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More dwarfs: a new microendemic species of *Vietnamophryne* (Anura, Microhylidae) from limestone karst of Quang Tri Province, Central Vietnam

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Abstract

A new species of the genus *Vietnamophryne* is described from limestone karst forests of Quang Tri Province, central Vietnam, based on two adult male specimens and integrative analyses of morphology and mitochondrial DNA. Morphologically, *Vietnamophryne abscondita* **sp**. **nov**. is distinguished from other congeners by the following combination of characters: (1) medium body size (snout-vent length 18.1–19.0 mm in males, $n = 2$); (2) head slightly wider than long; (3) snout short, rounded in dorsal view; (4) tympanum indistinct; (5) dorsal skin finely shagreened, lacking enlarged tubercles or a median dorsal ridge; (6) Finger I longer than half of Finger II; (7) toes without webbing, bearing rounded terminal discs; (8) forelimbs and hindlimbs slender; (9) dorsum dark brown to chocolate brown, with irregular lighter mottling; (10) flanks and limbs densely speckled with pale spots; (11) ventral surface bright yellow-orange to orange on chest and belly, with dark marbling on throat and ventral surfaces of limbs; (12) lower jaw region with scattered pale blotches; and (13) indistinct pale yellow suborbital spotting extending from the eye toward the mouth corner. Phylogenetic analyses based on the long mtDNA fragment recover *Vietnamophryne abscondita* **sp**. **nov**. as a well-supported independent lineage, showing uncorrected *p*-distances exceeding 3% from all currently recognized congeners of *Vietnamophryne*, based on the 16S rRNA gene fragment. Although shallow genetic divergences and limited sampling complicate species boundaries within the genus, further taxonomic changes are not proposed, and current species limits are considered provisional pending comprehensive integrative revision. The discovery of this species, together with a closely related undescribed lineage from the same province, highlights the underestimated diversity of *Vietnamophryne* and emphasizes the Annamites (Truong Son Mountains) as a priority region for future herpetological surveys.

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Key Words

Annamites, biogeography, Indochina, molecular phylogeny, morphology, systematics, Truong Son Mountains, *Vietnamophryne abscondita* sp. nov.

Introduction

Frogs of the family Microhylidae Günther, 1858 represent one of the most diverse amphibian lineages, with a pantropical distribution and high levels of morphological and ecological specialization (e.g., Poyarkov et al. 2018a, 2020, 2021a; Trofimets et al. 2024; Gorin et al. 2024). Within this family, the subfamily Asterophryinae Günther, 1858, is predominantly Australasian but includes several relictual lineages in mainland Southeast Asia, reflecting a complex biogeographic history (Suwannapoom et al. 2018). The genus *Vietnamophryne* Poyarkov, Suwannapoom, Pawangkhanant, Aksornneam, Duong, Korost & Che, 2018, was recently established to accommodate a group of miniaturized, semi-fossorial frogs from Indochina (Poyarkov et al. 2018a). To date, the genus comprises six species known primarily from montane evergreen forests and limestone karst habitats across northern Indochina (northern and central Vietnam, northern Thailand, and northern Laos) and adjacent southern Yunnan, China, most of which are known from extremely limited material: *Vietnamophryne aurantifusca* Ninh, Le, Bui, Nguyen, Orlov, Bezman-Moseyko, Le, Nguyen, Hoang, Ziegler & Nguyen, 2023; *V. cuongi* Nguyen, Hoang, Jiang, Orlov, Ninh, Nguyen, Nguyen & Ziegler, 2021; *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; and *V. vuquangensis* Hoang, Jiang, Nguyen, Orlov, Le, Nguyen, Nguyen, Nguyen, Nguyen & Ziegler, 2021 (Poyarkov et al. 2018a; Hoang et al. 2021; Nguyen et al. 2021; Wu et al. 2022; Ninh et al. 2023; Stuart et al. 2024).

