



A new species of *Theloderma* (Amphibia: Anura: Rhacophoridae) from Vietnam

TRUONG QUANG NGUYEN^{1,6}, CUONG THE PHAM¹, TAO THIEN NGUYEN^{2,5},
HAI NGOC NGO² & THOMAS ZIEGLER^{3,4}

¹*Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Hanoi, Vietnam. E-mails: nqt2@yahoo.com and cuong03091983@yahoo.com*

²*Vietnam National Museum of Nature, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Road, Hanoi, Vietnam. E-mails: nguyenthientao@gmail.com and ngohai2709@gmail.com*

³*AG Zoologischer Garten Köln, Riehler Strasse 173, D–50735 Cologne, Germany. E-mail: ziegler@koelnerzoo.de*

⁴*Institute of Zoology, University of Cologne, Zùlpicher Strasse 47b, D–50674 Cologne, Germany*

⁵*The Kyoto University Museum, Kyoto University, Yoshida Honmachi, Sakyoku, Kyoto, 606-8501, Japan*

⁶*Corresponding author. E-mail: nqt2@yahoo.com*

Abstract

We describe a new species of *Theloderma* from northwestern Vietnam based on morphological differences and molecular divergence. *Theloderma annae* sp. nov. is distinguishable from its congeners on the basis of a combination of the following characters: Size small, SVL 27.1–28.5 mm in males, 30.3–32.6 mm in females; head longer than wide; vomerine teeth absent; snout long (SL/SVL 0.16–0.19); spines on upper eyelid absent; tibiotarsal projection absent; dorsal skin smooth; dermal fringes on forearm and tarsus absent; dorsal surface grayish green; and throat and ventral surface of arms and thighs brown with white spots.

Key words: *Theloderma annae* sp. nov., karst forest, molecular phylogeny, taxonomy, Hoa Binh Province

Introduction

The genus *Theloderma* Tschudi, 1838 is considered as the most poorly known group of tree frogs due to their cryptic habits and rarity of representatives (Nguyen *et al.* 2014). This genus currently comprises 25 species, of which one-third have been described in the last ten years (Frost 2016). Vietnam contains the greatest number of species in the genus, with 13 species recorded from the country, six of which having been described since 2009, viz. *T. bambusicola* Orlov, Poyarkov, Vassilieva, Ananjeva, Nguyen, Nguyen & Geissler, *T. chuyangsinensis* Orlov, Poyarkov, Vassilieva, Ananjeva, Nguyen, Nguyen & Geissler, *T. lateriticum* Bain, Nguyen & Doan, *T. nebulosum* Rowley, Le, Hoang, Dau & Cao, *T. palliatum* Rowley, Le, Hoang, Dau & Cao, and *T. vietnamense* Poyarkov, Orlov, Moiseeva, Pawangkhanant, Ruangsuwan, Vassilieva, Galoyan, Nguyen, and Gogoleva (Bain *et al.* 2009; Nguyen *et al.* 2009; Orlov *et al.* 2006, 2012; Rowley *et al.* 2011; Poyarkov *et al.* 2015). However, Poyarkov *et al.* (2015) subsequently synonymized *T. bambusicola* with *T. laeve* and *T. chuyangsinensis* with *T. palliatum*. In addition, Nguyen *et al.* (2014) recently recorded *T. petilum* (Stuart & Heatwole) for the first time from Vietnam.

During recent field work in northwestern Vietnam, specimens of a small tree frog species were collected in the karst forest of Ngoc Son – Ngo Luong Nature Reserve in Hoa Binh Province. Molecular comparisons revealed this taxon being nested within the genus *Theloderma*. Closer morphological examination showed that the specimens from Hoa Binh are clearly distinguished from other known members of *Theloderma* by a combination of morphological features. The phylogenetic analyses revealed this taxon to be clustered within the *Theloderma truongsongense* group with a strong support value but being distinctly separated from congeners. Due to morphological and molecular differences of the newly-collected specimens to all known species in the genus, we herein describe the newly discovered *Theloderma* population from Hoa Binh as a new species.

Materials and methods

Sampling. Field surveys were conducted in April 2014 by C.T. Pham, H.T. An, H.N. Ngo (hereafter C.T. Pham *et al.*), in October 2014 and in April 2015 by T.Q. Nguyen, C.T. Pham, M.D. Le, H.N. Ngo (hereafter T.Q. Nguyen *et al.*) in Ngoc Son – Ngo Luong NR, Lac Son District, Hoa Binh Province, northwestern Vietnam. Specimens were collected between 19:00 and 23:30 h. After taking photographs, specimens were anaesthetized with ethyl acetate, fixed in 80% ethanol for 4–6 hours, and then later transferred to 70% ethanol for permanent storage. Tissue samples were preserved separately in 95% ethanol. Preserved specimens were deposited in the collection of the Institute of Ecology and Biological Resources (IEBR), Hanoi; Vietnam National Museum of Nature (VNMN), Hanoi, Vietnam; and the Zoologisches Forschungsmuseum Alexander Koenig (ZFMK), Bonn, Germany. Other specimens referred to in this paper were deposited at the American Museum of Natural History (AMNH), Australian Museum (AMS), Chengdu Institute of Biology (CIB), Hanoi National University of Education (HNUE), Graduate School of Human and Environmental Studies, Kyoto University (KUHE), Field ID of Zoological Museum, Moscow University (NAP).

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) and *Liuixalus romeri* (Smith) were selected as outgroups according to Nguyen *et al.* (2015) and Li *et al.* (2016) (Table 1).

Chromas Pro software (Technelysium Pty Ltd., Tewantin, Australia) was used to edit the sequences, which were aligned using MAFFT version 7 (Katoh & 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 by Kakusan 4 (Tanabe 2011) based on the Akaike information criterion (AIC). We compared both partitioned and non-partitioned models on our data set, and the latter was selected based upon AIC. The best model selected for ML was the general time reversible model (GTR: Tavaré 1986) with a gamma shape parameter (G: 0.319 in ML and 0.339 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 30,001 trees (burn-in = 3,000,000). We checked parameter estimates and convergence using Tracer version 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*.

Morphological characters. Measurements were taken with a digital caliper to the nearest 0.1 mm. Abbreviations are as follows: SVL: Snout-vent length, HL: Head length (from the back of mandible to the tip of snout), HW: Maximum head width (across angle of jaws), SNL: Snout length (from anterior corner of eye to the tip of snout), NS: Distance from nostril to the tip of snout, EN: Distance from anterior corner of the eye to the nostril, IN: Internarial distance, IOD: Interorbital distance, ED: Eye diameter, UEW: Maximum width of upper eyelid, DAE: Distance between anterior corner of eyes, DPE: Distance between posterior corners of eyes, MAE: Distance between angle of jaws and anterior corner of the eye, MPE: Distance between angle of jaws and posterior corner of the eye, MN: Distance from the back of mandible to the nostril, TYD: Tympanum diameter, TYE: Distance from anterior margin of tympanum to posterior corner of the eye, FLL: Forelimb length (from axilla to elbow), HAL: Hand length (from elbow to the tip of third finger), fd1–4: Width of discs of fingers I–IV, fw1–4: Width of fingers I–IV, TFL: Third finger length, OPT: Outer palmar tubercle length, FeL: Femur length (from vent to knee), TbL: Tibia length (from knee to tarsus), TbW: Maximum tibia width, FoL: Foot length (from tarsus to the tip of fourth toe), FTL: Fourth toe length, IMT: Inner metatarsal tubercle length, OMT: Outer metatarsal tubercle length, td1–4: Width of discs of toes I–IV, tw1–4: Width of toes I–IV. Terminology for describing eye coloration in life and webbing formula followed those of Glaw & Vences (1997, 2007). Sex was determined by the presence of nuptial pads and gonadal inspection.

