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A new species of *Vietnamophryne* from Vietnam

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Abstract: A new species of the genus *Vietnamophryne* is described from Vu Quang National Park in North Vietnam based on molecular phylogenetic and morphological analyses. The new species is morphologically distinct from congeners and can be easily distinguished from all other members of the genus *Vietnamophryne* by (1) body size males (n=4) 14.12-14.91 mm; females (n=9) 15.15-17.33 mm; (2) dorsal skin shagreened with small flat pustules loosely scattered on back in males, mostly smooth, slightly shagreened posteriorly, lacking enlarged tubercles in females; (3) belly color ivory-lemon to lemon-yellow with weak dark-brown marbling in males, bright lemon-yellow with weak dark-brown marbling in females; (4) tympanum relatively faint, comparatively small, rounded, 5.4-6.0% of SVL in males, 4.8-6.2% of SVL in females; (5) ratio snout length to eye length 119-128% in males, 100-130 % in females; (6) ratio head width to head length 117-124% in males, 110-131% in females; and (7) ratio first finger to second finger 43-49% in males, 44-50% in females. In the phylogenetic analysis the new species revealed to be sister to *V. inexpectata*, with 4.3-4.6% divergence in the 16SrRNA gene. So far, the new species is only known from the evergreen tropical forests of Vu Quang National Park at an elevation of 966 m a.s.l. This is the third species of *Vietnamophryne* reported from Vietnam.

Keywords: *Vietnamophryne vuquangensis* **sp. nov.** - MtDNA phylogeny - New species - Taxonomy - Vietnam.

INTRODUCTION

The genus *Vietnamophryne* Poyarkov, Suwannapoom, Pawangkhanant, Aksornneam, Duong, Korost & Che, 2018 was only recently erected. It belongs to the subfamily *Asterophryinae* and is sister taxon to *Siamophryn*e. *Vietnamophryne* consists of three species of small-sized frogs reportedly occurring at two localities in Vietnam (*V. inexpectata* from a primary montane tropical forest in Kon Chu Rang, Nature Reserve (NR) Gia Lai Province and *V. orlovi* from a primary montane subtropical forest on the southern slopes of Phia Oac Mt., Phia Oac-Phia Den National Park (NP), Cao Bang Province) and at one locality in northern Thailand (*V. occidentalis* from a primary montane subtropical forest on limestone outcrops of Doi Tung Mt., Pong Ngam District, Chiang Rai Province) (Frost, 2020; Poyarkov *et al.,* 2018).

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During recent field research in North Central Vietnam, a series of Indochinese Dwarf Frogs were collected from Vu Quang National Park (NP), in Ha Tinh Province, Vietnam. Based on morphological differences and molecular divergence, we herein describe this population as a distinct *Vietnamophryne* species.

MATERIAL AND METHODS

Sampling: Field surveys were conducted in Vu Quang NP, Ha Tinh Province, Vietnam (Fig. 1) on 12 October 2019 by T. T. Nguyen, Q. H. Nguyen and N. Orlov. Geographic coordinates and elevation were obtained using a Garmin GPSMAP 78S (WGS 84 data). Thirteen specimens (n = 9, females VNMN 010487, 010491, 010492, 010517-010521, 010528; n = 4, males VNMN 010488-010490, 010498) were collected in the field. After photographing animals in life, specimens were euthanized in a closed vessel with a piece of cotton wool containing ethyl acetate, fixed in 95% ethanol for 8-10 hours and subsequently stored in 70% ethanol (Simmons, 2002). Tissues for genetic analyses were taken from liver prior to preservation and stored in 95% ethanol. Specimens were subsequently deposited in the Vietnam National Museum of Nature (VNMN) Hanoi, Vietnam.

Molecular analyses

DNA extraction and sequencing

Extraction of genomic DNA from 13 tissue samples (Table 1) was carried out using TIANamp Genomic DNA kit (TIANGEN BIOTECH, Beijing, China), Tiangen following the manufacturer's instructions. Total DNA was amplified using an Eppendorf PCR machine. PCR total volume was 25 µl, consisting of 12 µl of Mastermix, 6 µl of water, 1 µl of each primer at a concentration of 10 pmol/µl, and 5 µl of DNA. Primers used in the PCR and sequencing were as follows: 12SAL (5'-AAACTGGGATTAGATACCCCACTAT-3'; forward), 16S2000H (5'-GTGATTAYGCTACCTTTGC-ACGGT-3'; reverse) (Zhang *et al.*, 2008) and LR-N-13398 (5'-CGCCTGTTTACCAAAAACAT -3'; forward), LR-J 12887 (5'-CCGGTCTGAACTCAGATCACGT -3'; reverse) (Simon, 1994). PCR conditions: 94°C for 5 minutes of initial denaturation; with 35 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 30 s, and extension at 72°C for 45 s; and the final extension at 72°C for 7 minutes. PCR products were sent to Tsingke Biological Technology company for sequencing (http://www.tsingke.net). The obtained sequences were deposited in GenBank under the accession numbers MW763017 to MW763029 (Table 1). We amplified a 1940 base pair (bp) length fragment of the 12S rRNA–