Recent herpetological surveys in Vietnam have revealed that the Central Annamites (Truong Son Mountains) represent an important center of amphibian and reptile diversity, characterized by high levels of micro-endemism and ongoing species discovery (e.g., Poyarkov et al. 2019, 2021b, 2022, 2023, 2025; Grismer et al. 2019; Nguyen and Vogel 2025; Vogel et al. 2025; Korolev et al. 2026). This region encompasses a heterogeneous landscape of limestone karst formations, montane evergreen forests, and lowland refugia, providing a wide range of microhabitats that promote localized diversification (Sterling et al. 2006; Poyarkov et al. 2021a, 2023). Despite increasing research efforts, large portions of this area remain poorly surveyed, and additional cryptic diversity is expected.

During recent fieldwork in limestone karst forests in the northern part of Quang Tri Province, central Vietnam (Fig. 1), two unidentified microhylid frogs were collected that were tentatively assigned to the genus *Vietnamophryne* based on a suite of externally observable characters consistent with the generic diagnosis: small body size (snout-vent length < 21 mm), rounded snout, round pupil, dorsal skin warty to finely shagreened, first finger shortened, absence of toe webbing, rounded digit tips lacking expanded discs, and absence of maxillary teeth. The specimens were found in leaf litter and soil microhabitats, consistent with the semi-fossorial lifestyle reported for congeners (see Poyarkov et al. 2018a). Subsequent detailed morphological examination revealed a combination of characters that clearly distinguish these specimens from all currently described species of *Vietnamophryne*. Molecular analyses of mitochondrial DNA (mtDNA) sequences further demonstrated that they represent a well-supported, genetically distinct lineage within the genus. Based on this integrative evidence, the specimens are concluded to represent a previously undescribed species of *Vietnamophryne*, which is formally described herein.

Material and methods

Sampling

Fieldwork was conducted in Quang Tri Province, central Vietnam, by D.T. Nguyen, T.V. Duong, and N.A. Poyarkov in May 2022 (Fig. 1). Geographic coordinates and elevation were obtained using a Garmin GPSMAP 60CSx (USA) and recorded in the WGS84 datum. The specimens were photographed in life and then euthanized using 20% benzocaine prior to fixation in 96% ethanol and subsequent storage in 70% ethanol. Tissue samples for genetic analysis were taken prior to preservation and stored in 95% ethanol. Specimens and tissues were subsequently deposited in the herpetological collections of the Joint Vietnam-Russia Tropical Science and Technology Research Center (VRTC), Hanoi, Vietnam, and the Zoological Museum of Moscow State University (ZMMU, Moscow, Russia).

DNA extraction and mtDNA fragment sequencing

Total genomic DNA was extracted from ethanol-preserved femoral muscle tissue using standard phenol-chloroform proteinase K (final concentration

Figure 1. Distribution of the genus *Vietnamophryne* in Indochina. The dot in the center of a symbol marks the type locality of a species; colors of symbols correspond to those used in Fig. 2. Localities: *V. inexpectata*: 1—Kon Chu Rang NR, Gia Lai, Vietnam (Poyarkov et al. 2018); *Vietnamophryne* sp.: 2—Bac Huong Hoa NR, Quang Tri, Vietnam (ITBCZ 5406); *V. abscondita* sp. nov.: 3—Kim Dien, Quang Tri, Vietnam (this study); *V. vuquangensis*: 4—Vu Quang NP, Ha Tinh, Vietnam (Hoang et al. 2021); *V. cuongi*: 5—Ba Vi NP, Ha Noi, Vietnam (Nguyen et al. 2021); *V. aurantifusca*: 6—Na Hang NR, Tuyen Quang, Vietnam (Ninh et al. 2023); *V. orlovi*: 7—Phia Oac–Phia Den NP, Cao Bang, Vietnam (Poyarkov et al. 2018); 8—Phu Luong NR, Thanh Hoa, Vietnam (Dau et al. 2025); 9—Pingbian Daweishan NP, Yunnan, China (Wu et al. 2022); *V. occidentalis*: 10—Mt. Doi Tung, Chiang Rai, Thailand (Poyarkov et al. 2018); *Vietnamophryne* sp.: 11—Hiem, Houaphanh, Laos (Stuart et al. 2024).

