# NEW RECORD OF *Theloderma annae* Nguyen, Pham, Ngo, Nguyen, Ziegler, 2016 (ANURA: RHACOPHORIDAE) FROM NINH BINH PROVINCE, VIETNAM

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#### SUMMARY

We herein report a new provincial record of *Theloderma annae* Nguyen, Pham, Nguyen, Ngo, Ziegler, 2016 from Ninh Binh Province based on five collected specimens. Three of them were found in Trang An Landscape Complex and the remaining specimens were collected in Cuc Phuong National Park. Most specimens were detected on limestone cliffs, rarely appeared on the leaves. Our specimens are specially identical with the original description of *Theloderma annae* from Ngoc Son - Ngo Luong Nature Reserve, Hoa Binh Province by the following characters: 1) A small size *Theloderma* species (SVL 27.2 - 29.9 mm in males, 32.2 - 36.7 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 very 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. *Theloderma annae*'s distribution range is expanded to lower 100 m a.s.l. Our research brought the species number of the genus *Theloderma* known from Trang An Landscape Complex to one and from Cuc Phuong National Park to four species.

Keywords: Distribution, new record, Ninh Binh province, Theloderma annae.

#### **1. INTRODUCTION**

The genus *Theloderma* Tschudi, 1838 is known as a most rarely group of tree frogs, containing 26 species in the world (Frost 2018). Vietnam currently represents the greatest number of species under this genus, with 16 species recorded up to now. Three of them have been described from this country in the last three years such as *T. vietnamense* Poyarkov, Orlov, Moiseeva, Pawangkhanant, Ruangsuwan, Vassilieva, Galoyan, Nguyen & Gogoleva 2015, *T. annae* Nguyen, Pham, Nguyen, Ngo & Ziegler 2016, *T. auratum* Poyarkov, Kropachev, Gogoleva & Orlov 2018 (Poyarkov et al. 2015, Nguyen et al. 2016, Poyarkov et al. 2018).

*Theloderma annae* was discovered two years ago by Nguyen et al. (2016) from Hoa Binh Province, Vietnam. The original description was based on eight specimens collected in the karst forest of Ngoc Son - Ngo Luong Nature Reserve (NR). The species was diagnosed as a representative of the genus *Theloderma* because of size small, SVL 27.1 -28.5 mm in males, 30.3 - 32.6 mm in females, head convex above and longer than wide, vomerine teeth absent, dorsal skin very smooth and sleek, tibiotarsal projection absent, dorsal surface in life gravish green, white spots appear in throat and ventral surface of arms and thighs. During our recent field surveys in Trang An Landscape Complex (LC) and Cuc Phuong National Park (NP) of Ninh Binh province, we collected a total of five specimens that were assignable to the genus Theloderma. morphological Based on examination revealed them to be representatives of the Anna's Mossy Frog Theloderma annae Nguyen, Pham, Ngo, Nguyen, Ziegler, 2016 which was first described by Nguyen et al. (2016) from limestone karst forest in Ngoc Son - Ngo Luong NR of Hoa Binh province. Thus we herein provide a new provincial record of Theloderma annae from Ninh Binh province.

#### 2. RESEARCH METHODOLOGY 2.1 Sampling

Field surveys were conducted by Vinh Q. Luu, Quang H. Nguyen, Oanh V. Lo, Ngoan V. Ha in Cuc Phuong NP in August 2017 and by Vinh Q. Luu, Quang H. Nguyen, Oanh V. Lo, Ngoan V. Ha, Chung V. Hoang in Trang An LC from May to June 2017 and in May 2018 (hereafter Vinh Q. Luu et al.). Specimens were collected between 19:00 and 23:30 h. The specimens were euthanized in a closed vessel with a piece of cotton wool containing ethyl acetate. After that, fixed in 80% ethanol for 4 - 6 hours, and then transferred to 70% ethanol for permanent storage. The specimens were subsequently deposited in the collection of the Vietnam National University of Forestry (VNUF), Hanoi, Vietnam.