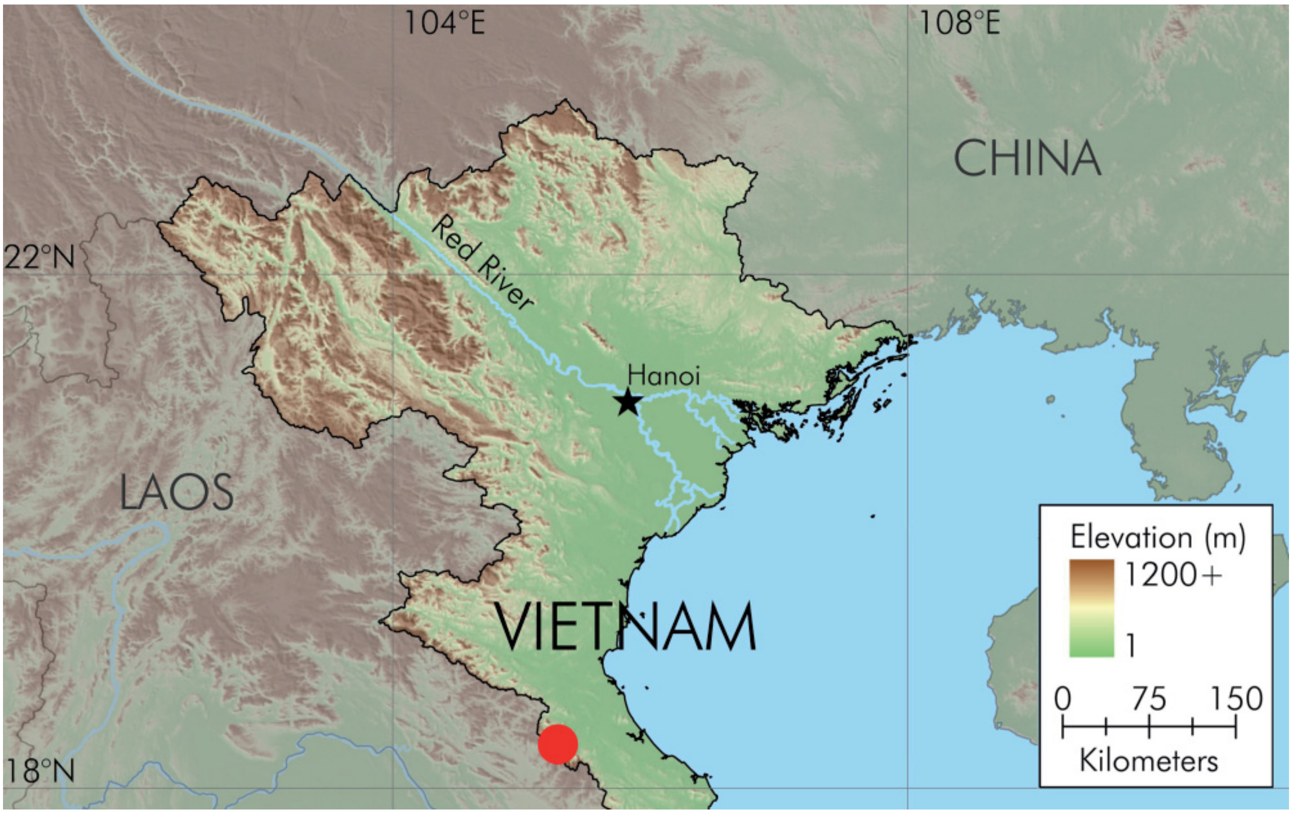

Fig. 1. Map showing the type locality of *Vietnamophryne vuquangensis* **sp. nov.** (red circle) in Vu Quang NP, Ha Tinh Province, Vietnam.

Table 1. GenBank accession numbers, and associated samples that were used in this study. All sequences generated by this study have accession numbers: MW73017-MW763029.

No.	Species	Locality	Voucher	Accession No.	Reference
1.	*Vietnamophryne vuquangensis* **sp. nov.**	Vu Quang NP, Ha Tinh Province, Vietnam	VNMN 010487 VNMN 010488 VNMN 010489 VNMN 010490 VNMN 010491 VNMN 010492 VNMN 010498 VNMN 010517 VNMN 010518 VNMN 010519 VNMN 010520 VNMN 010521 VNMN 010528	MW763017 MW763018 MW763019 MW763020 MW763021 MW763022 MW763023 MW763024 MW763025 MW763026 MW763027 MW763028 MW763029	This study
2.	*V. inexpectata*	Kon Chu Rang NR, Gia Lai Province, Vietnam	ZMMU A-5820	MH004403	Poyarkov *et al.* (2018)
3.	*V. occidentalis*	Doi Tung Mt., Chiang Rai, Thailand	ZMMU A-5822	MH004406	Poyarkov *et al.* (2018)
4.	*V. orlovi*	Phia Oac-Phia Den NP, Cao Bang Province, Vietnam	ZMMU A-5821	MH004404	Poyarkov *et al.* (2018)
	Outgroup				
5.	*Siamophryne troglodytes*	Sai Yok, Kanchanaburi, Thailand.	ZMMU NAP-06651	MG682553	Suwannapoom *et al.* (2018)

16S rRNA mitochondrial gene (457bp of 12S ribosomal RNA, 68bp of tRNA-Val, 1415bp of 16S ribosomal RNA).

Phylogenetic analysis

In addition to the newly collected series, all specimens belonging to the genus *Vietnamophryne* for which homologous sequences were available (in this publication for all 3 *Vietnamophryne* species) were included in the phylogenetic analysis. *Siamophryne troglodytes* was included in the analysis as an outgroup. Locality information and accession numbers for all sequences included in the analysis are shown in Table 1. Chromas Pro software (Technelysium Pty Ltd., Tewantin, Australia) was used to edit the sequences, which were aligned using the ClustalW (Thompson *et al.,* 1997) option in MEGA 7.0 (Kumar *et al.,* 2016) with default parameters and subsequently optimized manually in BioEdit 7.0.5.2 (Hall, 1999). We then checked the initial alignments by eye and adjusted slightly to exclude some positions of 12S rRNA and tRNA-Val. Only fragments of the 16S rRNA (707bp) gene were used for phylogenetic tree analysis. 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). 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 [burnin = 30 0010 (Nguyen *et al.,* 2014)]. We checked parameter estimations and convergence using Tracer version 1.7.1 (Rambaut *et al.,* 2018). 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 75% or greater as sufficiently resolved (Hillis & Bull, 1993; Huelsenbeck & Hillis, 1993), and nodes with a BPP of 95% or greater as significant in the BI analysis (Leaché & Reeder, 2002).

Morphological analysis

Sex of adult individuals was determined using gonadal dissection. All measurements were taken from fixed specimens using a digital caliper to the nearest 0.01 mm and subsequently rounded to a 0.1 mm precision; morphometrics followed Poyarkov *et al.* (2014), Suwannapoom *et al.* (2018) and Poyarkov *et al.* (2018): (1) snout-vent length (SVL; length from tip of snout to cloaca); (2) head length (HL; length from tip of snout to rear of jaw angle); (3) snout length (SL; length from anterior corner of eye to tip of snout); (4) eye length (EL; distance between anterior and posterior corners of eye); (5) nostril-eye length (N-EL; distance between anterior corner of eye and nostril center); (6) head width (HW;

maximum width of head at level of mouth angles in ventral view); (7) internarial distance (IND; distance between central points of nostrils); (8) interorbital distance (IOD; shortest distance between eyeballs in dorsal view); (9) upper eyelid width (UEW; maximum distance between eyeball and lateral edge of upper eyelid); (10) forelimb length (FLL; length of straightened forelimb from limb base to tip of third finger); (11) lower arm and hand length (LAL; distance between elbow and tip of third finger); (12) hand length (HAL; distance between proximal end of outer palmar (metacarpal) tubercle and tip of third finger); (13) inner palmar tubercle length (IPTL; maximum distance between proximal and distal ends of inner palmar tubercle); (14) outer palmar tubercle length (OPTL; maximum diameter of outer palmar tubercle); (15) hindlimb length (HLL; length of straightened hindlimb from groin to tip of fourth toe); (16) tibia length (TL; distance between knee and tibiotarsal articulation); (17) foot and tibiotarsus length (FTL; length from tibiotarsal joint to end of fourth toe); (18) foot length (FL; distance between distal end of tibia and tip of fourth toe); (19) inner metatarsal tubercle length (IMTL; maximum length of inner metatarsal tubercle); (20) outer metatarsal tubercle length (OMTL; maximum length of outer metatarsal tubercle); (21) tympanum length, maximum tympanum diameter (TYD); (22) tympanum-eye distance (TED); (23-26) finger lengths (1-3FLO, 4FLI; for outer side (O) of first, inner side (I) of fourth, distance between tip and junction of neighboring finger); (27) first finger width (1FW), measured at distal phalanx; (28-30) finger disk diameters (2-4FDW); (31) first toe length (1TOEL), distance between distal end of inner metatarsal tubercle and tip of first toe; (32-35) second to fifth toe lengths (outer lengths for toes II-IV, inner length for toe V); (36-40) toe disk diameters (1-5TDW).