TABLE 1. Samples used in molecular analyses.

No	Species	Voucher	Locality	Genbank number	References
1.	<i>Theلودerma sp. nov.</i>	IEBR 3732	Vietnam: Hoa Binh	LC168170	This study
2.	<i>Theلودerma sp. nov.</i>	IEBR 3733	Vietnam: Hoa Binh	LC168171	This study
3.	<i>Theلودerma sp. nov.</i>	IEBR 3734	Vietnam: Hoa Binh	LC168172	This study
4.	<i>T. asperum</i>	Pet trade	Malaysia: Perak	KT461929	Poyarkov <i>et al.</i> (2015)
5.	<i>T. albopunctatum</i>	VNMN J291	Vietnam: Vinh Phuc: Tam Dao	KJ802914	Nguyen <i>et al.</i> (2015)
6.	<i>T. albopunctatum</i>	VNMN 3540	Vietnam: Lao Cai: Sa Pa	KJ802913	Nguyen <i>et al.</i> (2014)
7.	<i>T. albopunctatum</i>	VNMN 4404	Vietnam: Kon Tum: Ngoc Linh	LC012854	Nguyen <i>et al.</i> (2015)
8.	<i>T. albopunctatum</i>	VNMN 4406	Vietnam: Thanh Hoa: Xuan Lien	LC012856	Nguyen <i>et al.</i> (2015)
9.	<i>T. albopunctatum</i>	VNMN PAE 262	Vietnam: Son La: Ta Xua	LC012857	Nguyen <i>et al.</i> (2015)
10.	<i>T. bicolor</i>	VNMN 3536	Vietnam: Lao Cai: Sa Pa	KJ802915	Nguyen <i>et al.</i> (2014)
11.	<i>T. corticale</i>	VNMN J2892	Vietnam: Vinh Phuc: Tam Dao	KJ802916	Nguyen <i>et al.</i> (2014)
12.	<i>T. corticale</i>	VNMN J2932	Vietnam: Tuyen Quang: Na Hang	KJ802917	Nguyen <i>et al.</i> (2014)
13.	<i>T. gordonii</i>	KUHE 32447	Laos: Houaphan	KJ802919	Nguyen <i>et al.</i> (2014)
14.	<i>T. gordonii</i>	VNMN PAE217	Vietnam: Son La: Ta Sua	KJ802918	Nguyen <i>et al.</i> (2014)
15.	<i>T. gordonii</i>	VNMN 4407	Vietnam: Kon Tum: Ngoc Linh	LC012852	Nguyen <i>et al.</i> (2015)
16.	<i>T. laeve</i>	VNMN 4403	Vietnam: Gia Lai: Mang Yang	LC012846	Nguyen <i>et al.</i> (2015)
17.	<i>T. lateriticum</i>	VNMN 1215	Vietnam: Bac Giang: Tay Yen Tu	LC012850	Nguyen <i>et al.</i> (2015)
18.	<i>T. lateriticum</i>	VNMN PAE 226	Vietnam: Son La: Ta Sua	LC012849	Nguyen <i>et al.</i> (2015)
19.	<i>T. lateriticum</i>	AMNH 168757 /IEBR A.0860	Vietnam: Lao Cai: Sa Pa	LC012848	Nguyen <i>et al.</i> (2015)
20.	<i>T. leporosum</i>	KUHE 52581	Malaysia: Negeri Sembilan: Kenaboi	AB847128	Matsui <i>et al.</i> (2014)
21.	<i>T. licin</i>	KUHE 19426	Thailand: Nakhon Sri Tammarat	LC012859	Nguyen <i>et al.</i> (2015)
22.	<i>T. licin</i>	KUHE 52599	Malaysia: Selangor	KJ802920	Nguyen <i>et al.</i> (2014)
23.	<i>T. nebulosum</i>	AMS R 173409	Vietnam: Kon Tum	JN688168	Rowley <i>et al.</i> (2011)
24.	<i>T. nebulosum</i>	AMS R 173877	Vietnam: Kon Tum	JN688168	Rowley <i>et al.</i> (2011)
25.	<i>T. nebulosum</i>	ROM 39588	Vietnam: Kon Tum	LC012845	Nguyen <i>et al.</i> (2015)
26.	<i>T. palliatum</i>	NAP 2735	Vietnam: Dak Lak	LC012843	Nguyen <i>et al.</i> (2015)
27.	<i>T. palliatum</i>	NAP 2736	Vietnam, Dak Lak	LC012844	Nguyen <i>et al.</i> (2015)
28.	<i>T. palliatum</i>	AMS R173130	Vietnam: Lam Dong	JN688172	Rowley <i>et al.</i> (2011)
29.	<i>T. palliatum</i>	AMS R173131	Vietnam: Lam Dong	JN688173	Rowley <i>et al.</i> (2011)
30.	<i>T. petilum</i>	HNUE MNA.2012.0001	Vietnam: Dien Bien: Muong Nhe	KJ802925	Nguyen <i>et al.</i> (2014)
31.	<i>T. rhododiscus</i>	CIB GX200807017	China: Guangxi	LC012842	Nguyen <i>et al.</i> (2015)
32.	<i>T. ryabovi</i>	VNMN 3924	Vietnam: Kon Tum: Mang Canh	LC012860	Nguyen <i>et al.</i> (2015)
33.	<i>T. stellatum</i>	stellatum-1	Thailand: Chanthaburi: Phliu	KT461918	Poyarkov <i>et al.</i> (2015)
34.	<i>T. truongsongnense</i>	VNMN 4402	Vietnam: Khanh Hoa: Hon Ba	LC012847	Nguyen <i>et al.</i> (2015)
35.	<i>T. vietnamense</i>	VNMN 3687	Vietnam: Phu Yen: Krong Trai	KJ802923	Nguyen <i>et al.</i> (2014)
36.	<i>T. vietnamense</i>	VNMN 3686	Vietnam: Phu Yen: Krong Trai	KJ802922	Nguyen <i>et al.</i> (2014)
37.	<i>Nyctixalus pictus</i>	KUHE 53517	Borneo: Sarawak: Bario	LC012863	Nguyen <i>et al.</i> (2015)
38.	<i>Liuixalus romeri</i>	CIB 20080048	China: Hong Kong	AB871412	Nguyen <i>et al.</i> (2014)