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

#### **3. RESULTS AND DISCUSSION**

#### New record of *Theloderma annae* Nguyen, Pham, Nguyen, Ngo, Ziegler, 2016

Specimens examined (n = 5): Two adult males and three adult females: VNUF A.2017.52 (field number TA 17.52), adult male, collected by Vinh Q. Luu et al. on 19 May 2017 in karst forest near Tran temple (20°15.423'N, 105°53.794'E) at an elevation of 67m, CP A.2017.06 (field number NHQ 17.06), adult male, collected by Vinh Q. Luu et al. on 25 August 2017 in the forest of Cuc Phuong NP (20°17.69'N, 105°40.00'E) at an elevation of 321m), VNUF A.2018.45 (field number TA 18.45), adult female, collected by Vinh Q. Luu et al. on 28 May 2018 in karst forest near Tran temple (20°15.418'N, 105°53.965'E) at an elevation of 94 m), VNUF A.2017.57 (field number TA 17.57), adult female, collected by Vinh Q. Luu et al. on 21 June 2017 in karst forest near Tran temple (20°15.565'N, 105°53.448'E) at an elevation of 75m) and CP A.2017.07 (field number NHQ 17.07), adult female, collected by Vinh Q. Luu et al. on 25 August 2017 in the forest of Cuc Phuong NP (20°17.69'N, 105°40.00'E) at an elevation of 321m, Ninh Binh Province, Vietnam.

Morphological character: Body (SVL 27.2 - 29.9 mm in males, 32.2 - 36.7 mm in females). Head wide convex above and smaller than head length (HW 10.4 - 12.3 mm in males, 11.3 - 13 mm in females; HL 11.5 -13.2 mm in males, 12.4 - 13.8 mm in females). The distance from the back of mandible to the nostril (MN 10.5 - 12 mm in males; 12.3 - 12.6 mm in females). In front of eyes, the snout round, it's length higher than horizontal diameter of eyes (SNL 4.7 - 5.9 mm in males, 5.2 - 6.3 mm in females; ED 4.1 - 4.5 mm in males, 3.4 - 5.2 mm in females); canthus rostral round; odontophores oblique and separated, between choanae; widely the distance of interorbital wider than internarial distance and upper eyelid (IOD 3.8 - 4.2 mm in males, 4.3 - 4.5 mm in females; IND 2.5 - 3.4 mm in males, 3.0 - 3.4 mm in females; UEW 2.3 - 3.1 mm in males, 2.6 - 3.4 mm in females); pupil oval, horizontal. Besides, the distance between angle of jaws and anterior corner of the eyes elder than distance between angle of jaws and posterior corner of the eyes (MAE 7.9 - 8.9 mm in males, 8.3 - 9.2 mm in females; MPE 4.7 - 5.5 mm in males, 4.3 - 5.7 mm in females); the rate of distance between interior corner of eyes (DAE 5.7 - 7.5 mm in males, 6.5 - 7.4 mm in females) about 60% compare to distance between posterior corner of eyes (DPE 9.1 - 10.8 mm in males, 9.0 -12.1 mm in females); nostril round, nearly snout (NS 1.6 - 1.8 mm in males, 1.9 mm in females; EN 3.2 - 4.1 mm in males, 3.9 - 4.6 mm in females) and far away than two eyes; tympanum distinct round and clearly (TYD 2.7 - 3.3 mm in males, 2.5 - 3.2 mm in females); the distance between tympanum and eyes smaller than eyes diameter (TYE 1.1 - 1.6 mm in males, 0.9 - 2.0 mm in females; ED 4.1 -4.5 mm in males, 3.4 - 5.2 mm in females) pineal ocellus absent; spinules on upper eyelid vomerine teeth absent; absent; tongue lanceolate; supratympanic fold distinct,

extending from behind the eye to a half of dorsal view.

