2013
26	<i>T. horridum</i>	LJT W45	Malaysia	KC465842	Li et al., 2013
27	<i>T. horridum</i>	ZMMU NAP-04015	Thailand,Satun	KT461890	Poyarkov et al., 2015
28	<i>T. lacustrinum</i>	NCSM84682	Laos,Vientiane Province,Feuang District	KX095245	Sivongxay et al., 2016
29	<i>T. lacustrinum</i>	NCSM84683	Laos,Vientiane Province,Feuang District	KX095246	Sivongxay et al., 2016
30	<i>T. laeve</i>	ZMMU NAP-01645	Vietnam Lam Dong, Cat Loc	KT461913	Poyarkov et al., 2015

Table 1/1. Samples and sequences used in this study. Generic allocation according to Frost (2017).

ID	Species	Voucher no.	Locality	GenBank no.	Reference
31	<i>T. laeve</i>	ZMMU NAP-02906 AMNH	Vietnam Binh Phuoc, Bu Gia Map	KT461883	Poyarkov et al., 2015
32	<i>T. lateriticum</i>	168757/IEBR A. 0860	Vietnam, Lao Cai, SaPa	LC012848	Nguyen et al., 2015
33	<i>T. lateriticum</i>	VNMN PAE 226	Vietnam, Son La, Ta Sua	LC012849	Nguyen et al., 2015
34	<i>T. lateriticum</i>	VNMN 215	Vietnam, Ban Giang, Yen Tu	LC012850	Nguyen et al., 2015
35	<i>T. lateriticum</i>	VNMN 216	Vietnam, Ban Giang, Yen Tu	LC012851	Nguyen et al., 2015
36	<i>T. lateriticum</i>	NHMG201704001	China, Guangxi, Shangsi	MH521262	This study
37	<i>T. leporosum</i>	LJT W46	Malaysia	KC465841	Li et al., 2013
38	<i>T. leporosum</i>	leporosum-1	Malaysia, Selangor	KT461922	Poyarkov et al., 2015
39	<i>T. leporosum</i>	KUHE 52581	Malaysia, Negeri Sembilan	AB847128	Nguyen et al., 2014
40	<i>T. licin</i>	KUHE 19426	Tailand, Nakon Sri Tamarat	LC012859	Li et al., 2016
41	<i>T. licin</i>	KUHE 52599	Malaysia, Selangor	KJ802920	Nguyen et al., 2014
42	<i>T. moloch</i>	SDBDU 2011.345	Arunachal Pradesh, India	KU169993	Biju et al., 2016
43	<i>T. nebulosum</i>	ROM 39588	Vietnam, Kon Tum, Ngoc Linh	KT461887	Poyarkov et al., 2015
44	<i>T. nebulosum</i>	AMS R 173409	Vietnam, Kon Tum, Ngoc Linh	JN688168	Rowley et al., 2011
45	<i>T. nebulosum</i>	AMS R 173877	Vietnam, Kon Tum, Ngoc Linh	JN688169	Rowley et al., 2011
46	<i>T. palliatum</i>	AMS R 173130	Vietnam Lam Dong, Bi Doup-Nui Ba	JN688172	Rowley et al., 2011
47	<i>T. palliatum</i>	ZMMU NAP-01846	Vietnam Lam Dong, Bi Doup-Nui Ba	KT461901	Poyarkov et al., 2015
48	<i>T. petilum</i>	HNUE MNA.2012.0001	Vietnam Dien Bien, Muong Nhe	KJ802925	Nguyen et al., 2014
49	<i>T. phrynoderma</i>	CAS 243920	Myanmar, Tanintharyi	KJ128282	Dever, 2017
50	<i>T. phrynoderma</i>	CAS 247910	Myanmar, Tanintharyi	KJ128283	Dever, 2017
51	<i>T. pyaukkya</i>	CAS 236133	Myanmar, Kachin	KU244360	Dever, 2017
52	<i>T. pyaukkya</i>	CAS 234869	Myanmar, Chin	KU244370	Dever, 2017
53	<i>T. rhododiscum</i>	KIZ060821063	China, Guangxi, Jinxiu	EF564533	Yu et al., 2008
54	<i>T. rhododiscum</i>	KIZ060821170	China, Guangxi, Jinxiu	EF564534	Yu et al., 2008
55	<i>T. rhododiscum</i>	SCUM 061102L	China, Guangxi, Jinxiu	EU215530	Li et al., 2008
56	<i>T. rhododiscum</i>	CIB GX200807048	China, Guangxi, Jinxiu	KJ802921	Nguyen et al., 2014
57	<i>T. ryabovi</i>	ryabovi-1	Vietnam Kon Tum, Kon Plong, Mang Canh	KT461914	Poyarkov et al., 2015