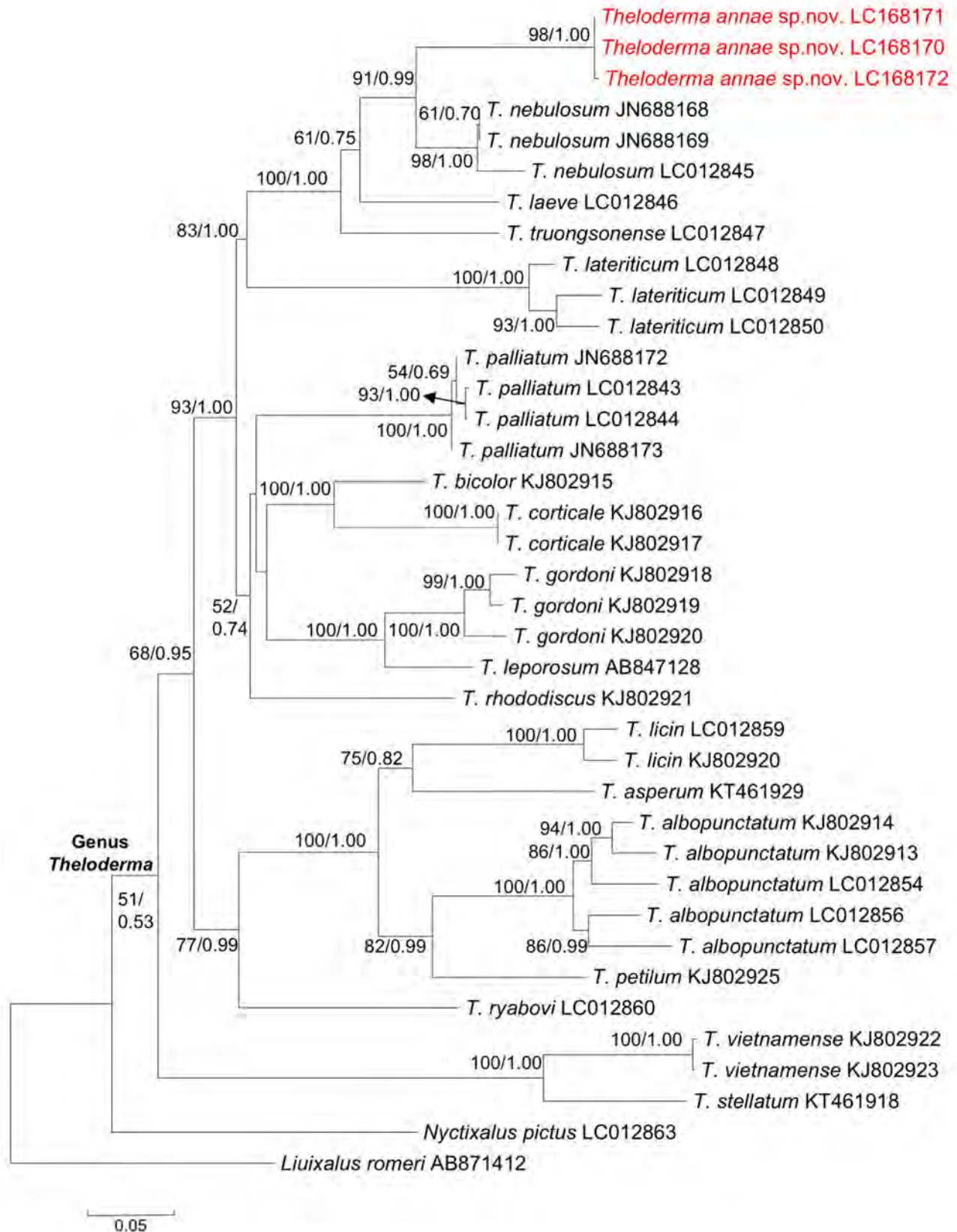


FIGURE 1. Maximum likelihood (ML) tree based on partial sequence of mitochondrial genes (12S rRNA, tRNAval, and 16S rRNA) for samples of *Theلودerma* and referenced species. Numbers above and below branches represent bootstrap support for ML and Bayesian posterior probabilities (MLBS/ BPP).

TABLE 2. Mean uncorrected (“p”) distance (%) among 489 bp fragments of 16S rRNA from species of the genus *Theلودerma*.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
1. <i>Theلودerma annae</i> sp. nov.	-																		
2. <i>T. albopunctatum</i>	15.9	-																	
3. <i>T. asperum</i>	14.8	11.7	-																
4. <i>T. bicolor</i>	10.5	11.1	12.1	-															
5. <i>T. corticale</i>	11.0	13.8	13.6	4.8	-														
6. <i>T. gordonii</i>	14.8	16.0	16.8	11.4	12.8	-													
7. <i>T. laeve</i>	10.7	13.0	13.8	10.2	12.7	15.6	-												
8. <i>T. lateriticum</i>	11.7	15.2	16.4	8.4	11.0	14.7	11.6	-											
9. <i>T. leporosum</i>	12.3	13.6	13.9	6.5	8.7	10.3	11.5	10.2	-										
10. <i>T. licin</i>	17.3	11.3	11.3	13.3	15.4	17.9	17.0	17.5	14.6	-									
11. <i>T. nebulosum</i>	7.4	12.3	12.1	7.7	9.0	12.0	7.2	11.2	8.9	13.9	-								
12. <i>T. palliatum</i>	13.1	14.2	15.1	7.2	8.9	13.0	13.3	11.2	10.4	16.5	11.7	-							
13. <i>T. petilum</i>	14.8	8.5	10.8	10.6	14.7	16.4	14.2	14.5	12.7	11.4	12.7	14.0	-						
14. <i>T. ryabovi</i>	13.3	13.7	12.6	10.1	10.7	14.3	10.0	13.0	11.0	15.1	10.7	11.7	13.3	-					
15. <i>T. rhododiscus</i>	13.9	12.4	12.5	6.9	9.5	13.0	11.0	11.4	10.1	15.5	10.2	9.1	11.8	10.2	-				
16. <i>T. stellatum</i>	18.1	17.9	21.1	15.2	18.3	21.3	18.6	17.7	19.3	19.3	16.3	17.7	16.4	21.3	16.7	-			
17. <i>T. truongsongense</i>	9.7	13.4	13.4	10.0	12.7	16.7	8.9	11.3	13.0	14.9	7.4	12.8	13.4	13.4	11.8	17.7	-		
18. <i>T. vietnamense</i>	18.5	18.7	20.5	14.7	16.5	20.1	18.3	17.2	18.2	19.1	17.3	17.4	18.2	18.5	16.8	8.8	18.0	-	

Results

Phylogenetic analyses. Aligned, combined sequences of 12S rRNA, tRNA^{val}, and 16S rRNA yielded a total of 1628 bp (104 bp of 12S, 70 bp of tRNA^{val} and 1454 bp of 16S). The data set also included nine shorter sequences (ca. 530 bp) obtained from three samples of *Theلودerma* sp. nov. from Hoa Binh, one sample of *T. asperum* from Genbank (KT461929), two samples of *T. nebulosum* from GenBank (JN688168, JN688169), two samples of *T. palliatum* from GenBank (JN688172, JN688173), and one sample of *T. stellatum* from Genbank (KT461918). Of 1628 nucleotide sites, 799 were variable and 685 were parsimony informative within the ingroup. The ML and Bayesian analyses produced topologies with $-\ln L = 14948.102$ and 15000.616 , respectively.