1 mg/mL) extraction with subsequent isopropanol precipitation (as per Hillis et al. 1996). The isolated DNA was visualized using agarose electrophoresis in the presence of ethidium bromide. The resulting DNA concentration in 1 µL was measured using a NanoDrop 2000 (Thermo Scientific, USA) and consequently adjusted to 100 ng DNA/µL. mtDNA fragments were amplified, covering partial sequences of the 12S rRNA and 16S rRNA mtDNA genes and the complete sequence of the tRNAVal mtDNA gene, to obtain a 2,591-bp-long continuous fragment of mtDNA. These mtDNA markers have been used for comprehensive phylogenetic studies on Microhylidae frogs (De Sá et al. 2012; Matsui et al. 2011; Peloso et al. 2016; Pyron and Wiens 2011; Van Der Meijden et al. 2007; Poyarkov et al. 2018a), including molecular taxonomic research on the subfamily Asterophryinae (Blackburn et al. 2013; Frost et al. 2006; Köhler and Günther 2008; Kurabayashi et al. 2011; Oliver et al. 2013; Rittmeyer et al. 2012; Suwannapoom et al. 2018). PCR was performed in 20 µL reactions using 50 ng of genomic DNA, 10 nmol of each primer, 15 nmol of each dNTP, 50 nmol of additional $MgCl_2$, Taq PCR buffer (10 mmol/L of Tris-HCl, pH 8.3, 50 mmol/L of KCl, 1.1 mmol/L of $MgCl_2$, and 0.01% gelatin), and 1 U of Taq DNA polymerase. The PCR conditions, as well as primers used for PCR procedures and sequencing, followed Suwannapoom et al. (2018). The PCR products were loaded onto 1.5% agarose gels in the presence of ethidium bromide. Visualization was carried out using agarose electrophoresis. If distinct bands were obtained, products were purified prior to cycle sequencing using 2 µL of ExoSapIt (Amersham, UK), diluted at a 1:4 ratio, per 5 µL of PCR product. The 10 µL sequencing reaction included 2 µL of template, 2.5 µL of sequencing buffer, 0.8 µL of 10 pmol primer, 0.4 µL of BigDye Terminator v3.1 Sequencing Standard (Applied Biosystems, USA), and 4.2 µL of water. The cycle sequencing reaction included 35 cycles with the following steps: 10 s at 96 °C, 10 s at 50 °C, and 4 min at 60 °C. Cycle sequencing products were then purified by ethanol precipitation. Sequencing was performed on an ABI 3730xl automated sequencer (Applied Biosystems, USA). The obtained sequences were deposited in GenBank under accession numbers PZ134128–PZ134130 (Table 1).

Table 1. Samples of *Vietnamophryne* and other Microhylidae species used for molecular analysis in this study.