Forelimbs: arm very short, nearly one per three times on hand length (FLL 5.1 - 7.01 mm in males, 5.5 - 6.5 mm in females; HAL 14.2 - 18 mm in males, 15.3 - 18.2 mm in females), dermal fringe along outer side of forearm absent; full webbing, the fingers relative are I<II<IV<III; fingers tips with discs with distinct circummarginal grooves; nuptials pads is oval shape.

Hind limbs: Tibia length about five times than tibia width (TbL 16.9 - 18.9 mm in males, 17.1 - 18.6 mm in females; TbW 3.7 - 3.7 mm in males, 3.9 - 4.1 mm in females). It's higher than thigh length (Fel 15.7 - 15.9 mm in males, 16.3 - 17.6 mm in females) but shorter than foot length (FoL 21.6 - 25.1 mm in males; 22.1 - 24.7 mm in females); relationship between length of toes I<II<III≤V<IV; therefore, tips of toes with enlarged discs with distinct circummarginal grooves, width of discs slightly smaller than those of fingers; webbing formula I1-1/2II1-2III1-2IV2-1V; dermal ridge along outer side of tibia, tarsal fold absent.





Figure 1. (A) Dorsal and (B) ventral views of the adult male *Theloderma annae* (VNUF A.2018.45) in life (*Photos: Vinh Q. Luu*)

*Skin texture in life*: Doral surface of head and body are very smooth; behind the part of dorsum, dorsal surface and both sides of limb have lots of small granules; dorsolateral folds absent; similar to dorsal surface, throat, chest, belly and ventral surface of thighs granular also smooth; dermal appendage at vent absent. *Coloration in life:* Greyish green, pupil round; dorsal surface upper at the beginning was grey green; tympanum brown; dorsum and above part of flanks dark and small blotches make a network; forelimb surface was yellow green with some dark spots; throat and chest with brown color.



Figure 2. Microhabitat of *Theloderma annae* in the limestone karst forest of Trang An LC (*Photo: Vinh Q. Luu*)

*Coloration in preservative:* dorsal surface of head, dorsum change from yellow green into dark yellow with makings and blotches are grey green; lip above and tympanum light brown; dorsal surface of thigh, tibia and foot have some light dark bands; forelimbs ventral have some white spots similar to ventral surface of thighs; tibia and webbing change from yellow grey into dark brown.

*Ecological notes*: In Trang An LC, one adult male (field number VNUF A.2017.52) collected at gloaming (6:35 p.m) and two adult females (field number VNUF A.2018.45 and VNUF A.2017.57) on the karst cliff, approximately 1.8 - 2 m above the ground, at elevations 67 - 90 m a.s.l. (*versus* 343 - 650 in type series). The surrounding habitat was

limestone karst forest. The air temperature about 28 - 28.6°C, the relative humidity 68 -82%. In Cuc Phuong NP, two specimens were collected, one adult male (field number CP A.2017.06) and one adult female (CP A.2017.07) on the karst cliff, approximately 0.7 - 0.8 m above the ground, at elevation 67 m a.s.l. The habitat also limestone karst forest. The air temperature was 28.1°C and the relative humidity was 67%.

*Remarks:* In the surface of ventral view, there were two glands below axillary and greyish green below snout (appear in two adult females in Trang An LC). The size of adult females in Trang An LC is slightly larger than that of adult females in Ngoc Son - Ngo Luong NR (SVL  $36.2\pm0.70$  mm; SVL  $31.5\pm1.63$  mm,

respectively). Besides, snout vent length of adult males in Cuc Phuong NP is longer than adult males in Ngoc Son - Ngo Luong NR (SVL 29.9 mm; 27.7±0.34 mm).

Distribution: Theloderma annae were originally found in Ngoc Son - Ngo Luong

NR, Hoa Binh Province, Viet Nam. Thus the species was recorded for the first time from Ninh Binh province and for Trang An Landscape Complex as well as Cuc Phuong National Park as well.