Phylogenetic analyses employing ML and BI methods yielded slightly different topologies only among referenced species, and only the ML tree is presented in Fig. 1. The unidentified species of *Theلودerma* from Hoa Binh was placed in the clade containing *T. laeve* (Smith), *T. nebulosum*, and *T. truongsongense* (Orlov & Ho) with strong support (MLBS = 100%, BPP = 1.00) (Fig. 1).

The interspecific uncorrected genetic p-distances at the fragment of 16S rRNA gene examined between the unnamed *Theلودerma* species from Hoa Binh and all congeners analysed varied from approximately 7.4% (compared with *T. nebulosum*) to 18.5% (compared with *T. vietnamense*) (Table 2).

Theلودerma annae sp. nov.

(Figs. 2, 3)

Holotype. IEBR 3732 (Field number HB 2014.185), adult male, collected by T. Q. Nguyen *et al.* on 9 October 2010 in karst forest near Cho Village (20°24.909'N, 105°19.102'E, at an elevation of 343 m), Tu Do Commune, Lac Son District, Hoa Binh Province, Vietnam.

Paratypes. Seven specimens collected from Ngoc Son – Ngo Luong Nature Reserve in Lac Son District, Hoa Binh Province, Vietnam: IEBR 3733–3735 (Field numbers HB 2014.92–2014.94), adult males, collected by C. T. Pham *et al.* on 19 April 2014 in the karst forest near Khu Village (20°27.976'N, 105°18.421'E, at an elevation of 650 m) Ngoc Son Commune; ZFMK 97289 (Field number HB 2014.121), adult female, collected by T.Q. Nguyen *et al.* on 2 October 2014 in the karst forest near Khu Village (20°26.862'N, 105°20.144'E, at an elevation of 553 m) Ngoc Son Commune; ZFMK 97290 (Field number HB 2014.147), adult male, collected by T.Q. Nguyen *et al.* on 6 October 2014 in the karst forest near Khang Village (20°23.778'N, 105°20.847'E, Vietnam, at an elevation of 219 m) Tu Do Commune; IEBR 3736 (Field number HB 2014.186), adult female, collected by T.Q. Nguyen *et al.* on 9 October 2010 in the karst forest near Cho Village (20°24.964'N, 106°19.116'E, at an elevation of 279 m), Tu Do Commune; VNMN A.2016.5 (Field number HB 2014.211), adult male, collected by T.Q. Nguyen *et al.* on 10 October 2014 in the karst forest near Khu Village (20°26.517'N, 105°20.147'E, at an elevation of 570 m) Ngoc Son Commune.

Diagnosis. Morphologically, the frog specimens from Hoa Binh Province showed diagnostic characters of the genus *Theلودerma*, 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 (see Liem 1970; McLeod & Norhayati 2007; Rowley *et al.* 2011). However, because no morphological synapomorphy is known for the genus *Theلودerma*, and its monophyly is not certain (Bain *et al.* 2009; Li *et al.* 2009; Rowley *et al.* 2011) we used molecular evidence to provide independent support of our generic placement.

The new species is distinguished from its congeners and other small rhacophorid species by a combination of the following characters: 1) Size small (SVL 27.1–28.5 mm in males, 30.3–32.6 mm in females); 2) head longer than wide; 3) vomerine teeth absent; 4) snout long (SNL/SVL 0.16–0.19); 5) spines on upper eyelid absent; 6) tibiotarsal projection absent; 7) dorsal skin smooth; 8) dermal fringes on forearm and tarsus absent; 9) dorsal surface greyish green; and 10) throat and ventral surfaces of arms and thighs brown with white spots.

Description of holotype. Adult male, body robust (SVL 27.4 mm), dorsoventrally compressed. Head slightly longer than wide (HL 11.7 mm, HW 10.8 mm), convex above; snout round in dorsal view, slightly protruding, its length longer than horizontal diameter of eye (SNL 5.1 mm, ED 4.1 mm); canthus rostralis round, loreal region oblique, concave; interorbital distance wider than internarial distance and upper eyelid (IOD 3.6 mm, IN 3.3 mm, UEW 2.9 mm); distance between anterior corner of eyes (DAE 6.2 mm) about 68% distance between posterior

corner of eyes (DPE 9.2 mm); nostril round, without a lateral flap of skin, closer to tip of snout than to the eye (NS 2.4 mm, EN 3.4 mm); pupil oval, horizontal; tympanum distinct (TYD 2.7 mm), round, smaller than eye diameter (ED 4.1 mm), greater than the distance between tympanum and eye (TYE 1.1 mm); pineal ocellus absent; spinules on upper eyelid absent; vomerine teeth absent; choanae small, oval; tongue cordate, deeply notched posteriorly; vocal sac absent; supratympanic fold distinct, extending from behind the eye to beyond level of axilla.

Forelimbs: Arm short, about half of hand length (FLL 5.8 mm, HAL 14.2 mm), dermal fringe along outer side of forearm absent; fingers free of webbing, relative lengths of fingers $I < II \leq V < III$; tips of fingers with enlarged discs with distinct circummarginal grooves; disc of finger III approximately two times of width of finger III (fd3/fw3 1.99) but smaller than tympanum diameter (fd3/TYD 0.58); subarticular tubercles distinct, blunt, round, formula 1, 1, 2, 2; nuptial pads prominent, oval; outer palmar tubercle divided into two.

Hindlimbs: Heels overlapping when held at right angles to the body; tibia length about five times greater than tibia width (TbL 16.7 mm, TbW 3.5 mm), longer than thigh length (FeL 14.7 mm) but shorter than foot length (FoL 20.8 mm); relative length of toes $I < II < III \leq V < IV$; tips of toes with enlarged discs with distinct circummarginal grooves, discs slightly smaller than those of fingers; webbing formula $II-1\frac{1}{2} III-2 IIII-2 IV2-1V$; subarticular tubercles distinct, blunt, round, formula 1, 1, 2, 3, 2; inner metatarsal tubercle small (IMT 1.7 mm); dermal ridge along outer side of tibia and tarsal fold absent; projection at tibiotarsal articulation absent.

Skin texture in life: Dorsal surface of head and body smooth; posterior part of dorsum, flanks, dorsal surface and lateral sides of limbs with small granules; dorsolateral folds absent; throat and chest smooth, belly and ventral surface of thighs granular; dermal appendage at vent absent.

Coloration in life: Iris greyish green, pupil round; background of dorsal surface greyish green; head with a mossy green blotch between eyes, in triangular shape; tympanum brown; dorsum and upper part of flanks with dark markings and blotches, forming a network; dorsal surface of forelimbs yellowish green with some mossy green spots and bars; throat and chest white with brown marbling; belly immaculate white. The coloration is brighter at night.

Coloration in preservative: Dorsal surface of head, dorsum, and upper part of flanks greyish brown with dark markings and blotches; upper lip and tympanum light brown; forelimb, dorsal surface of thigh, tibia and foot grey with dark bands, posterior part of thigh below the vent greyish brown with small white spots; chin and throat brown with small white spots; belly immaculate cream to white; ventral part of forelimbs white to grey with small white spots; ventral surface of thighs white to grey with small white spots; tibia and webbing dark brown.