Table 1/2. Samples and sequences used in this study. Generic allocation according to Frost (2017).

ID	Species	Voucher no.	Locality	GenBank no.	Reference
58	<i>T. ryabovi</i>	ryabovi-2	Vietnam Kon Tum, Kon Plong, Mang Canh	KT461915	Poyarkov et al., 2015
59	<i>T. stellatum</i>	stellatum-1	Thailand Chanthaburi, Phliu	KT461918	Poyarkov et al., 2015
60	<i>T. stellatum</i>	ZMMU NAP-03961	Thailand Nakhon Nayok, Nang Rong	KT461917	Poyarkov et al., 2015
61	<i>T. truongsongense</i>	ROM 39363	Vietnam Khanh Hoa, Hon Ba	KT461925	Poyarkov et al., 2015
62	<i>T. truongsongense</i>	AMS R 171510	Vietnam Quang Nam	JN688174	Rowley et al., 2011
63	<i>T. vietnamense</i>	ZMMU NAP-00707	Vietnam Dong Nai, Nam Cat Tien	KT461889	Poyarkov et al., 2015
64	<i>T. vietnamense</i>	ZMMU NAP-03680	Vietnam Tay Ninh, Lo Go-Xa Mat	KT461921	Poyarkov et al., 2015
65	<i>T. vietnamense</i>	ZMMU NAP-03723	Vietnam Kien Giang, Phu Quoc	KT461919	Poyarkov et al., 2015
66	<i>Nasutixalus medogensis</i>	6255Rao	China, Motuo, Xizang	GQ285679	Jiang et al., 2016
67	<i>Liuixalus romeri</i>	CIB20080048	China, Hong Kong	AB871412	Nguyen et al., 2014
68	<i>Kurixalus idiotocus</i>	SCUM 061107L	China, Taiwan, Lianhuachi	EU215547	Li et al., 2008

Table 1/3. Samples and sequences used in this study. Generic allocation according to Frost (2017).

Character	Male NHMG1704001	Male NHMG1706010	Male NHMG1706011	Female NHMG1706012
SVL	24.6	23.8	23.3	24.8
HL	7.7	7.2	6.6	8.2
HW	9.3	8.3	8.2	9.3
SNT	4.2	3.9	3.8	4.1
ED	3.6	3.0	3.1	3.6
IOD	3.0	2.9	3.1	3.5
TD	2.1	1.9	2.0	1.8
UEW	2.1	1.9	2.2	2.4
TED	0.3	0.6	0.6	0.6
IN	2.5	2.2	2.2	2.7
EN	2.0	2.2	2.1	2.7
TIB	12.7	12.8	12.0	13.4
FLL	11.8	11.5	10.9	13.2
THL	13.3	13.1	12.9	13.8
PL	10.3	10.0	9.3	10.8
ML	7.3	6.8	6.2	7.0
FTD ₃	1.3	1.5	1.3	1.2
HTD ₄	1.2	1.3	1.2	1.1

Table 2. Measurements (mm) of *Theloderma lateriticum*. Abbreviations defined in text.