 Table 1. Morphological characters of *Theloderma annae* from Trang An LC and Cuc Phuong NP in comparison with the type series of the species from Ngoc Son - Ngo Luong NR

Place	Trang An LC			Cuc Phuong NP		Ngoc Son-Ngo Luong NR	
Number	VNUF	VNUF	VNUF	СР	СР	Mean±SD	Mean±SD
	A.2017.52	A.2017.57	A.2018.45	A.2017.06	A.2017.07	(n=6)	(n=2)
Sex	8	4	<b>P</b>	2	Ŷ	8	4
SVL	27.2	35.7	36.7	29.9	32.2	27.7±0.34	31.5±1.63
HW	12.3	11.7	13.0	10.4	11.3	10.5±0.26	12.3±0.64
HL	13.3	13.8	13.7	11.5	12.4	11.4±0.23	12.7±0.42
MN	12.0	12.6	12.2	10.5	12.3	$2.2 \pm 0.08$	$2.4 \pm 0.35$
MAE	8.93	8.7	9.2	7.9	8.3	4.8±0.13	4.7±0.71
MBE	5.45	4.6	5.7	4.7	4.3	8.4±0.27	$8.6 \pm 0.49$
SNL	5.49	5.8	6.3	4.7	5.2	4.9±0.16	5.2±0.28
ED	4.52	5.2	4.3	4.1	3.4	4.3±0.11	$4.9 \pm 0.0.7$
UEW	3.1	3.4	3.1	2.3	2.6	$2.8 \pm 0.07$	3.1±0.21
IN	3.4	2.3	3.4	2.5	3.0	3.1±0.18	$3.5 \pm 0.07$
IOD	3.8	4.5	3.7	4.2	4.3	3.7±0.09	3.9±0.14
DAE	7.5	7.1	7.4	5.7	6.5	6.0±0.16	6.5±0.14
DPE	10.8	11.3	12.1	9.1	9.0	8.8±0.16	9.8±0.57
NS	1.6	1.8	1.9	1.8	1.9	$2.2 \pm 0.08$	$2.5 \pm 0.07$
EN	4.1	4.6	3.5	3.2	3.9	3.2±0.11	3.3±0.21
TYD	3.3	3.0	3.2	2.7	2.5	2.6±0.12	$3.2{\pm}0.07$
TYE	1.6	1.3	2.0	1.1	0.9	01.0±0.10	$1.0{\pm}0.07$
FLL	7.0	6.5	6.5	5.1	5.5	4.9±0.36	$5.9{\pm}0.57$
HAL	18.0	16.8	18.2	14.2	15.3	$13.7{\pm}~0.26$	15.1±1.06
TFL	6.8	6.9	6.4	6.7	6.7	5.7±0.20	6.1±0.28
fd3	1.7	1.8	1.1	0.9	1.2	$1.6 \pm 0.06$	$1.6 \pm 0.07$
FeL	16.0	17.0	17.6	15.7	16.3	$14.4\pm\!\!0.48$	15.4±0.57
TbL	18.9	18.4	18.6	16.9	17.1	16.3±0.38	18.2±1.06
TbW	3.7	3.5	4.1	3.7	3.9	$3.1 \pm 0.14$	3.3±0.28
FoL	25.1	24.7	24.5	21.6	22.1	$20.3{\pm}~0.33$	22.7±0.99
FTL	15.3	15.0	13.2	15.1	14.9	$11.8\pm\!\!0.63$	13.4±0.14
SNL/SVL	0.2	0.2	0.2	0.2	0.2	$0.2 \pm 0.01$	$0.2{\pm}0.01$
ED/SNL	0.8	0.9	0.7	0.9	0.7	$0.9 \pm 0.03$	$0.9{\pm}0.05$
TYE/TYD	0.5	0.4	0.6	0.4	0.4	$0.3 \pm 0.03$	$0.3 \pm 0.02$
fd3/TYD	0.5	0.6	0.3	0.3	0.5	$0.6 \pm 0.04$	$0.5 \pm 0.03$