Variation and sexual dimorphism. Measurements and morphological characters of the type series are provided in Table 3. The dorsal markings are indistinct in the specimen IEBR 3735, more prominent in the specimens IEBR 3734, 3736, ZFMK 97289, 97290 and consisting of three dark blotches in the individual IEBR 3733. The males are smaller in size than the females (SVL 27.1–28.6 mm, $n = 6$ vs. 30.3–32.6 mm, $n = 2$, respectively).

Etymology. We name this new species in honor of our colleague and friend, Anna Rauhaus, section animal keeper of the terrarium division of the Cologne Zoo, Germany, in recognition of her support of our research and conservation work in Vietnam. As common names we suggest Anna's Mossy Frog (English),Ếch cây sần an-na (Vietnamese), and Annas Moosfrosch (German).

Ecological notes. *Theلودerma annae* sp. nov. appears to be closely associated with karstic environment. Specimens were found at night between 19:00–23:30 h near cave entrances and in valleys surrounded by limestone cliffs, relatively far from water sources (Fig. 4). Advertisement calls, eggs and tadpoles of the species have not been recorded during our field surveys. The main habitat at the type locality is secondary karst forest of medium and small hardwoods mixed with shrubs and vines. Most of specimens were found on leaves, about 0.3–1.2 m above the ground but some specimens were collected on a limestone cliff. The air temperatures at the times of collection ranged from 20.4–30.2°C and relative humidity from 59–87%. In Ngoc Son – Ngo Luong Nature Reserve, several species of *Theلودerma* have been recorded, viz. *T. albopunctatum* (Liu & Hu), *T. corticale* (Boulenger), and *T. lateriticum*, but they were found in tree holes filled with water or on tree leaves near streams in the valleys. Other species of amphibians and reptiles found at the site were *Rhacophorus* sp., *Raorchestes parvulus* (Boulenger), *Cyrtodactylus bobrovi* Nguyen, Le, Ngo, Pham, Hoang, Pham & Ziegler, and *Gekko* cf. *palmatus* Boulenger.

Distribution. *Theلودerma annae* sp. nov. is currently known only from the type locality in Ngoc Son – Ngo Luong Nature Reserve, Hoa Binh Province, Vietnam. Several field surveys were conducted in the adjacent karst forest of Hang Kia – Pa Co Nature Reserve in Hoa Binh Province but no records of this species have been made so far.



FIGURE 2. Dorsal and ventral views of the holotype (IEBR 3732, adult male) of *Theloderma annae* sp. nov. from Hoa Binh Province, Vietnam.



FIGURE 3. Ventral side of hand (A) and foot (B) of the holotype (IEBR 3732) of *Theلودerma annae* sp. nov. in preservative.

TABLE 3. Measurements (in mm) and proportions of the type series of *Theلودerma annae* sp. nov. (H = holotype, P = paratype, other abbreviations defined in text).

	IEBR 3732	VNMN A.2016.5	IEBR 3733	IEBR 3734	IEBR 3735	ZFMK 97290		ZFMK 97289	IEBR 3736	
Sex	♂	♂	♂	♂	♂	♂	Min-Max	Mean±SD	♀	♀
Type status	H	P	P	P	P	P	(n=6)	(n=6)	P	P
SVL	27.4	27.7	28.0	28.5	27.8	27.1	27.1–28.5	27.7±0.34	30.3	32.6
HW	10.8	10.2	10.2	10.9	10.4	10.3	10.2–10.9	10.5±0.26	11.8	12.7
HL	11.7	11.2	11.1	11.2	11.6	11.6	11.1–11.7	11.4±0.23	12.4	13.0
MN	2.2	2.3	2.1	2.3	2.0	2.1	2.0–2.3	2.2±0.08	2.0	2.5
MFE	5.2	4.8	4.9	4.8	4.8	4.6	4.6–5.2	4.8±0.13	4.2	5.2
MBE	7.8	8.0	8.4	8.6	9.7	8.7	7.8–9.7	8.4±0.27	8.2	8.9
SNL	5.1	4.9	4.6	5.0	4.6	5.0	4.6–5.1	4.9±0.16	5.0	5.4
ED	4.1	4.2	4.2	4.5	4.2	4.4	4.1–4.5	4.3±0.11	4.8	4.9
UEW	2.9	2.8	2.9	2.8	2.7	2.6	2.6–2.9	2.8±0.07	2.9	3.2
IN	3.3	3.4	3.1	3.2	3.2	3.0	3.0–3.4	3.1±0.18	3.4	3.5
IOD	3.6	3.8	3.8	3.4	3.7	3.7	3.4–3.8	3.7±0.09	3.8	4.0
DAE	6.2	6.1	5.8	5.8	6.3	6.1	5.8–6.2	6.0±0.16	6.4	6.6
DPE	9.2	8.8	8.5	8.9	8.8	8.6	8.5–9.2	8.8±0.16	9.4	10.2
NS	2.4	2.1	2.2	2.1	2.2	2.3	2.1–2.4	2.2±0.08	2.4	2.5
EN	3.4	3.3	3.1	3.2	3.2	3.0	3.0–3.4	3.2±0.11	3.1	3.4
TYD	2.9	2.6	2.4	2.5	2.6	2.7	2.4–2.9	2.6±0.12	3.1	3.2
TYE	1.1	1.2	0.9	0.9	1.0	1.1	0.9–1.2	0.10±0.10	0.9	1.0
FLL	5.8	5.1	4.4	4.8	4.7	4.	4.4–5.8	4.9±0.36	5.5	6.3
HAL	14.2	13.3	13.6	13.9	13.8	13.4	13.3–14.2	13.7±0.26	14.3	15.8
TFL	6.2	5.6	5.7	5.8	5.8	5.3	5.3–6.2	5.7±0.20	5.9	6.3
fd3	1.7	1.5	1.6	1.6	1.7	1.5	1.5–1.7	1.6±0.06	1.5	1.6
FeL	14.7	15.1	13.8	14.8	14.1	13.8	13.8–15.1	14.4±0.48	15.0	15.8
TbL	16.7	16.2	15.8	15.7	16.4	17.0	15.7–17.0	16.3±0.38	17.4	18.9
TbW	3.5	3.0	3.1	3.0	3.1	2.9	2.9–3.5	3.1±0.14	3.1	3.5
FoL	20.8	21.1	19.7	20.2	20.0	20.7	19.7–20.8	20.3±0.33	22.0	23.4
FTL	12.9	12.3	12.1	11.7	10.7	11.0	10.7–12.9	11.8±0.63	13.5	13.3
SNL/SVL	0.19	0.18	0.18	0.17	0.17	0.18	0.17–0.19	0.18±0.01	0.16	0.17
ED/SNL	0.80	0.87	0.91	0.90	0.90	0.88	0.80–0.91	0.88±0.03	0.97	0.90
TYE/TYD	0.40	0.45	0.38	0.34	0.37	0.39	0.34–0.45	0.30±0.03	0.33	0.30
fd3/TYD	0.58	0.60	0.66	0.62	0.67	0.55	0.55–0.67	0.61±0.04	0.52	0.48

Comparisons. We compared the new species with other members of the genus *Theلودerma* based on morphological specimen examination (see Appendix I) and data obtained from the literature (e.g., Ahl 1927, 1931; Bain & Nguyen 2004; Bain *et al.* 2009; Boulenger 1903; Bourret 1937, 1942; Chanda 1994, Chan-ard 2003; Chan & Norhayati 2009; Fei *et al.* 2009, 2012; Inger *et al.* 1999; Jiang *et al.* 2009; Kunz *et al.* 2010; Liu & Hu 1962; McLeod & Norhayati 2007; Nguyen *et al.* 2014; Orlov *et al.* 2005, 2006, 2012; Smith 1924; Stuart & Heatwole 2004; Taylor 1962; Tschudi 1838; Rowley *et al.* 2011).