Species	Locality	Specimen voucher	Accession No.	Reference
Vietnamophryne abscondita sp. nov.	Kim Dien, Quang Tri, Vietnam	VRTC NAP-12097	PZ134128	This study
Vietnamophryne abscondita sp. nov.	Kim Dien, Quang Tri, Vietnam	ZMMU A-8446	PZ134129	This study
Vietnamophryne cuongi	Ba Vi NP, Hanoi, Vietnam	VNMN 010357	MT819964	Nguyen et al. (2021)
Vietnamophryne cuongi	Ba Vi NP, Hanoi, Vietnam	VNMN 010359	MT819967	Nguyen et al. (2021)
Vietnamophryne cuongi	Ba Vi NP, Hanoi, Vietnam	VNMN 010358	MT819968	Nguyen et al. (2021)
Vietnamophryne inexpectata	Kon Chu Rang NR, Gia Lai, Vietnam	ZMMU A-5820	MH004403	Poyarkov et al. (2018a)
Vietnamophryne occidentalis	Mt. Doi Tung, Chiang Rai, Thailand	ZMMU A-5822	MH004406	Poyarkov et al. (2018a)
Vietnamophryne occidentalis	Mt. Doi Tung, Chiang Rai, Thailand	ZMMU NAP-12814	PZ134130	This study
Vietnamophryne orlovi	Phia Oac-Phia Den NP, Cao Bang, Vietnam	ZMMU A-5821	MH004404	Poyarkov et al. (2018a)
Vietnamophryne orlovi	Phia Oac-Phia Den NP, Cao Bang, Vietnam	VNMN 011309	MW837544	Nguyen et al. (2021)
Vietnamophryne orlovi	Pingbian Daweishan NP, Yunnan, China	Yuan13749	OK377338	Wu et al. (2022)
Vietnamophryne sp.	Bac Huong Hoa NR, Quang Tri, Vietnam	ITBCZ 5406	MK239028	Unpublished
Vietnamophryne vuquangensis	Vu Quang NP, Ha Tinh, Vietnam	VNMN 010487	MW763017	Hoang et al. (2021)
Vietnamophryne vuquangensis	Vu Quang NP, Ha Tinh, Vietnam	VNMN 010488	MW763018	Hoang et al. (2021)
Vietnamophryne vuquangensis	Vu Quang NP, Ha Tinh, Vietnam	VNMN 010489	MW763019	Hoang et al. (2021)
Vietnamophryne vuquangensis	Vu Quang NP, Ha Tinh, Vietnam	VNMN 010490	MW763020	Hoang et al. (2021)
Vietnamophryne vuquangensis	Vu Quang NP, Ha Tinh, Vietnam	VNMN 010491	MW763021	Hoang et al. (2021)
Vietnamophryne vuquangensis	Vu Quang NP, Ha Tinh, Vietnam	VNMN 010492	MW763022	Hoang et al. (2021)
Vietnamophryne vuquangensis	Vu Quang NP, Ha Tinh, Vietnam	VNMN 010498	MW763023	Hoang et al. (2021)
Vietnamophryne vuquangensis	Vu Quang NP, Ha Tinh, Vietnam	VNMN 010517	MW763024	Hoang et al. (2021)
Vietnamophryne vuquangensis	Vu Quang NP, Ha Tinh, Vietnam	VNMN 010518	MW763025	Hoang et al. (2021)
Vietnamophryne vuquangensis	Vu Quang NP, Ha Tinh, Vietnam	VNMN 010519	MW763026	Hoang et al. (2021)
Vietnamophryne vuquangensis	Vu Quang NP, Ha Tinh, Vietnam	VNMN 010520	MW763027	Hoang et al. (2021)
Vietnamophryne vuquangensis	Vu Quang NP, Ha Tinh, Vietnam	VNMN 010521	MW763028	Hoang et al. (2021)
Vietnamophryne vuquangensis	Vu Quang NP, Ha Tinh, Vietnam	VNMN 010528	MW763029	Hoang et al. (2021)
Outgroups				
Aphantophryne pansa	Papua New Guinea	ABTC 49605	DQ283195	Frost et al. (2006)
Austrochaperina sp.	Papua New Guinea	BSFS 11377	KC822485	Blackburn et al. (2013)
Barygenys exsul	Papua New Guinea	BPBM 20128	EU100119; EU100235	Köhler and Günther (2008)
Callulops robustus	Papua New Guinea	PT-506	KM509105	Peloso et al. (2016)
Choerophryne sp.	Papua New Guinea	ABTC 47720	DQ283207	Frost et al. (2006)
Cophixalus sphagnicola	Papua New Guinea	ABTC 47881	DQ283206	Frost et al. (2006)
Copiula sp.	New Guinea	AMS R124417	DQ283208	Frost et al. (2006)
Gastrophrynoides immaculatus	Negeri Sembilan, Malaysia	UKMHC 279	AB634647; AB634705	Matsui et al. (2011)
Genyophryne thomsoni	Papua New Guinea	ABTC 49624	DQ283209	Frost et al. (2006)
Hylophorbus rufescens	Papua New Guinea	LSUMZ 94943	JX119248; JX119392	Oliver et al. (2013)
Kaloula rugifera	China	N/a	KP682314	Deng et al. (2015)
Liophryne rhododactyla	Papua New Guinea	ABTC 49566	DQ283199	Frost et al. (2006)
Mantophryne lateralis	Papua New Guinea	LSUMZ 92102	JN048989; JN049014	Rittmeyer et al. (2012)
Metamagnusia slateri	Papua New Guinea	PT-507	KM509160	Peloso et al. (2016)
Oreophryne anulata	Philippines	PNMCMNHH 1366	KC822488	Blackburn et al. (2013)
Oreophryne brachypus	Papua New Guinea	ABTC 50081	DQ283194	Frost et al. (2006)
Oreophryne variabilis	Indonesia	TNHC 58922	KC822489	Blackburn et al. (2013)
Sphenophryne cornuta	Papua New Guinea	LSUMZ 94793	JX119386; JX119242	Oliver et al. (2013)
Siamophryne troglodytes	Sai Yok, Kanchanaburi, Thailand	ZMMU NAP-06651	MG682553	Suwannapoom et al. (2018)
Xenorhina obesa	Papua New Guinea	PT-529	KM509212	Peloso et al. (2016)