Figure 3. Distribution map of *Theloderma annae*: new records from Ninh Binh province and the type locality from Hoa Binh province

### 4. CONCLUSIONS

The morphological characters of the collected specimens from Ninh Binh province completely with the original matched description of Theloderma annae from Ngoc Son - Ngo Luong Nature Reserve, Hoa Binh province by also having small size, head longer than wide, vemerine teeth absent, dorsal skin smooth, dermal fringes absent, dorsal surfaces of head and body greyish green, and ventral surfaces of head, forelimbs, and thighs brown with white spots. The main habitat of Theloderma annae was limestone karst forest in pretty high elevation. In previous research, this species has seen at the elevation from 219 - 650 m a.s.l (Nguyen et al. 2016) in Ngoc Son - Ngo Luong NR. However, in this research, there was a significant difference in distribution found from that species. The results pointed out that Theloderma annae can live at a lower elevation range, from 67 - 94 m a.s.l., which means this species have adapted with lower altitude in order to survive. Besides, the discovery increases the number of the genus Theloderma in Trang An LC to one species and in Cuc Phuong NP to four species. Therefore, additional studies on the diversity of the genus Theloderma in Ninh Binh

province are needed for proposing effective conservation solutions.

#### Acknowledgements

We are grateful to directorates of the Cuc Phuong National Park and Trang An Landscape Complex for supporting our field work and issuing relevant species. We thank Hoang V. Chung, Ha V. Ngoan, Lo V. Oanh, Pham T.K. Dung, Nguyen H. Quang for their assistance in the field. This research is supported by the National Foundation for Technology Science and Development (NAFOSTED, Grant No. 106.06-2017.18) and the Nagao Natural Environment Foundation (No. 20170824).

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# GHI NHẬN MỚI CỦA LOÀI ẾCH CÂY SẦN AN-NA (*Theloderma annae* Nguyen, Pham, Ngo, Nguyen, Ziegler, 2016) TẠI TỈNH NINH BÌNH, VIỆT NAM

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#### TÓM TẮT

Loài Éch cây sần an-na (*Theloderma annae*) lần đầu tiên được ghi nhận tại tỉnh Ninh Bình, dựa trên đặc điểm hình thái học của 2 mẫu đực và 3 mẫu cái trưởng thành được thu vào tháng 5, tháng 6 năm 2017 và tháng 5 năm 2018 tại khu vực rừng trên núi đá vôi của Quần thể danh thắng Tràng An và Vườn quốc gia Cúc Phương tỉnh Ninh Bình. Mẫu vật thu được phù hợp với mô tả gốc của loài bởi Nguyễn Quảng Trưởng và cộng sự (2016) bởi các đặc điểm hình thái sau: kích thước nhỏ, chiều dài thân của con đực trưởng thành 27,2 – 29,9 mm, con cái trưởng thành 32,2 - 36,7 mm. Chiều dài đầu lớn hơn chiều rộng đầu, mõm dài, không xuất hiện răng lá mía, không xuất hiện túi kêu, không có mí mắt trên, khuyết ở gốc lưỡi, lưng có màu xanh xám và nhẵn, xuất hiện nhiều đốm trắng nhỏ li ti ở cổ họng, bụng, cánh tay và chân, không có rìa da ở trên cẳng tay và chân. Nghiên cứu này đã ghi nhận số loài ếch cây sần thuộc giống *Theloderma* ở Quần thể danh thắng Tràng An tỉnh Ninh Bình là 1 loài và ở Vườn quốc gia Cúc Phương lên 4 loài và sự phân bố của loài này được biết đến dưới 100 m so với mực nước biến.

Từ khoá: Éch cây sần an-na, ghi nhận mới, phân bố, tỉnh Ninh Bình.

Received	: 14/8/2018
Revised	: 12/9/2018
Accepted	: 21/9/2018