Theلودerma annae sp. nov. differs from *T. bicolor* (Bourret), *T. corticale*, *T. gordonii* Taylor, *T. (Stelladerma* in Poyarkov *et al.* 2015) *horridum* (Boulenger), *T. kwangsiense* (Liu & Hu), *T. leporosum* Tschudi, *T. moloch* (Annandale), *T. nagalandense* Orlov, Dutta, Ghate & Kent, and *T. phrynoderma* (Ahl) by having a smaller size (SVL 27.1–28.6 mm in males and 30.3–32.6 mm in females vs. SVL ≥ 40 mm in the other species) and smooth dorsal skin (vs. with large warts in other species).



FIGURE 4. *Theloderma annae* sp. nov. (IEBR 3734) *in situ* on vegetation in Ngoc Son – Ngo Luong Nature Reserve, Hoa Binh Province, Vietnam.

The new species differs from *T. andersoni* (Ahl) by having smooth dorsal skin (vs. with small tubercles in *T. andersoni*), in dorsal color pattern (greyish green vs. dark olive with two yellow spots on middle of flanks and a large black spot on groin in *T. andersoni*) and ventral color (white vs. yellow with fine brown spots anteriorly in *T. andersoni*); from *T. albopunctatum* (Liu & Hu) and *T. asperum* (Boulenger) by having smooth dorsal skin (vs. with small tubercles in latter species), in dorsal color pattern (greyish green vs. greyish brown with large conspicuous white pattern in latter species), in ventral color pattern (white vs. marbled black and bluish grey in latter species), and iris color (greyish green vs. reddish brown in latter species); from *T. baibengense* Jiang, Fei & Huang by having smooth dorsal skin (vs. with small tubercles in *T. baibengense*), in dorsal color pattern (greyish green vs. back with large conspicuous white pattern in *T. baibengense*), and ventral color pattern (white vs. dark with white pattern in *T. baibengense*); from *T. lateriticum* in dorsal color pattern (greyish green vs. brick red with single black mid-dorsal spots in *T. lateriticum*), in ventral color pattern (white vs. uniform grey-brown with white spots in *T. lateriticum*), and iris color (greyish green vs. brick-red in *T. lateriticum*); from *T. laeve* (Smith) in dorsal color pattern (greyish green vs. golden beige to light brown above with small dark brown spots and blotches forming an x-shaped figure, and dark longitudinal streaks on flanks in *T. laeve*), in ventral color pattern (white vs. light greyish pink to violet), and iris color (greyish green vs. grey above and dark brown below in *T. laeve*); from *T. licin* McLeod & Ahmad by having dorsal skin smooth (vs. with small tubercles in *T. licin*), in dorsal color pattern (greyish green vs. white to pale brown in *T. licin*), in ventral color pattern (white vs. white with brown reticulations in *T. licin*), and iris color (greyish green vs. red in *T. licin*); from *T. nebulosum* by having smooth dorsal skin (vs. with small tubercles in *T. nebulosum*), in dorsal color pattern (greyish green vs. brown with dark marking in *T. nebulosum*), in ventral color pattern (white vs. dark brownish black with pale blue in *T. nebulosum*), and iris color (greyish green vs. pale gold above, reddish brown below in *T. nebulosum*); from *T. palliatum* by having smooth



FIGURE 5. Type locality (black square) of *Theloderma annae* sp. nov. in Hoa Binh Province, Vietnam.

dorsal skin (vs. with small tubercles in *T. palliatum*), in dorsal color pattern (greyish green vs. pale brown or pale yellow to light straw brown with dark blotches forming an x-shaped figure in *T. palliatum*), in ventral color pattern (white vs. dark brownish black with pale blue or greyish with light bluish and dark spots and blotches in *T. palliatum*), and iris color (greyish green vs. pale gold above, reddish brown below in *T. palliatum*); from *T. petilum* (Stuart & Heatwole) in dorsal color pattern (greyish green vs. light brown with dark brown reticulations and scattered black spots in *T. petilum*), and the iris color (greyish green vs. upper part reddish brown, lower part grey in *T. petilum*); from *T. rhododiscus* (Liu & Hu) in dorsal color pattern (greyish green vs. brown in *T. rhododiscus*), in ventral color pattern (white vs. brown black scattered with grey white network in *T. rhododiscus*), and iris color (greyish green vs. red brown in *T. rhododiscus*); from *T. ryabovi* Orlov, Dutta, Ghate & Kent by having a smaller size (SVL 27–33 mm vs. 44 mm in *T. ryabovi*), in dorsal color pattern (greyish green vs. light beige with lilac pattern and black spots in *T. ryabovi*), in ventral color pattern (white vs. dark grey in *T. ryabovi*), and iris color (greyish green vs. dark brown in *T. ryabovi*); from *T. (Stelladerma* in Poyarkov *et al.* 2015) *stellatum* Taylor and *T. (Stelladerma* in Poyarkov *et al.* 2015) *vietnamense* in dorsal color pattern (greyish green vs. brown with light markings in the latter), in ventral color pattern (white vs. dark brown reticulated with cream in in the latter), and iris color (greyish green vs. brown with black reticulations in the latter); and from *T. truongsongense* (Orlov & Ho) in dorsal color pattern (greyish green vs. pale brown with small, distinct darker brown markings in *T. truongsongense*), in ventral color pattern (white vs. dark grey with black round speckles in *T. truongsongense*), and iris color (greyish green vs. pale gold above, reddish brown below in *T. truongsongense*).

In terms of dorsal color pattern *Theلودerma annae* **sp. nov.** is similar to *Gracixalus quyeti* (Nguyen, Hendrix, Böhme, Vu & Ziegler) from Quang Binh Province and *G. waza* Nguyen, Le, Pham, Nguyen, Bonkowski & Ziegler from Cao Bang Province. In addition, *Theلودerma annae* **sp. nov.** and the two afore mentioned *Gracixalus* species also have similar life histories, all inhabiting limestone karst forest far from water sources. However, *Theلودerma annae* differs from *G. quyeti* by having dorsal skin smooth (vs. with small sharp tubercles in *G. quyeti*), the absence of a dark inverse Y-marking on dorsum (vs. present in *G. quyeti*), and the outer palmar tubercle prominent (vs. indistinct in *G. quyeti*). *Theلودerma annae* **sp. nov.** differs from *G. waza* in the ratio of TYD/TDE (0.39 in males and 0.32 in females vs. 0.87 in males and 0.98 in females in *G. waza*), and the absence of a dark inverse Y-marking on dorsum (vs. present in *G. waza*) (Nguyen *et al.* 2008, 2012).