We obtained comparative morphological data from the original descriptions of species of *Vietnamophryne*: *V. inexpectata* Poyarkov, Suwannapoom, Pawangkhanant, Aksornneam, Duong, Korost & Che, 2018; *V. occidentalis* Poyarkov, Suwannapoom, Pawangkhanant, Aksornneam, Duong, Korost & Che, 2018; *V. orlovi* Poyarkov, Suwannapoom, Pawangkhanant, Aksornneam, Duong, Korost & Che, 2018.

RESULTS

Sequence variation

Results of the phylogenetic analyses are shown in Fig. 2. The 16S rRNA gene fragments consisted of 707 sites: 650 sites were conserved and 57 sites were variable, of which 35 were parsimony-informative. The transition–transversion bias was estimated as 6.098. Nucleotide frequencies were A=34.09%, T=23.10%, C=24.45%, and G=18.36%. The Bayesian and Maximum likelihood analyses produced topologies with –ln L = 1581.393 and 1463.9769, respectively. The best model selected for BI was the general time reversible model (GTR: Tavaré, 1986) with a gamma shape parameter (G: 0.418 in BI; 0.282in ML).

Phylogenetic analyses employing BI methods, with most well-supported nodes on the Bayesian tree are presented in Fig. 2. In the analyses, the newly collected *Vietnamophryne* specimens were recovered as sister to *V. inexpectata*, which was recently described from southern Vietnam (Poyarkov *et al.,* 2018).

Interspecific uncorrected p-distances of the *Vietnamophryne* (Table 2) ranged from 1.9% (between *V. orlovi* and *V. occidentalis*) to 6.2% (between the new form and *V. orlovi*). The genetic divergence of the new form from Vu Quang NP and its congeners ranged from 4.3-4.6% (*V. inexpectata*) to 5.8-6.2% (*V. orlovi*), which was distinctly higher than genetic distances between *V. orlovi* and *V. occidentalis* (1.9%), and distances between *V. occidentalis* and *V. inexpectata* (4%). Individuals in populations of the new form have genetic differences from 0.01% to 0.03%. This is indicating that the differentiation between the new form and other species of *Vietnamophryne* has reached specific level, which was supported by morphological distinctiveness.

Vietnamophryne vuquangensis sp. nov.

Figs 3-4; Table 3

Holotype: VNMN 010489, adult male, collected in evergreen tropical forest at Vu Quang National Park, Ha Tinh Province, Vietnam (18°15’47.9”N 105°20’25.1”E at an elevation of 966 m a.s.l.) on 12 October 2019 by T. T. Nguyen and Q. H. Nguyen.

Paratypes (n=12): Three adult males (VNMN 010488, VNMN 010490, VNMN 010498), nine adult females (VNMN 010487, VNMN 010491, VNMN 010492, VNMN 010517 to VNMN 010521, VNMN 010528) same location of holotype, collected on 12 October 2019 by Q. H. Nguyen from a forest (18°15’47.9”N 105°20’25.1”E at an elevation of 966 m a.s.l.)

Diagnosis: (1) Body size small SVL (n=4 males, 14.12-14.91 mm; n=9 female, 15.15-17.33 mm); (2) body habitus stout FLL/SVL and HLL/SVL ratios 52-55% and 149-157% in males; 50-58% and 136-158% in females, respectively; (3) Head length greater than width, HW/HL ratio 117-124% in males, 110-131% in females; (4) snout comparatively long, rounded in dorsal view, snout length greater than eye length (SL/EL ratio 119-128% in males; 103-130% in females); (5) eye medium-sized, eye length/snout-vent length ratio 11% in males; 10-12% in females; eye to nostril distance 6-7% of SVL; (6) tympanum relatively blurred in males, comparatively small, rounded, TYD/SVL ratio 5.4-6.0% in males, 4.8-6.2% in females; (7) first finger (F1) well developed, less than half of second finger (F2) (1FLO/2FLO ratio 43-49% in