Phylogenetic analyses

For phylogenetic analysis, the 12S rRNA and 16S rRNA Microhylidae dataset of Poyarkov et al. (2018a) was used, with the addition of the newly obtained sequences of *Vietnamophryne*. Data on sequences and specimens used in molecular analyses are summarized in Table 1. In total, sequences of the 12S rRNA and 16S rRNA mtDNA fragments of 47 microhylid representatives were included in the final analysis, including 27 samples of *Vietnamophryne*, 46 samples of the subfamily Asterophryinae (17 specimens of Australasian asterophryine genera and two specimens of *Gastrophrynoides* Noble, 1926 and *Siamophryne* Suwannapoom, Sumontha, Tunprasert, Ruangsuwan, Pawangkhanant, Korost & Poyarkov, 2018 from Sundaland and Tenasserim,

respectively), while the sequence of *Kaloula rugifera* Stejneger was used as an outgroup.

Nucleotide sequences were initially aligned using ClustalX 1.81 software (Thompson et al. 1994) with default parameters and then optimized manually in BioEdit 7.0.5.2 (Hall 1999) and MEGA 6.0 (Tamura et al. 2013). Mean uncorrected genetic distances (*p*-distances) between sequences were determined using MEGA 6.0. Species delimitation algorithms used in this study include ABGD and ASAP with default parameters (Miralles et al. 2022) and PTP and bPTP with default parameters as well (Zhang et al. 2013). MODELTEST v3.06 (Posada and Crandall 1998) was applied to estimate the optimal evolutionary models to be used for dataset analysis. The best-fitting model was the GTR+I+G model of DNA evolution, as suggested by the Akaike Information Criterion (AIC). Phylogenetic trees were inferred using maximum likelihood (ML) and Bayesian inference (BI). The ML analysis was conducted using IQ-TREE 3.0.1 software (Nguyen et al. 2015). Confidence in tree topology was assessed by 10,000 ultrafast bootstrap replications for ML analysis (UFB) (Minh et al. 2013). The BI analysis was conducted using MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). Metropolis coupled Markov chain Monte Carlo (MCMCMC) analyses were run with one cold chain and three heated chains for ten million generations and were sampled every 1,000 generations. Two independent MCMCMC runs were performed, and 1,000 trees were discarded as burn-in. Confidence in tree topology was assessed using posterior probability (PP) (Huelsenbeck and Ronquist 2001). Tree nodes with UFB values of 95 or greater and PP values over 0.95 were regarded as sufficiently resolved; those with UFB values between 95 and 90 and PP between 0.95 and 0.90 as tendencies; and those with UFB values below 90 and PP below 0.90 as unresolved (Huelsenbeck and Hillis 1993; Minh et al. 2013).