Discussion

In our phylogenetic analysis *Theلودerma annae* is assigned to the *T. truongsongense* group (or the *T. laeve* group in Poyarkov *et al.* 2015) with significant values of nodal support. The mean genetic distance (16S gene) between the new species and other members of this species group varied from 7.4% (compared with *T. nebulosum*) to 10.7% (compared with *T. laeve*) (Table 2). Morphologically, the new species can be clearly distinguished from members of the *T. truongsongense* group in having smooth dorsal skin and the dorsal surface being greyish green with mossy markings. The greenish brown or 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 (see also Nguyen *et al.* 2012). However, biodiversity of karst forests in Ngoc Son – Ngo Luong Nature Reserve as well as in northwestern Vietnam is currently threatened due to the effects of road construction, expanding agriculture, and illegal timber logging (T. Nguyen pers. obs.).

The discovery of additional new species of *Theلودerma* 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 *Theلودerma* in the poorly studied regions of northern Vietnam.

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References

- Ahl, E. (1927) Zur Systematik der asiatischen Arten der Froschgattung *Rhacophorus*. *Sitzungsberichte der Gesellschaft Naturforschender Freunde zu Berlin*, 1927, 35–47.
- Ahl, E. (1931) Amphibia, Anura III, Polypedatidae. *Das Tierreich*, 55, 1–477.
- Bain, R.H. & Nguyen, T.Q. (2004) Herpetofaunal diversity of Ha Giang Province in northeastern Vietnam, with description of two new species. *American Museum Novitates*, 3453, 1–42.
[http://dx.doi.org/10.1206/0003-0082\(2004\)453<0001:HDOHGP>2.0.CO;2](http://dx.doi.org/10.1206/0003-0082(2004)453<0001:HDOHGP>2.0.CO;2)
- 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.
- Boulenger, G.A. (1903) Descriptions of three new batrachians from Tonkin. *Annals and Magazine of Natural History*, Series 7, 12, 186–188.
<http://dx.doi.org/10.1080/00222930308678835>
- Bourret, R. (1937) Notes herpétologiques sur l'Indochine française. XIV. Les batraciens de la collection du Laboratoire des Sciences Naturelles de l'Université. Descriptions de quinze espèces ou variétés nouvelles. *Annexe au Bulletin Général de l'Instruction Publique*. Hanoi, 1937, 5–56.
- Bourret, R. (1942) *Les Batraciens de l'Indochine*. Hanoi, Institut Océanographique de l'Indochine. Hanoi, Vietnam.
- Chan, K.O. & Norhayati, A. (2009) Distribution and natural history notes on some poorly known frogs and snakes from Peninsular Malaysia. *Herpetological Review*, 40, 294–301.
- Chan-ard, T. (2003) *A photographic guide to amphibians in Thailand*. Darnsutha Press, Bangkok, Thailand.
- Chanda, S.K. (1994) Anura (Amphibia) of north-eastern India. *Memoirs of the Zoological Survey of India*, 18: 1–143
- Fei, L., Hu, S., Ye, C., & Huang, Y. (2009) *Fauna Sinica. Amphibia. Volume 2. Anura*. Science Press, Beijing, 957 pp.
- Fei, L., Ye, C. & Jiang, J. (2012) *Colored Atlas of Chinese Amphibians*. Sichuan Publishing Group, Sichuan, China, 519 pp.
- Fouquet, A., Gilles, A., Vences, M., Marty, C., Blance, M. & Gemmel, N.J. (2007) Underestimation of species richness in neotropical frogs revealed by mtDNA analysis. *PLoS ONE*, 2, e1109.
<http://dx.doi.org/10.1371/journal.pone.0001109>
- Frost D.R. (2016) *Amphibian Species of the World: an on-line reference. Version 6.0 (15 July 2016)*. American Museum of Natural History, New York, USA. Available from: <http://research.amnh.org/herpetology/amphibia> (Accessed 13 Sept. 2016)
- Glaw, F. & Vences, M. (1997) Anuran eye colouration: definitions, variation, taxonomic implication and possible functions. In: Böhme, W., Bischoff, W. & Ziegler, T. (Eds.), *Herpetologia Bonnensis*. SHE Proceedings, Bonn, pp. 125–138.
- Glaw, F. & Vences, M. (2007) *A field guide to the amphibians and reptiles of Madagascar*. Third Edition, Frosch Verlag, Cologne.
- Huelsenbeck, J.P. & Hillis, D.M. (1993) Success of phylogenetic methods in the four taxon case. *Systematic Biology*, 42, 247–264.
<http://dx.doi.org/10.2307/2992463>
- Inger, R.F., Orlov, N.L. & Darevsky, I. (1999) Frogs of Vietnam: A report on new collections. *Fieldiana: Zoology*, 92, 1–46.
- Jiang, J., Fei, L. & Huang, Y. (2009) *Aquixalus beibengensis* Jiang, Fei & Huang, sp. nov. In: Fei, L., Hu, S., Ye, C. & Huang, Y. (Eds.), *Fauna Sinica. Amphibia. Volume 2. Anura*. Chinese Academy of Science, Science Press, Beijing, pp. 708–711.
- Katoh, K. & Standley, D.M. (2013) MAFFT multiple sequence alignment software, version 7: improvements in performance and usability. *Molecular Phylogenetics and Evolution*, 30, 772–780.
<http://dx.doi.org/10.1093/molbev/mst010>
- Kunz, K., Honigs, S. & Eisenberg, T. (2010) *Moosfrösche: die Gattung Theloderma*. Natur und Tier Verlag, Münster, Germany. 125 pp
- Kuraishi, N., Matsui, M., Hamidy, A., Belabut, D.M., Ahmad, N., Panha, S., Sudin, A., Yong, H.-S., Jiang, J.-P., Ota, H., Thong, H.T. & Nishikawa, K. (2013) Phylogenetic and taxonomic relationships of the *Polypedates leucomystax* complex (Amphibia). *Zoologica Scripta*, 42, 54–70.
<http://dx.doi.org/10.1111/j.1463-6409.2012.00562.x>
- Leaché, A.D. & Reeder, T.W. (2002) Molecular systematics of the eastern fence lizard (*Sceloporus undulatus*): a comparison of