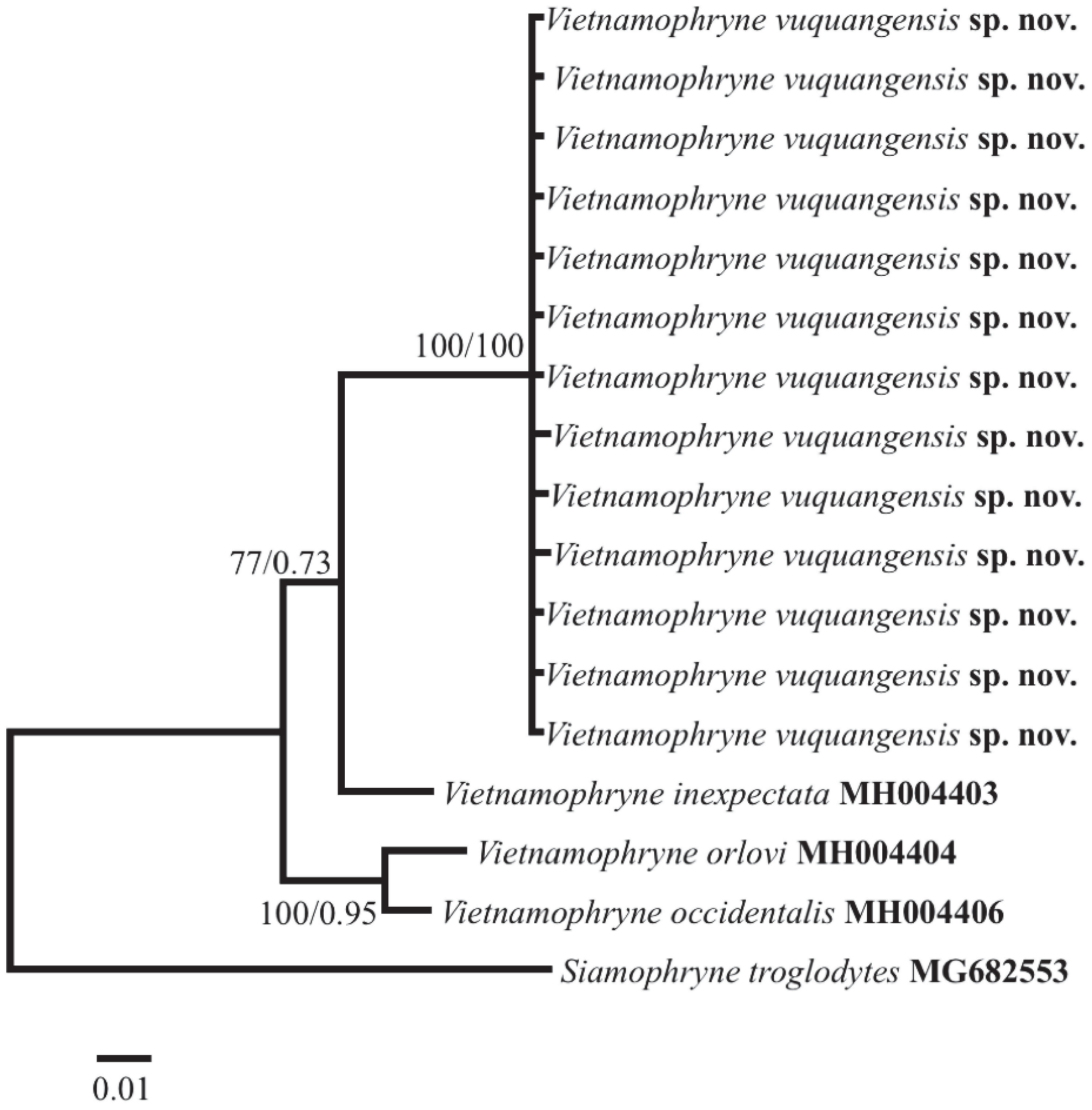

Fig. 2. Bayesian inference (BI) tree based on the partial 16S rRNA mitochondrial gene. Values at nodes correspond to BI/ML support values, respectively. Numbers above and under branches are ML bootstrap values and Bayesian posterior probabilities. *Siamophryne troglodytes* are used as the outgroup.

Table 2. Uncorrected ("p") distance matrix showing percentage pairwise genetic divergence 16S rRNA between members of the *Vietnamophryne* species group and outgroup (*Siamophryne*).

		1	2	3	4
1	*Vietnamophryne vuquangensis* **sp. nov.**	0-0.1			
2	*Vietnamophryne inexpectata*	4.3-4.6			
3	*Vietnamophryne occidentalis*	5.7-6.0	0.04		
4	*Vietnamophryne orlovi*	5.8-6.2	0.048	0.019	

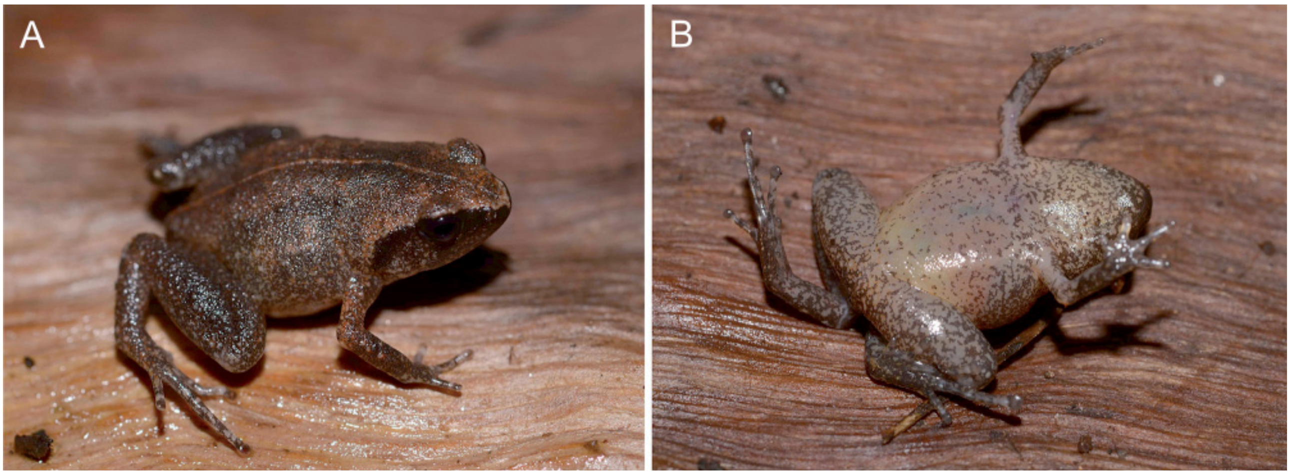

Fig. 3. Holotype (VNMN 010489, male) of *Vietnamophryne vuquangensis* **sp. nov.** in life: dorsolateral view (A) and ventral view (B).

male, 44-50% in females), relative finger lengths: I<IV<II<III, relative toe lengths: I<II<V<III<IV; (8) tips of all digits rounded, not expanded in F1-F4, T1, T2, and T5, weakly expanded in T3 and T4; (9) subarticular tubercles under fingers and toes weak, indistinct; (10) outer metatarsal tubercle absent, inner metatarsal tubercle small, flattened (3-5% of SVL); (11) skin of ventral surface completely smooth, dorsal skin shagreened with small flat pustules loosely scattered on dorsum in males, dorsal skin mostly smooth, slightly shagreened posteriorly, lacking enlarged tubercles in females; (12) belly color ivory-lemon to lemon-yellow with weak dark-brown marbling in males, bright lemon-yellow with weak dark-brown marbling in female.

Description of holotype: Body size small, habitus stout with SVL 14.91 mm an, head wider than long (HW/HL 117%), snout comparatively long, obtuse, rounded in dorsal view (Fig. 4A), truncate in lateral view (Fig. 4E), snout length greater than eye length (SL/EL ratio 128%); nostril small, rounded, lateral, much nearer to tip of snout than to eye (N-EL/EL ratio 63%); dorsal surface of head slightly convex; canthus rostralis distinct, rounded; loreal region concave; eyes medium-sized (EL/SVL ratio 11%); eye to nostril distance 7% of SVL; eyes slightly protuberant in dorsal and lateral views; pupil round; tympanum relatively faint, comparatively small, rounded, 5% of SVL; supratympanic fold present, distinct, glandular (Fig. 4E); vomerine teeth and spikes absent; tongue spatulate and free behind, papillae on tongue absent and vocal sac opening indiscernible.

Forelimbs comparatively short, around one-third of hindlimb length (FLL/HLL 35%); hand shorter than lower arm, almost one-third of forelimb length (HAL/FLL 42%); fingers short, round in cross-section, first finger well developed, almost a third of length of second finger (1FL/2FL 45%); relative finger lengths: I<IV<II<III (Fig. 4C). Finger webbing absent; dermal fringes present, weak. Tips of fingers rounded, not dilated, finger disks absent, terminal grooves absent; longitudinal furrow on dorsal surface of fingers absent; subarticular tubercles under fingers indistinct; nuptial pad absent; two palmar tubercles: inner palmar tubercle small, oval; outer palmar tubercle oval, slightly longer than inner palmar tubercle (IPTL/OPTL 78%); palmar surface smooth, supernumerary palmar tubercles absent.

Hindlimbs short and thick, tibia length less than half of snout-vent length (TL/SVL 48.0%); tibiotarsal articulation of adpressed limb reaching eye level; foot length equal to tibia length (FL/TL 99%); relative toe lengths: I<II<V<III<IV; tarsus smooth, tarsal fold absent; tips of toes rounded, tips of toes III and IV slightly dilated (Fig. 4D), terminal grooves absent; toes rounded in cross-section; toe webbing absent between all toes; dermal fringes on toes present, weak; subarticular tubercles under toes indistinct; single metatarsal tubercle: inner metatarsal tubercle rounded, flattened (IMTL/SVL ratio 5%).