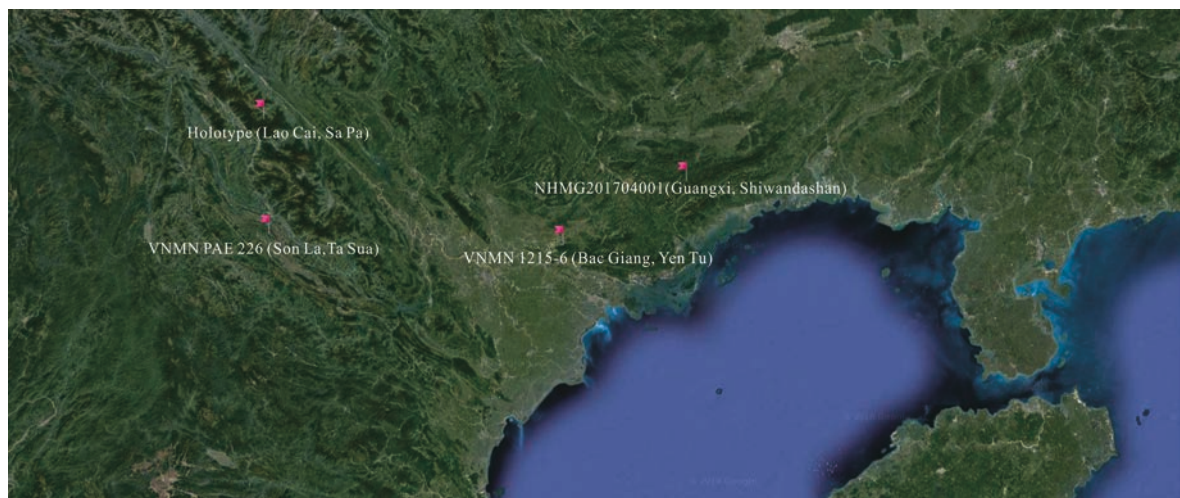
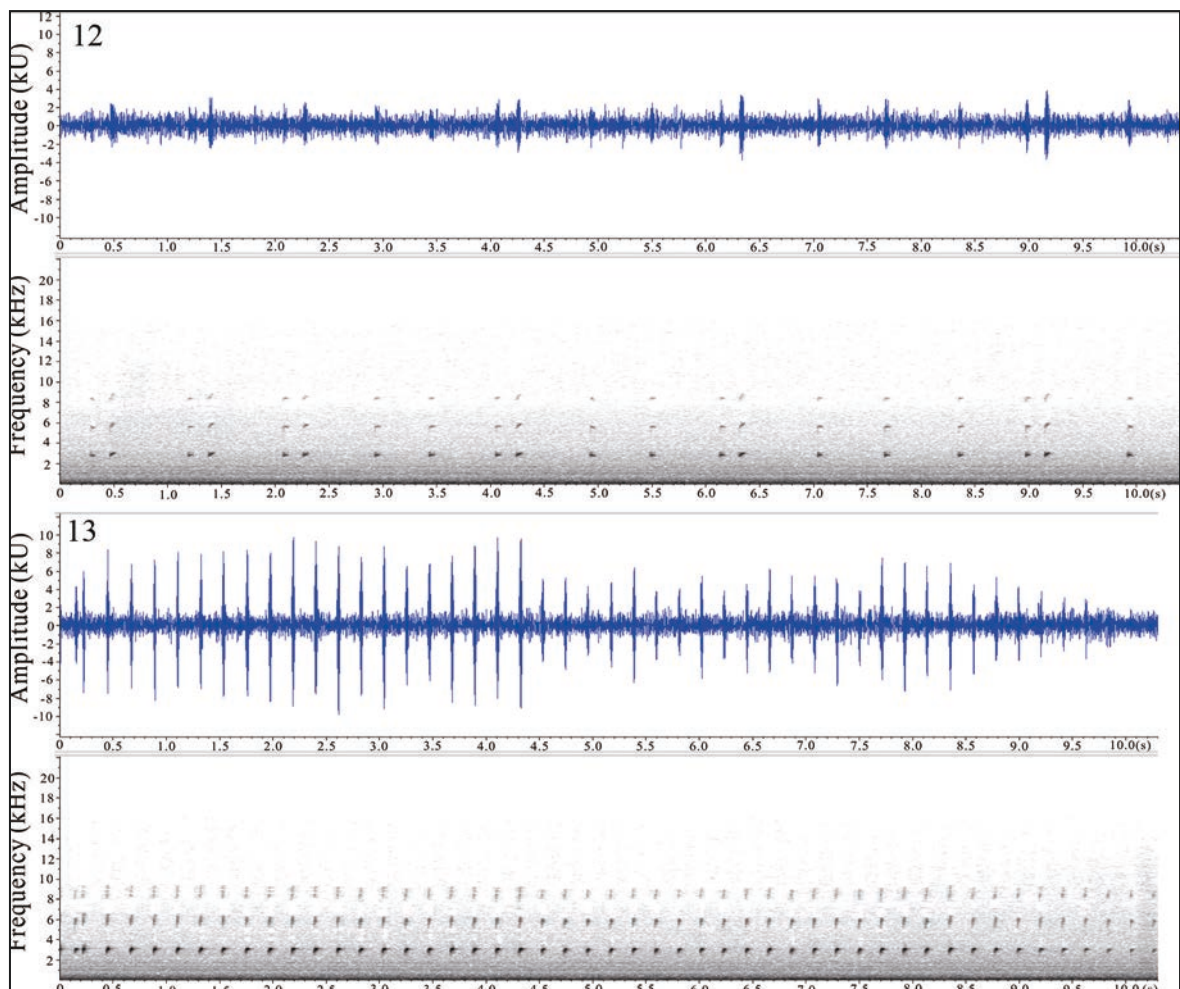