Morphological analysis

The sex of adult individuals was determined using gonadal dissection. All measurements were taken to the nearest 0.02 mm and subsequently rounded to 0.1 mm precision from preserved specimens using a digital caliper under a light dissecting microscope. Measurements included the following 38 morphometric characters (see Poyarkov et al. 2018a): (1) snout-vent length (SVL; length from tip of snout to cloaca); (2) head length (HL; length from tip of snout to hind border 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 medial edges of eyeballs in dorsal view); (9) upper eyelid width (UEW; maximum distance between medial edge of 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); (21–24) finger lengths (1–3FLO, 4FLI; measured as the distance between tip and junction of neighboring finger for outer side (O) for F1–F3, and for inner side (I) for F4); (25) first finger width (1FW, measured at distal phalanx); (26–28) finger disk diameters (2–4FDW); (29) first toe length (1TOEL, distance between distal end of inner metatarsal tubercle and tip of first toe); (30–33) second to fifth toe lengths (outer lengths for toes II–IV, inner length for toe V); (34–38) toe disk diameters (1–5TDW).

A principal component analysis (PCA) using the *prcomp* function from the *stats* R package and visualized using *ggplot2* (Slowikowski et al. 2026), *ggrepel* (Wickham 2016), and *factoextra* (Kassambara and Mundt 2026) packages in R 4.5.2 (R Core Team 2025) was used to compare differences and similarities in the morphospace of individuals of the genus *Vietnamophryne*. Given the limited number of specimens examined, PCA was conducted as a pooled analysis combining the sex-specific size-corrected datasets for males and females.

The PCA comprised 23 size-corrected morphometric characters (SVL, HL, SL, EL, N-EL, HW, IND, IOD, UEW, FLL, LAL, HAL, 1FL, TL, FL, 1TOEL, 2FL, 3FL, 4FL, 2TOEL, 3TOEL, 4TOEL, and 5TOEL) (Suppl. material 1). To remove potential effects of allometry in the morphometric characters, the size-correction algorithm of Chan and Grismer (2022) was used. Two operational taxonomic units (hereafter, OTUs) were represented by a single specimen (*V. inexpectata* and *V. occidentalis*); these singletons were retained in the ordination dataset by ln-transforming their raw metric values and appending them back to the size-corrected matrix. All differences were considered statistically significant at $P \leq 0.05$.

For comparisons with other taxa, original species descriptions and previously published data for the genus *Vietnamophryne* were used, including Poyarkov et al. (2018a), Hoang et al. (2021), Nguyen et al. (2021), and Ninh et al. (2023). Other abbreviations: Mt. = Mountain; NR = Nature Reserve; NP = National Park; asl = above sea level.

Results

Phylogenetic relationships

Phylogenetic analyses with ML and BI algorithms resulted in essentially similar topologies, with the differences between trees explained by computational characteristics—all the polytomies in BI were resolved dichotomously in ML, remarkably without significant nodal support (Fig. 2). Although the monophyly of the subfamily Asterophryinae and its two radiations—Australo-Papuan and Asian—was strongly supported (100/1.0 in all three nodes; hereafter, node support values are given for PP/UFB), relationships between genera remain largely unresolved. According to the analyses, *Gastrophrynoides* groups with *Siamophryne*, without significant nodal support (0.61/64). The genus *Vietnamophryne* is monophyletic (1.0/100) and consists of two clades. The first clade is composed of species *V. aurantifusca*, *V. cuongi*, *V. occidentalis*, and *V. orlovi* (1.0/100), with the relationships between them being unresolved. The second clade includes *V. inexpectata*, *V. vuquangensis*, *Vietnamophryne* sp. from northern Quang Tri Province, and another undescribed lineage of *Vietnamophryne* sp. from southern Quang Tri Province, Vietnam, also without significant resolution.