Skin on anterior dorsal and dorsolateral surfaces shagreened with numerous small flat tubercles (Fig. 3A); tubercles small or pustules finely scattered and more prominent along the posterior sides of dorsum, sacral area, and dorsal surfaces of hindlimbs; dorsal surface of forelimbs smooth; upper eyelids smooth, supratympanic folds with low glandular ridges; ventral sides of trunk, head and limbs completely smooth (Fig. 3B); dorsomedial vertebral skin ridge weak, well discernible on the midline of dorsum and head.

Color of holotype in life: Dorsum reddish-gray on black tone, black markings mixed with small speckles densely scattered on dorsal surfaces of head, body, and limbs (Fig. 3A); posterior parts of dorsum and dorsal surfaces of hindlimbs with gray-brown pustules; canthus rostralis with narrow faded stripe formed by tiny flat tubercles;

Table 3. Measurements (in mm) and proportions of the type series of *Vietnamophryne vuquangensis* **sp. nov.**

Specimen ID	VNMN 010489 Holotype	VNMN 010488	VNMN 010490	VNMN 010498	VNMN 010517	VNMN 010518	VNMN 010519	VNMN 010520	VNMN 010521	VNMN 010528	VNMN 010491	VNMN 010492	VNMN 010487
Sex	M	M	M	M	F	F	F	F	F	F	F	F	F
SVL	14.91	14.12	14.85	14.12	16.48	17.33	15.64	15.49	16.69	15.72	16.67	15.15	15.51
HL	3.73	3.51	3.65	3.54	3.91	4.65	3.85	3.78	4.4	3.92	4.4	3.63	4.36
SL	2.09	1.95	1.94	1.79	2.1	2.18	1.99	1.91	2.13	1.99	2.1	2.03	1.88
EL	1.63	1.62	1.6	1.51	1.88	1.94	1.74	1.86	1.94	1.67	1.61	1.64	1.66
N-EL	1.03	0.99	1.01	0.85	1.15	1.24	1.06	1.08	1.07	1.04	1.14	1	1.07
HW	4.35	4.35	4.44	4.15	5.13	5.82	4.3	4.79	4.93	4.54	5.19	4.41	4.78
IND	1.48	1.47	1.54	1.24	1.69	1.74	1.51	1.62	1.53	1.5	1.59	1.49	1.51
IOD	1.87	1.97	2.16	1.71	2.06	2.13	1.93	1.8	1.9	1.84	1.89	1.84	1.91
UEW	0.92	0.85	0.91	0.8	1.11	1.34	0.91	0.91	0.93	0.85	0.88	0.78	0.79
FLL	8	7.66	7.73	7.79	8.83	8.71	8.86	8.54	8.99	8.61	8.5	7.91	8.99
LAL	6.2	5.67	5.77	5.75	6.41	6.29	6.64	6.22	6.87	6.28	6.47	6.02	6.24
HAL	3.34	3.03	3.01	3.16	3.66	3.52	3.52	3.1	3.67	3.24	3.29	2.98	3.53
IPTL	0.25	0.2	0.25	0.48	0.58	0.59	0.57	0.49	0.52	0.51	0.5	0.48	0.49
OPTL	0.32	0.25	0.31	0.56	0.62	0.67	0.63	0.59	0.61	0.65	0.62	0.56	0.59
HLL	22.84	20.99	22.43	22.2	24.72	23.53	24.71	24.23	25.39	24.07	24.31	23.74	23.29
TL	7.11	6.68	6.85	6.79	7.41	7.33	7.61	7.49	7.52	7.23	7.4	7.06	7.15
FTL	10.44	9.66	10.21	9.94	11.59	10.93	11.44	11.32	11.93	10.45	10.99	10.57	11
FL	7.06	5.87	6.59	6.47	7.69	7.15	7.14	7.39	8	6.86	6.88	6.76	7.57
IMTL	0.68	0.58	0.64	0.45	0.75	0.56	0.72	0.59	0.62	0.58	0.58	0.47	0.59
TYD	0.87	0.85	0.86	0.76	0.92	1.05	0.89	0.76	0.99	0.83	0.81	0.82	0.83
TED	0.62	0.64	0.68	0.59	0.64	0.74	0.67	0.65	0.64	0.68	0.55	0.68	0.71
1FLO	0.59	0.57	0.58	0.58	0.61	0.53	0.59	0.56	0.65	0.69	0.63	0.57	0.6
2FLO	1.31	1.17	1.28	1.36	1.31	1.11	1.32	1.18	1.38	1.41	1.34	1.14	1.35
3FLO	1.99	1.85	1.89	1.98	1.98	1.61	1.95	1.78	2.01	2.19	1.96	1.74	1.99
4FLI	1.29	1.09	1.18	1.13	1.04	1.06	1.07	1.04	1.26	1.24	1.06	1.09	1.16
1FW	0.22	0.16	0.22	0.2	0.22	0.27	0.22	0.22	0.22	0.23	0.27	0.18	0.22
2FDW	0.28	0.23	0.25	0.24	0.31	0.38	0.28	0.28	0.31	0.29	0.33	0.26	0.29
3FDW	0.3	0.26	0.3	0.31	0.39	0.41	0.39	0.38	0.36	0.31	0.36	0.3	0.3
4FDW	0.27	0.23	0.28	0.26	0.32	0.35	0.26	0.25	0.33	0.28	0.28	0.28	0.27
1TOEL	0.59	0.34	0.52	0.33	0.25	0.28	0.3	0.35	0.38	5	0.23	0.3	0.33
2TOEL	1.45	1.01	1.25	1.1	1.21	1.16	1.47	1.39	1.42	1.48	1.38	1.05	1.15
3TOEL	2.74	2.42	2.58	2.36	2.53	2.35	2.93	2.51	2.72	2.8	2.87	2.24	2.16
4TOEL	4.07	3.84	4.01	4.39	4.22	3.92	4.14	4.03	4.59	4.37	3.95	3.89	3.92
5TOEL	2.08	1.64	1.58	1.63	1.82	1.7	1.55	1.91	1.79	1.54	1.44	1.71	1.46
1TDW	0.25	0.25	0.25	0.3	0.23	0.26	0.19	0.2	0.4	0.22	0.18	0.23	0.21
2TDW	0.36	0.31	0.33	0.38	0.34	0.4	0.33	0.39	0.52	0.31	0.31	0.43	0.33
3TDW	0.39	0.38	0.39	0.43	0.43	0.43	0.35	0.45	0.56	0.4	0.48	0.55	0.38
4TDW	0.43	0.41	0.43	0.49	0.57	0.53	0.44	0.57	0.69	0.43	0.47	0.55	0.4
5TDW	0.38	0.36	0.38	0.35	0.4	0.38	0.33	0.35	0.34	0.35	0.33	0.44	0.37