Figure 11. Distribution of *Theloderma lateriticum*.



Figures 12, 13. Two types of advertisement calls of *Theloderma lateriticum* recorded at ambient temperature of 21°C. Figure 12 and figure 13 showed variable durations between calls.

	1	2	3	4
Holotype (Lao Cai, Sa Pa)				
VNMN PAE 226 (Son La, Ta Sua)	4.1			
VNMN 1215 (Bac Giang, Yen Tu)	4.3	3.9		
VNMN 1216 (Bac Giang, Yen Tu)	4.1	3.7	0.6	
NHMG201704001 (Guangxi, Shiwandashan)	4.4	4.3	2.4	2.2

Table 3. Uncorrected p-distances (in %) in *Theloderma lateriticum* group based on mitochondrial 16S rRNA sequences (~530 bp).

Nguyen et al. (2015) and Huang et al. (2017) (Fig. 10). All *T. lateriticum* specimens form a monophyletic group. Holotype is closer to Ta Sua specimen than Yen Tu and Shiwandashan specimens, matching their geographical distance (Fig. 11). Uncorrected sequence divergences at the 16S rRNA gene between type locality and other three sites range from 4.1–4.4% (Table 3), but Shiwandashan specimens and Yen Tu specimens range from 2.2–2.4% (Table 3). Genetic variation between *T. lateriticum* and all available homologous 16S rRNA sequences is great than 8.5%.

Advertisement call. We only recorded the calls of a single male (Voucher no. NHMG1704001) near PVC buckets at an ambient temperature of 21°C. We detected two typical calls, but these calls have the same dominant frequency and harmonics (Fig. 12, 13). The dominant frequency ranges from 2.5–3.5 kHz, and harmonics present at 5.8–6.8 kHz and 8.0–9.0 kHz. Figures 13, 13 showed waveforms and corresponding spectrograms for 10 s. For figure 12, the durations between calls are variable, ranging from 200–700 ms; for figure 13, the durations are relatively stable, presenting around 220 ms.