Genetic distances

The genetic divergence between *Vietnamophryne* sp. from the northern part of Quang Tri Province, Vietnam, and all other congeners ranged from p = 3.01% (with

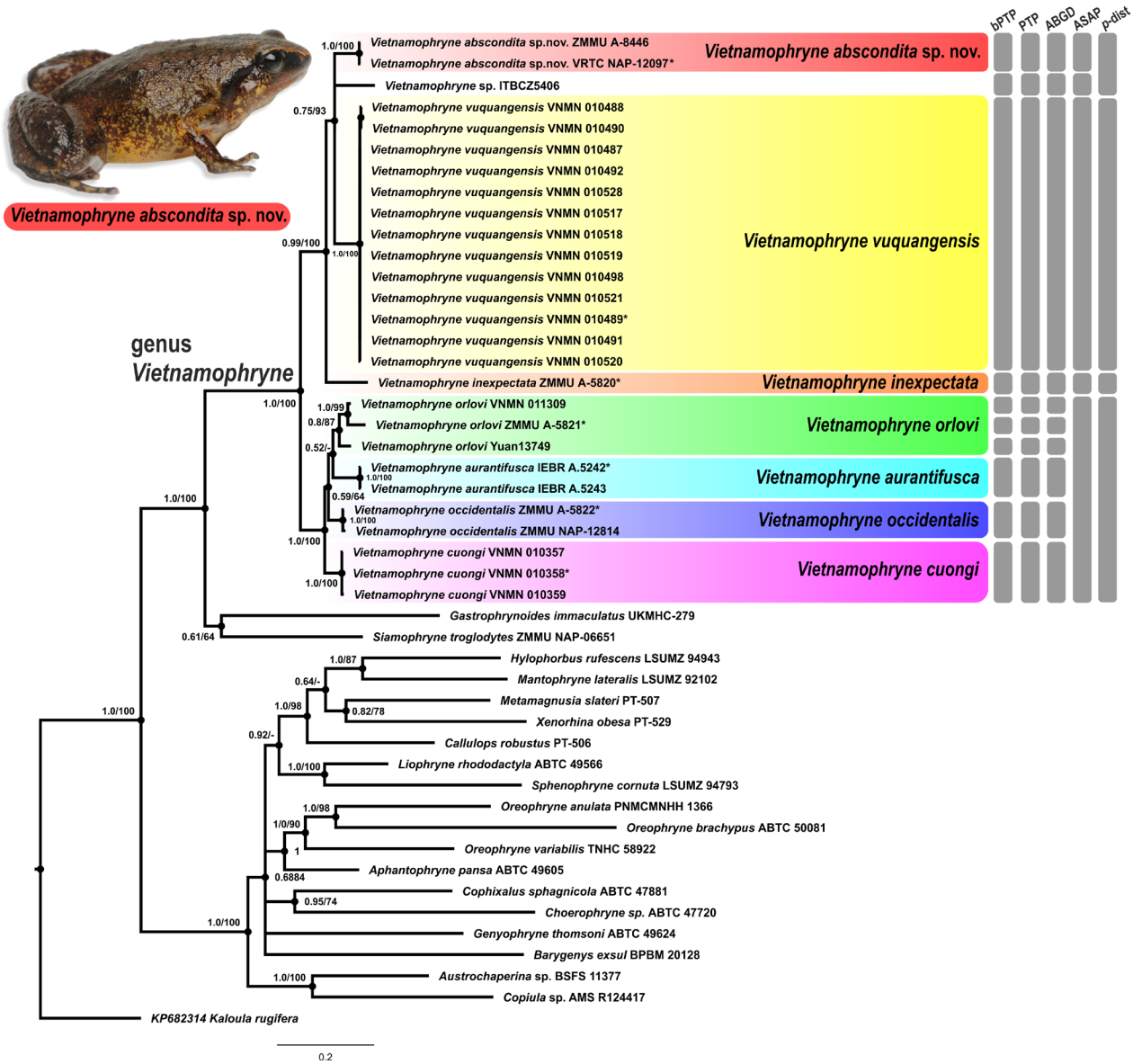

Figure 2. Genealogical relationships of the subfamily Asterophryinae based on the analysis of the 12S–16S mtDNA fragment. Numbers at tree nodes correspond to PP/UFB support values, respectively. Numbers after the sample names correspond to voucher numbers in Table 1. Grey bars on the left of the *Vietnamophryne* species correspond to the results of species delimitation analyses. An asterisk (*) denotes the holotype of a species. Photograph in the thumbnail by N.A. Poyarkov.

V. vuquangensis) to p = 4.01% (with *V. occidentalis*) (Table 2). These values correspond to or are higher than the formal p = 3% threshold for a fragment of the 16S rRNA gene, which is considered a putative indicator of species-level divergence in anurans (Vences et al. 2005; Vieites et al. 2009). A below-threshold divergence was also identified between the new population from northern Quang Tri Province and an unpublished sequence of *Vietnamophryne* sp. from the southern part of the same province (MK239028; p = 2.74%). Finally, several relatively low p-distance values were observed between *V. occidentalis* and *V. orlovi* (1.67%), *V. occidentalis* and *V. cuongi* (1.75%), *V. orlovi* and *V. cuongi* (1.59%), *V. orlovi* and *V. aurantifusca* (1.92%), and *V. aurantifusca* and *V. cuongi* (2.01%); all these divergence values are lower than the formal p = 3% threshold for the 16S rRNA gene.

Morphological differentiation