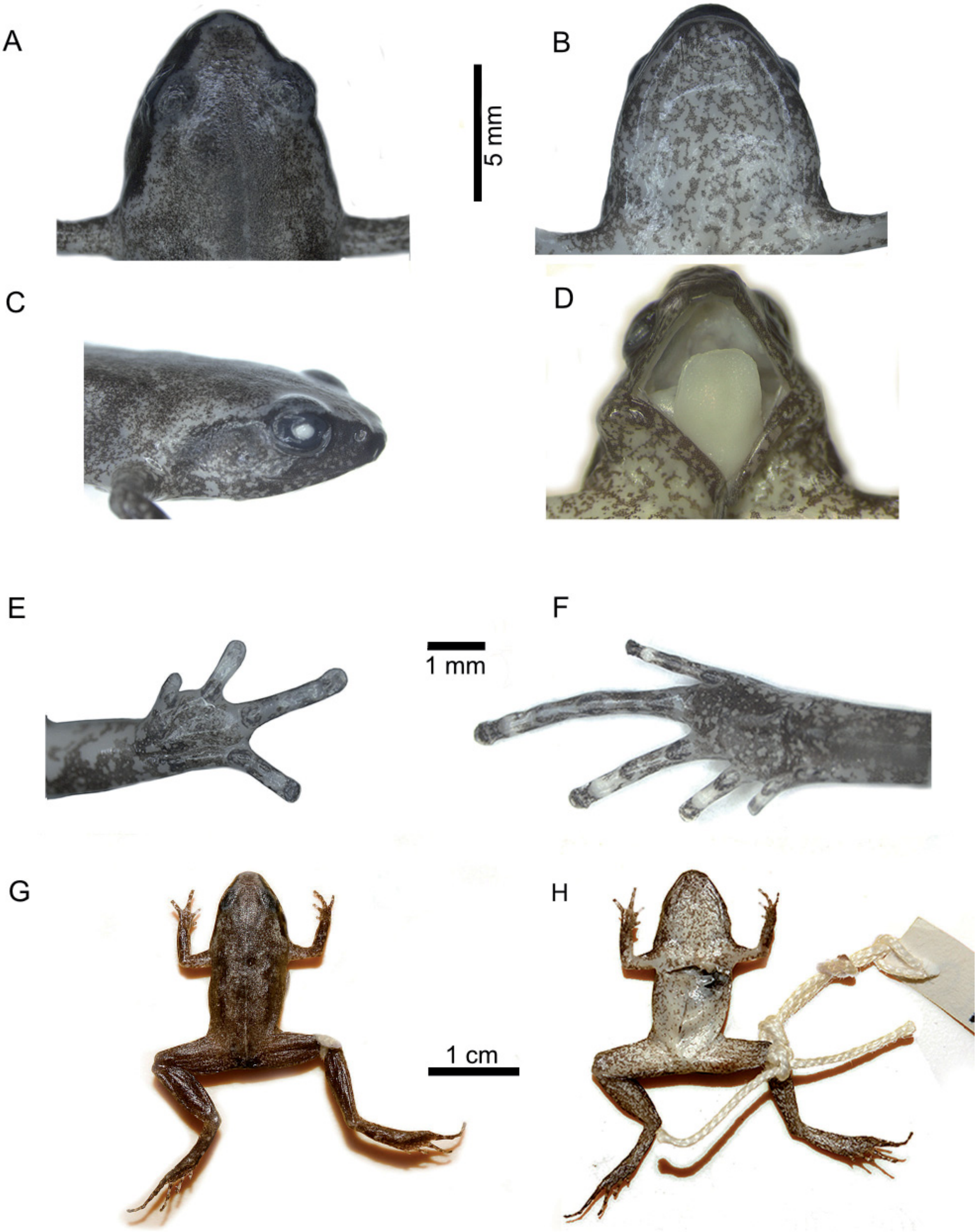

Fig. 4. Dorsolateral view of head (A), ventral view of head (B), lateral view of head (C), tongue (D), left hand (E), left foot (F), dorsolateral view (G) and ventral view (H) of the holotype (VNMN 010489) of *Vietnamophryne vuquangensis* **sp. nov.** in preservative.

loreal region dark with whitish mottling on lower jaw and mouth corners from snout tip toward eye along canthus rostralis (Fig. 3A); lateral sides of head dark brown with whitish mottling on upper jaw and mouth corners; white mid-dorsal line present, from near tip of snout to sacral area; dorsal surfaces of forearms orange-brown, interspersed with grey-brown; dorsal surfaces of hindlimbs reddish-brown with rare dark-brown tubercles and pustules; loreal region dark brown with whitish mottling; supratympanic fold with brick-red glandular tubercles with some whitish pustules; ventrally ivory-lemon with weak dark-brown marbling, more scarce on belly, denser on chest, throat, and ventral surfaces of limbs; fingers and toes dorsally gray-brown with indistinct reddish blotches, ventrally gray-brown with irregular beige or yellowish blotches (Fig. 3B). Pupil round, black, iris uniform dark brown (Fig. 3A)

Color of holotype in preservative: Dorsal surface changes to grayish-brown with white-gray spots made of rare dark-brown tubercles and collapsed pustules; underside of limbs, chin, and vent area grayish-white with denser dark-gray marbling. Underside of throat, chest and anterior portion of thighs white with scarce marbling (Fig. 4A, B).

Variation: The females have larger body sizes compared with males; dorsum reddish-gray on black ground in males and brownish-gray in females; tympanum distinct in females, relatively faint in males. The mid-dorsal line is well discernible in specimens VNMN 010492 and VNMN 010521, from near tip of snout to sacral area; mid-dorsal line absent in specimens VNMN 010487, VNMN 010491, VNMN 010517 to VNMN 010520, and VNMN 010528. For morphological variation see Table 1, and for color pattern variation see Fig. 5.

Etymology: The specific epithet is in reference to the type locality (Vu Quang National Park). We recommend "Vu Quang Dwarf Frog" as the common English name of the new species and the common name in Vietnamese as "Nhái lùn vũ quang".

Distribution and ecology: *Vietnamophryne vuquangensis* **sp. nov.** is currently known only from the type locality, Vu Quang NP, Vu Quang District, Ha Tinh Province. Specimens of the new species were found on the soil or under large tree-trunks and under leaf litter in evergreen tropical forests of Vu Quang NP at an elevation of 966 m a.s.l. (Fig. 6). The air temperature at the time of the finding was not recorded, but was 27°C at 19:40h. Other species of amphibians were found at night in the same habitat comprising *Microhyla mukhlesuri* Boulenger, 1884, *M.* cf. *heymonsi* Vogt, 1911, *Leptobrachium* cf. *chapaensis* (Bourret, 1937) and *Polypedates mutus* (Smith, 1940).