DISCUSSION

Morphologically, Shiwandashan, Yen Tu and Ta Sua specimens are similar to holotype. Nguyen et al. (2015) were convinced that Yen Tu and Ta Sua specimens had a vocal slit, but they lacked a vocal slit in holotype as well as our specimens. For color model, our specimens are more similar to Ta Sua specimens than Yen Tu specimens and holotype (Hecht et al., 2013; Pham & Nguyen, 2018). Toe

webs also display subtle differences, webbing formula: I 1- – 1- II 1+ – 1- III 1+ – 1+ IV 1+ – 2- V in our specimens vs. I1½ – 2II1½ – 2III1 – 2½IV2½ – 2V in Ta Sua specimens. For the skin texture, holotype and Yen Tu specimens are granular, but Shiwandashan and Ta Sua specimens are obviously smooth (Hecht et al., 2013; Pham & Nguyen, 2018). Holotype was collected on 10 September, but other specimens were collected in April or June. In Shiwandashan, we found that the breeding season of *T. lateriticum* ranges from April to June. Whether some morphological differences are caused by the breeding season and non-breeding season need further investigation.

Genetic variations between holotype and other specimens range from 4.1% to 4.4% based on the part of 16S rRNA (~530 bp); genetic variations greater than 3% represents differentiation at the species level in frogs (Vences et al., 2005). However, genetic variation between our specimens and Yen Tu specimens is about 2.2%. Other examples of high intraspecific genetic variation included *T. albopunctatum* (2.5%), *T. gordonii* (2.1–4.6%), *T. licin* (3.8%), *T. pyaukkya* (3.9%), *T. truongsongense* (3.8%) and *T. stellatum* (0.4–3.0%) (Nguyen et al., 2015; Pham & Nguyen, 2018). High genetic variation indicated the possible presence of cryptic species in these group. For *T. lateriticum* group, because *T. lateriticum* was described based on a single specimen and lacked variation data, we consider these specimens as a single species despite presence of subtle morphological variations and relative high genetic variation. If we want to determine the *T. lateriticum* species complex, it is necessary to collect more specimens from type locality.

ACKNOWLEDGEMENTS

This work was supported by the Natural Science Foundation of Guangxi, China (Grant No: 2016GXNSFAA380007) and the Opening Foundation of Key Laboratory of Environment Change and Resources Use in Beibu, Gulf Ministry of Education (Nanning Normal University) and Guangxi Key Laboratory of Earth Surface Processes and Intelligent Simulation (Nanning Normal University) (Grant No: GTEU-KLOP-X1812).