- parsimony, likelihood, and Bayesian approaches. *Systematic Biology*, 51, 44–68.
<http://dx.doi.org/10.1080/106351502753475871>
- Li, C., Hou, M., Yu, G.-h., Yan, F., Li, B.-z., Jiang, K., Li, P.-p. & Orlov, N.L. (2016) Rediscovery of *Theلودerma moloch* (Amphibia: Anura: Rhacophoridae) from Southeast Tibet, China after 102 years. *Russian Journal of Herpetology*, 23 (1), 41–54.
- Liem, S.S. (1970) The morphology, systematic, and evolution of the old world treefrogs (Rhacophoridae and Hyperoliidae). *Fieldiana Zoology*, 57, 1–145.
<http://dx.doi.org/10.5962/bhl.title.2939>
- Liu, C.-c. & Hu, S.-q. (1962) A herpetological report of Kwangsi. *Acta Zoologica Sinica*, 14 (Supplement), 73–104.
- Matsui, M., Shimada, T. & Sudin, A. (2014) First record of the tree-frog genus *Chiromantis* from Borneo with the description of a new species (Amphibia, Rhacophoridae). *Zoological Science*, 31, 45–51.
<http://dx.doi.org/10.2108/zsj.31.45>
- McLeod, D.S. & Ahmad, N. (2007) A new species of *Theلودerma* (Anura: Rhacophoridae) from southern Thailand and peninsular Malaysia. *Russian Journal of Herpetology*, 14, 65–72.
- Nguyen, S.V., Ho, C.T. & Nguyen, T.Q. (2009) *Herpetofauna of Vietnam*. Edition Chimaira, Frankfurt am Main, Germany, 768 pp.
- Nguyen, T.Q., Hendrix, R., Böhme, W., Vu, N.T. & Ziegler, T. (2008) A new species of the genus *Philautus* (Amphibia: Anura: Rhacophoridae) from the Truong Son Range, Quang Binh Province, central Vietnam. *Zootaxa*, 1925, 1–13.
- Nguyen, T.Q., Le, M.D., Pham, C.T., Nguyen, T.T., Bonkowski, M. & Ziegler, T. (online: 2012/printed: 2013) A new species of *Gracixalus* (Amphibia, Anura, Rhacophoridae) from northern Vietnam. *Organisms Diversity & Evolution*, 13, 203–214.
<http://dx.doi.org/10.1007/s13127-012-0116-0>
- 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 (Amphibia: Anura: Rhacophoridae) from Vietnam and its phylogenetic position. *Current Herpetology*, 33, 112–120.
<http://dx.doi.org/10.5358/hsj.33.112>
- Nguyen, T.T., Matsui M. & Eto, K. (2015) Mitochondrial phylogeny of an Asian tree frog genus *Theلودerma* (Anura: Rhacophoridae). *Molecular Phylogenetics and Evolution*, 85, 59–67.
<http://dx.doi.org/10.1016/j.ympev.2015.02.003>
- Orlov, N.L., Dutta, S.K., Ghate, H.V. & Kent, Y. (2006) New species of *Theلودerma* from Kon Tum Province (Vietnam) and Nagaland State (India) (Anura: Rhacophoridae). *Russian Journal of Herpetology*, 13, 165–175
- Orlov, N.L. & Ho, C.T. (2005) A new species of *Philautus* from Vietnam (Anura: Rhacophoridae). *Russian Journal of Herpetology* 12, 135–142.
- Orlov, N.L., Poyarkov, N.A., Vassilieva, A.B., Ananjeva, N.B., Nguyen, T.T., Nguyen, S.N. & Geissler, P. (2012) Taxonomic notes on rhacophorid frogs (Rhacophorinae: Rhacophoridae: Anura) of southern part of Annamite Mountains (Truong Son, Vietnam), with description of three new species. *Russian Journal of Herpetology*, 19, 23–64.
- Poyarkov, N.A. Jr., Orlov, N.L., Moiseeva, A.V., Pawangkhanant, P., Ruwangsuwan, T., Vassilieva, A.B., Galoyan, E.A., Nguyen, T.T. & Gogoleva, S.S. (2015) Sorting out moss frogs: mtDNA data on taxonomic diversity and phylogenetic relationships of the Indochinese species of the genus *Theلودerma* (Anura, Rhacophoridae). *Russian Journal of Herpetology*, 22 (4), 241–280.
- Rambaut, A. & Drummond, A. (2009) TRACER, version 1.5. Available from: <http://beast.bio.ed.ac.uk/Tracer> (Accessed 13 Sept. 2016)
- Rowley, J.J.L., Le, D.T.T., Hoang, H.D., Dau, V.Q. & Cao, T.T. (2011) Two new species of *Theلودerma* (Anura: Rhacophoridae) from Vietnam. *Zootaxa*, 3098, 1–20.
- Smith, M.A. (1924) New tree-frogs from Indo-China and the Malay Peninsula. *Proceedings of the Zoological Society of London*, 1924, 225–234.
<http://dx.doi.org/10.1111/j.1096-3642.1924.tb01499.x>
- Stuart, B.L. & Heatwole, H.F. (2004) A new *Philautus* (Amphibia: Rhacophoridae) from northern Laos. *Asiatic Herpetological Research*, 10, 17–21.
- Tanabe, A.S. (2011) Kakusan 4 and Aminosan: two programs for comparing nonpartitioned, proportional and separate models for combined molecular phylogenetic analyses of multilocus sequence data. *Molecular Ecology Resources*, 11, 914–921.
<http://dx.doi.org/10.1111/j.1755-0998.2011.03021.x>
- Tavare, S. (1986) Some probabilistic and statistical problems in the analysis of DNA sequences. *Lectures on Mathematics in the Life Sciences* (American Mathematical Society), 17, 57–86.
- Taylor, E.H. (1962) The amphibian fauna of Thailand. *University of Kansas Science Bulletin*, 43, 265–599.
<http://dx.doi.org/10.5962/bhl.part.13347>
- Tschudi, J.J.v. (1838) *Classification der Batrachier mit Berücksichtigung der fossilen Thiere dieser Abtheilung der Reptilien*. Petitpierre, Neuchâtel, Switzerland.
<http://dx.doi.org/10.5962/bhl.title.59545>

APPENDIX I. Examined specimens.

- Gracixalus quyeti* (2): Vietnam: Quang Binh Province: ZFMK 82999 (holotype), VNUH 160706 (paratype).
G. waza (6): Vietnam: Cao Bang Province: Ha Lang District: IEBR A.2012.2 (holotype), IEBR A.2012.3, VNMN A.2012.2, A.2012.3, ZFMK 93666, 93667 (paratypes).
Theloderma albopunctatum (8): Vietnam: Bac Giang Province: Son Dong District: IEBR A. 2013.82, A.2013.83; Vietnam: Hoa Binh Province: Mai Chau District: IEBR 3760, 3761; Vietnam: Hoa Binh Province: Lac Son District: IEBR 3762–3765.
T. bicolor (2): Vietnam: Lao Cai Province: Sa Pa District: IEBR 3740, 3741.
T. corticale (4): Vietnam: Bac Giang Province: Son Dong District: IEBR A. 2013.84, A.2013.85; Vietnam: Hoa Binh Province: Lac Son District: IEBR 3758, 3759.
T. gordonii (3): Vietnam: Hoa Binh Province: Lac Son District: IEBR 3737; Vietnam: Vinh Phuc Province: Tam Dao NP: IEBR 3738, 3739.
T. lateriticum (8): Vietnam: Lao Cai Province: Sa Pa District: IEBR 3745–3747; Vietnam: Hoa Binh Province: Lac Son District: IEBR 3748–3753.
T. truongsongense (4): Vietnam: Quang Binh Province: Le Thuy District: IEBR 3754–3757.
T. vietnamense (3): Vietnam: Kien Giang Province: Phu Quoc District: IEBR 3742–3744.