Comparisons: *Vietnamophryne vuquangensis* **sp. nov**. differs from *V. inexpectata*, *V. orlovi* and *V. occidentalis* by body size, dorsal skin, belly color, tympanum diameter, ratio snout length to eye length, ratio head width to head length and ratio first finger length to second finger (for details see Table 4). *Vietnamophryne vuquangensis* **sp. nov.,** having small body size, with SVL 14.12-14.91 mm in males (n=4), SVL 15.15-17.33 mm in females (n=9), can be distinguished based on size from the larger *V. orlovi*, with medium sized body, SVL 15.40 mm in the single known male individual, and *V. occidentalis*, with large sized body, SVL 20.5 mm in the single known male individual. The single known male individual of *V. inexpectata* has SVL of 14.2 mm. *Vietnamophryne vuquangensis* **sp. nov.** has warty skin in the dorsolateral fold area and is shagreened posteriorly with numerous small flat tubercles on the anterior dorsum (vs. warty skin on posterior and shagreened skin on anterior dorsum in *V. inexpectata*, vs. mostly smooth skin, slightly shagreened posteriorly, lacking enlarged tubercles in *V. orlovi*, and vs. mostly smooth skin with rare flat tubercles in *V. occidentalis*). *Vietnamophryne vuquangensis* **sp. nov.** ventrally is ivory-lemon to lemon-yellow with weak dark-brow marbling (vs. grayish-beige ventral coloration with gray marbling in *V. inexpectata*, vs. lemon-yellow belly with dark brown marbling in *V. orlovi*, and vs. bright orange-red belly with sparse dark brown marbling in *V. occidentalis*). *Vietnamophryne vuquangensis* **sp. nov.** has the F1 well developed, one-third of length of second finger (1FLO/2FLO 33-38%, 0.27-0.48 mm) and thus can be further distinguished from *V. inexpectata* having the F1 reduced to nub (1FL/2FL 29.6%). In *V. orlovi* it is well developed, half of length of second finger, (1FLO/2FLO 50%), and in *V. occidentalis* it is also well developed, less than half of second finger (1FLO/2FLO 42%). *Vietnamophryne vuquangensis* **sp. nov.** has the head length notably shorter than wide (HW/HL 117-124%, 110-131%) and thus can be further distinguished from *V. inexpectata* and *V. occidentalis* which have head length almost equal to head width, (HW/HL 101.1%) and (HW/HL 99.0%), and from *V. orlovi*, having head length longer than wide (HW/HL 86.5%). *Vietnamophryne vuquangensis* **sp. nov.** has the snout length longer than eye diameter (SL/EL 119-128%) and can be further distinguished from *V. inexpectata*, having snout length subequal to eye length, (SL/EL 96.8%), from *V. occidentalis*, having snout length shorter than eye diameter (SL/EL 85.5%) and *V. orlovi*, having snout length notably longer than eye diameter (SL/EL 139%). In having an in part deficient or faint, indistinct tympanum *Vietnamophryne vuquangensis* **sp. nov.** differs from *V. inexpectata* (TYD/EL 58%) and *V. orlovi* (TYD/EL 50%), having slightly larger tympanum, and from *V. occidentalis* (TYD/EL 40%), having slightly smaller tympanum. *Vietnamophryne vuquangensis* **sp. nov.** having eye to nostril distance twice as long as eye length (N-EL/EL 56-63%), and thus differs from

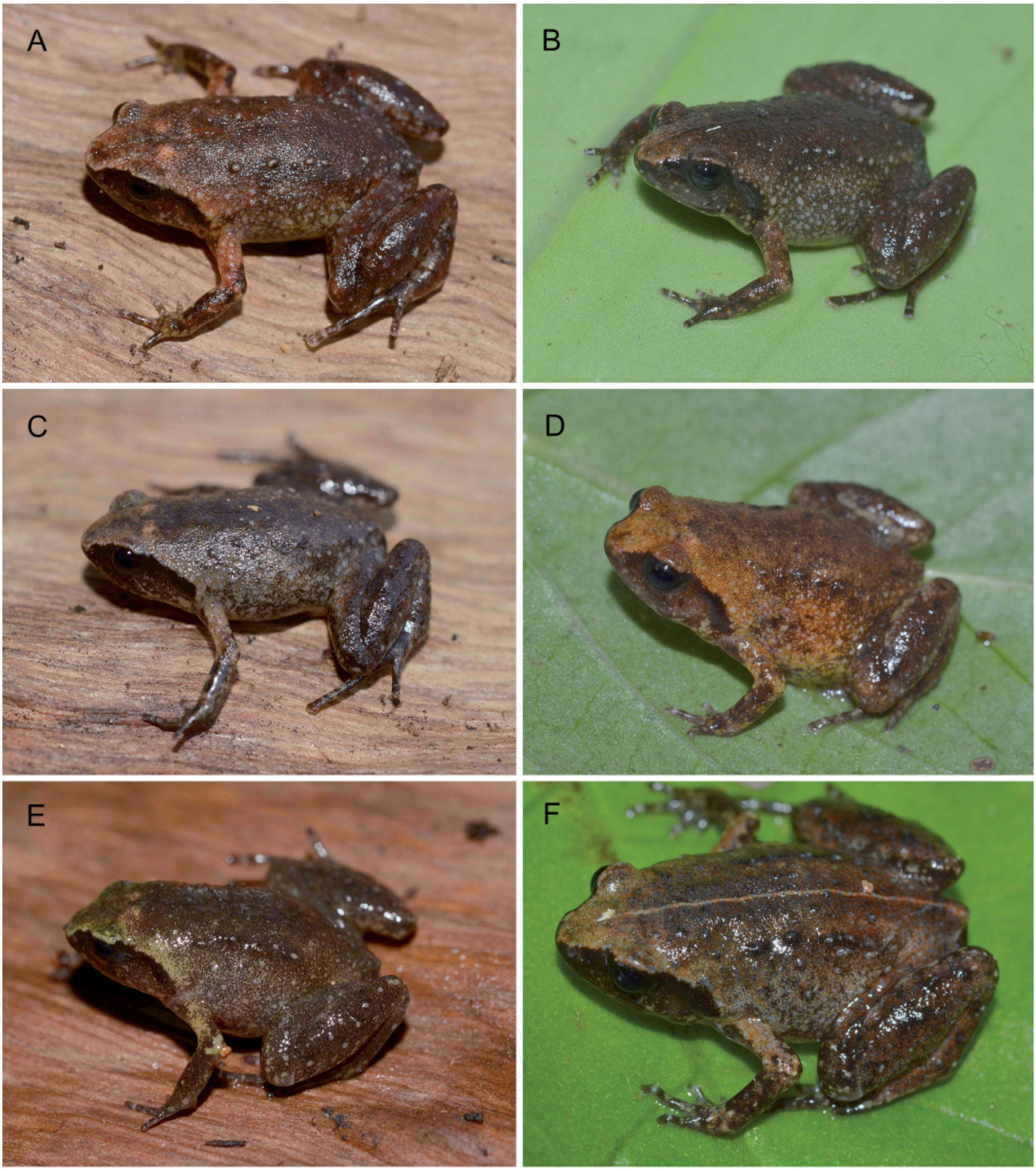

Fig. 5. Paratypes of *Vietnamophryne vuquangensis* **sp. nov**. (A) VNMN 010487, (B) VNMN 010491, (C) VNMN 010519, (D) VNMN 010518, (E) VNMN 010490, (F) VNMN 010521.

Table 4. Selected diagnostic characters for the species in the genus *Vietnamophryne*.