REFERENCES

- Altig R. & McDiarmid R.W., 1999. Body plan: development and morphology. In: McDiarmid, R.W. & Altig R. (Eds.), *Tadpoles: the biology of anuran larvae*. The University of Chicago Press, Chicago, pp. 24–51.
- Altschul S.F., Madden T.L., Schäffer A.A., Zhang J., Zhang Z., Miller W. & Lipman D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research*, 25: 3389–3402. <https://doi.org/10.1093/nar/25.17.3389>
- Bain R.H., Nguyen T.Q. & Doan K.V., 2009. A new species of the genus *Theloderma* Tschudi, 1838 (Anura: Rhacophoridae) from northwestern Vietnam. *Zootaxa*, 2191: 58–68.
- Biju S.D., Roelants K. & Bossuyt F., 2008. Phylogenetic position of the montane treefrog *Polypedates variabilis* Jerdon, 1853 (Anura: Rhacophoridae), and description of a related species. *Organisms Diversity and Evolution*, 8: 267–276. <https://doi.org/10.1016/j.ode.2007.11.004>
- Biju S., Senevirathne D., Garg G., Mahony S., Kamei S., Thomas R.G., Shouche A., Raxworthy Y., Mee-gaskumbura C.J. & Van Bocxlaer M., 2016. *Frankixalus*, a new rhacophorid genus of tree hole breeding frogs with oophagous tadpoles. *PLoS ONE*, 11: e0145727. <https://doi.org/10.1371/journal.pone.0145727>
- Chen W., Liao X., Zhou S., Mo Y. & Huang Y., 2018. Rediscovery of *Rhacophorus yaoshanensis* and *Theloderma kwangsiensis* at their type localities after five decades. *Zootaxa*, 4379: 484–496. <http://dx.doi.org/10.11646/zootaxa.4379.4.2>
- Das I. & Haas A., 2010. New species of *Microhyla* from Sarawak: Old World's smallest frogs crawl out of a miniature pitcher plants on Borneo (Amphibia: Anura: Microhylidae). *Zootaxa*, 1571: 37–52. <http://dx.doi.org/10.11646/zootaxa.2571.1.2>
- Dever J.A., 2017. A new cryptic species of the *Theloderma asperum* complex (Anura: Rhacophoridae) from Myanmar. *Journal of Herpetology*, 51: 425–436. <https://doi.org/10.1670/17-026>
- Frost D.R., Grant T., Faivovich J.N., Bain R.H., Haas A., Haddad C.F.B., de Sá R.O., Channing A., Wilkinson M., Donnellan S.C., Raxworthy C.J., Campbell J.A., Blotto B.L., Moler P., Drewes R.C., Nussbaum R.A., Lynch J.D., Green D.M. & Wheeler W.C., 2006. The amphibian tree of life. *Bulletin of the American Museum of Natural History*, 297: 1–370.
- Gawor A., Chapuis S., Pham T.C., Nguyen T.Q., Schmitz A. & Ziegler T., 2012. Larval morphology of two species of the genus *Theloderma* (Tschudi, 1838) from Vietnam (Anura: Rhacophoridae: Rhacophorinae). *Zootaxa*, 3395: 59–64.
- Gosner K.L., 1960. A simple table for staging anuran embryos and larvae with notes on identification. *Herpetologica*, 16: 183–190. <https://www.jstor.org/stable/3890061>
- Hecht V.L., Pham C.T., Nguyen T.T., Nguyen T.Q., Bonkowski M. & Ziegler T., 2013. First report on the herpetofauna of Tay Yen Tu Nature Reserve, northeastern Vietnam. *Biodiversity Journal*, 4: 507–552.
- Huang H., Chen Z., Wei Z., Bu R. & Wu Z., 2017. DNA barcoding revises a misidentification on mossy frog: new record and distribution extension of *Theloderma corticale* Boulenger, 1903 (Amphibia: Anura: Rhacophoridae). *Mitochondrial DNA Part A*: 1–8. <https://doi.org/10.1080/24701394.2016.1275601>
- Jiang K., Yan F., Wang K., Zou D., Li C. & Che J., 2016. A new genus and species of treefrog from Medog, southeastern Tibet, China (Anura, Rhacophoridae). *Zoological Research*, 37: 15–20. <https://doi.org/10.13918/j.issn.2095-8137.2016.1.15>
- Yu G., Rao D., Yang J. & Zhang M., 2008. Phylogenetic relationships among Rhacophorinae (Rhacophoridae, Anura, Amphibia): with an emphasis on the Chinese species. *Zoological Journal of the Linnean Society*, 153: 733–749. <https://doi.org/10.1111/j.1096-3642.2008.00404.x>
- Kumar S., Stecher G. & Tamura K., 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 33: 1870–1874. <https://doi.org/10.1093/molbev/msw054>
- Li J.T., Che J., Bain R.H., Zhao E.M. & Zhang Y.P., 2008. Molecular phylogeny of Rhacophoridae (Anura): A framework of taxonomic reassignment of species within the genera *Aquixalus*, *Chiromantis*, *Rhacophorus*, and *Philautus*. *Molecular Phylogenetics and Evolution*, 48: 302–312. <https://doi.org/10.1016/j.ympev.2008.03.023>
- Li J.T., Li Y., Klaus S., Rao D.Q., Hillis D.M. & Zhang Y.P., 2013. Diversification of rhacophorid frogs provides evidence for accelerated faunal exchange between India and Eurasia during the Oligocene. *Proceedings of the National Academy of Sciences of the United States of America*, 110: 3441–3446. <https://doi.org/10.1073/pnas.1300881110>