Species	*Vietnamophryne vuquangensis* **sp. nov.**		*V. inexpectata*	*V. orlovi*	*V. occidentalis*
	Male	Females	Male	Male	Male
Body size	14.12-14.91 mm	15.15-17.33 mm	14.2 mm	15.40 mm	20.5 mm
Skin of dorsum	Dorsal skin shagreened with small flat pustules loosely scattered on dorsum	Dorsal skin mostly smooth, slightly shagreened posteriorly, lacking enlarged tubercles	Dorsal skin shagreened anteriorly, distinctly warty posteriorly with large flat tubercles or pustules finely scattered on posterior dorsum	Dorsal skin mostly smooth skin, slightly shagreened posteriorly, lacking enlarged tubercles	Dorsal skin smooth, posteriorly with loosely scattered small flat tubercles present on surface of posterior dorsum
Belly color	ivory-lemon to lemon-yellow with weak dark-brown marbling	bright lemon-yellow with weak dark-brown marbling	grayish-beige with gray marbling	lemon-yellow belly with dark brown marbling	bright orange-red belly with sparse dark brown marbling
Tympanum	tympanum relatively faint, comparatively small, rounded, 5.4–6.0% of SVL	tympanum comparatively small, rounded, 4.8–6.2% of SVL	tympanum comparatively large, rounded, 7.9% of SVL	tympanum comparatively small, rounded, 5.5% of SVL	tympanum comparatively small, rounded, 5.0% of SVL
Snout length	snout comparatively long, rounded in dorsal and lateral views, snout length greater than eye length (SL/EL 119–128%)	snout comparatively long, rounded in dorsal and lateral views, snout length greater than eye length (SL/EL 100–130%)	snout short, obtuse in dorsal view, rounded in lateral view, subequal to eye length (96.8% of eye length)	snout comparatively long, rounded in dorsal and lateral views, snout length greater than eye length (SL/EL ratio 141.3%)	snout short, obtuse in dorsal view, rounded in lateral view, shorter than eye length (85.5% of eye length)
Head	Head wider than long, HW/HL ratio 117–124%	Head wider than long, HW/HL ratio 110–131%	head as long as wide, HW/HL ratio 101.1%	Head longer than wide, HW/HL ratio 86.5%	head as long as wide, HW/HL ratio 99.0%
Fist finger	Well developed, less than half of second finger (1FLO/2FLO 43-49%)	well developed, less than half of second finger (1FLO/2FLO 44-50%)	Reduced to nub, less than one-third of second finger (1FLO/2FLO 30%)	well developed, half of length of second finger (1FLO/2FLO 50%)	well developed, less than half of second finger (1FLO/2FLO 42%)

Fig. 6. Habitat of *Vietnamophryne vuquangensis* **sp. nov.** at the type locality in Vu Quang National Park, Ha Tinh Province, Vietnam.

V. orlovi by eye to nostril distance almost equal to eye length (N-EL/EL 111%). Eye to nostril distance in *V. inexpectata* (N-EL/EL 48.1%) and *V. occidentalis* (N-EL/EL 56%) is equivalent.

DISCUSSION

This study adds another new species to the only recently erected microhylid anuran genus *Vietnamophryne*. This highlights unpredicted species richness in *Vietnamophryne* and that probably further species will be discovered in other areas of Indochina, including central and northern Vietnam, Laos and northern Myanmar (see also Poyarkov *et al.,* 2018). Diversification within the genus *Vietnamophryne* was, most likely, a comparatively recent event due to the in part low genetic distances observed among species. Interspecific uncorrected p-distances of the *Vietnamophryne* species group were 3.1-5.1 in Poyarkov *et al.* (2018). We could extend the range from 1.9% (*V. occidentalis* vs. *V. orlovi*) to 5.8-6.2% (*V. orlovi* vs. *V. vuquangensis* **sp. nov.**) (Table 2). The new species of *Vietnamophryne* from Vu Quang NP differed clearly from all other group members in 16S rRNA sequences and it was most closely related genealogically to *V. inexpectata* from Kon Chu Rang NR (4.3-4.6%) This value was also distinctly higher than distances between *V. orlovi* and *V. occidentalis* (1.9%), as well as between *V. occidentalis* and *V. inexpectata* (4%). There is certainly more research required to have a better understanding of the genus *Vietnamophryne* and its interspecific distances and intraspecific variation range.

The species of the genus *Vietnamophryne* are tiny, little-known frogs, with narrow range and habitats in the evergreen forests. Therefore, even the loss of a small part of the habitat can lead to a serious decline among *Vietnamophryne vuquangensis* representatives. Given the small distribution range known for *V. vuquangensis*, we propose an IUCN Red List status of Endangered.

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REFERENCES

Frost D. R. 2020. Amphibian Species of the World: An Online Reference. Version 6.0. Electronic Database accessible. Available from: http://research.amnh.org/herpetology/amphibia/index.html (accessed 28 February 2020).

Hall T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium* Series, 41 (1999): 95-98.

Hillis D.M & Bull J.J. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* 42: 182-192.

Huelsenbeck J.P. & Hillis D.M. 1993. Success of Phylogenetic Methods in the Four-Taxon Case. *Systematic Biology*, 42(3): 247-264.

Kumar S., Stecher G. & Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33(7): 1870-1874.

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.

Nguyen T. T., Matsui M., Eto K. & Orlov N.L. 2014. A preliminary study of phylogenetic relationships and taxonomic problems of Vietnamese *Rhacophorus* (Anura: Rhacophoridae). *Russian Journal of Herpetology* 21(4): 274-280.

Poyarkov J. N. A., Vassilieva A. B., Orlov N. L., Galoyan E. A., Tran D., Le D. T. T., Kretova V. D. & Geissler P. 2014. Taxonomy and distribution of narrow-mouth frogs of the genus *Microhyla* Tschudi, 1838 (Anura: Microhylidae) from Vietnam with descriptions of five new species. *Russian Journal of Herpetology* 21(2): 89-148.

Poyarkov N. A., Suwannapoom C., Pawangkhanant P., Aksornneam A., Duong V. T., Korost D. V. & Che J. 2018. A new genus and three new species of miniaturized microhylid frogs from Indochina (Amphibia: Anura: Microhylidae: Asterophryinae). *Zoological Research* 38(3): 1-26.

Rambaut A., Drummond A.J., Xie D., Baele G. & Suchard M.A. 2018. Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7. *Systematic Biology* 67(5): 901-904.

Simmons J. E. 2002. Herpetological collecting and collections management. Revised edition. *Society for the Study of Amphibians and Reptiles, Herpetological Circular* 31: 1-153.

Suwannapoom C., Sumontha M., Tunprasert J., Ruangsuwan T., Pawangkhanant P., Korost D. V. & Poyarkov N. A. 2018. A striking new genus and species of cave-dwelling frog (Amphibia: Anura: Microhylidae: Asterophryinae) from Thailand. *PeerJ 6:*e4422. DOI: 10.7717/peerj.4422

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.

Tavaré S. 1986. Some probabilistic and statistical problems in the analysis of DNA sequences. *Lectures on Mathematics in the Life Sciences* 17: 57-86.

Thompson J. D., Gibson T. J., Plewniak F., Jeanmougin F. & Higgins D. G. 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic acids research* 25(24): 4876-4882.