- Li C., Hou M., Yu G.H., Yan F., Li B.Z., Jiang K., Li P.P. & Orlov N.L., 2016. Rediscovery of *Theloderma moloch* (Amphibian: Anura: Rhacophoridae) from south-east Tibet, China after 102 years. *Russian Journal of Herpetology*, 23: 41–54.
- Myers C.W. & Duellman W.E., 1982. A new species of *Hyla* from Cerro Colorado and other tree frog records and geographical notes from western Panama. *American Museum Novitates*, 275: 32.
- Nguyen T.T., Le D.T., Nguyen S.H.L., Matsui M. & Nguyen T.Q., 2014. First record of *Philautus petilus* Stuart and Heatwole, 2004 (Amphibian: Anura: Rhacophoridae) from Vietnam and its phylogenetic position. *Current Herpetology*, 33: 112–120. <https://doi.org/10.5358/hj.33.112>
- Nguyen T.T., Matsui M. & Eto K., 2015. Mitochondrial phylogeny of an Asian tree frog genus *Theloderma* (Anura: Rhacophoridae). *Molecular Phylogenetics and Evolution*, 85: 59–67. <https://doi.org/10.1016/j.ympev.2015.02.003>
- Nguyen T.Q., Pham C.T., Nguyen T.T., Ngo H.N. & Ziegler T., 2016. A new species of *Theloderma* (Amphibia: Anura: Rhacophoridae) from Vietnam. *Zootaxa*, 4168: 171–186. <http://dx.doi.org/10.11646/zootaxa.4168.1.10>
- Nylander J.A.A., 2004. MrModeltestv2. Program Distributed by the Author. Evolutionary Biology Centre, Uppsala University.
- Palumbi S.R., Martin A., Romano S., McMillan W.O., Stice L. & Grabowski G., 1991. The simple fool's guide to PCR. Department of Zoology, University of Hawaii, Honolulu, 47 pp.
- Pham V.A. & Nguyen Q.T., 2018. Diversity of the genus *Theloderma* (Amphibia: Anura: Rhacophoridae) from Son La Province. *VNU Journal of Science: Natural Sciences and Technology*, 34: 48–54. http://repository.vnu.edu.vn/handle/VNU_123/61629
- Poyarkov N.A.Jr., Orlov N.L., Moiseeva A.V., Pawangkhanant P., Ruangsuan T., Vassilieva A.B. & Gogoleva S.S., 2015. Sorting out moss frogs: mtDNA data on taxonomic diversity and phylogenetic relationships of the Indochinese species of the genus *Theloderma* (Anura, Rhacophoridae). *Russian Journal of Herpetology*, 22: 241–280.
- Poyarkov N.A. Jr., Kropachev I.I., Gogoleva S.I. & Orlov N.L., 2018. A new species of the genus *Theloderma* Tschudi, 1838 (Amphibia: Anura: Rhacophoridae) from Tay Nguyen Plateau, central Vietnam. *Zoological Research*, 39: 156–180. <https://doi.org/10.24272/j.issn.2095-8137.2018.018>
- Ronquist F.R. & Huelsenbeck J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19: 1572–1574. <https://doi.org/10.1093/bioinformatics/btg180>
- Rowley J.J.L., Le D.T.T., Hoang H.D., Dau V.Q. & Cao T.T., 2011. Two new species of *Theloderma* (Anura: Rhacophoridae) from Vietnam. *Zootaxa*, 3098: 1–20.
- Sivongxay N., Davankham M., Phimmachak S., Phoumixay K. & Stuart B.L., 2016. A new small-sized *Theloderma* (Anura: Rhacophoridae) from Laos. *Zootaxa*, 4147: 433–442. <http://dx.doi.org/10.11646/zootaxa.4147.4.5>
- Vences M., Thomas M., Bonett R.M. & Vieites D.R., 2005. Deciphering amphibian diversity through DNA barcoding: chances and challenges. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*, 360: 1859–1868. <https://doi.org/10.1098/rstb.2005.1717>
- Wilkinson J.A., Drewes R.C. & Tatum O.L., 2002. A molecular phylogenetic analysis of the family Rhacophoridae with an emphasis on the Asian and African genera. *Molecular Phylogenetics and Evolution*, 24: 265–273. [https://doi.org/10.1016/S1055-7903\(02\)00212-9](https://doi.org/10.1016/S1055-7903(02)00212-9)