The PCA of the seven OTUs based on 23 morphological characters is shown in Fig. 3; the dataset used for the analysis is presented in Suppl. material 1. The first two principal components together explained 72.98% of the total variance, with PC1 accounting for 54.41% and PC2 for 18.57%. OTUs of *V. aurantifusca*, *V. occidentalis*, *V. inexpectata*, and of *Vietnamophryne* sp. from the northern part of Quang Tri Province, Vietnam, occupied distinct regions of morphospace. However, the degree of separation varied among taxa, and the OTUs of *V. orlovi*, *V. cuongi*, and *V. vuquangensis* were placed together, although with no substantial overlap between them. The highest loadings on PC1 were observed for HW, TL, SVL, 2TOEL, and LAL. PC2 was dominated by a different suite of variables, especially FLL, EL, 5TOEL, 2FL, and HAL. The contribution of all morphometric characters to the PCA is presented in Suppl. material 2.

Species delimitation

Results of automatic species delimitation vary substantially between the analyses (Fig. 2). The ABGD, PTP, and bPTP take a rather divisional stance, indicating the presence of a total of ten species in the genus *Vietnamophryne*. These include all six currently recognized species, the newly discovered population of *Vietnamophryne* sp. from the northern part of Quang Tri Province, an undescribed *Vietnamophryne* sp. from southern Quang Tri Province, and all three lineages of *V. orlovi* reconstructed as independent "species." The ASAP algorithm results in a more conservative picture (Fig. 2), with only five supported species, including *V. inexpectata*; *V. orlovi* + *V. occidentalis* + *V. cuongi* + *V. aurantifusca* merged as a single "species"; *V. vuquangensis*; an undescribed *Vietnamophryne* sp. from southern Quang Tri Province; and a *Vietnamophryne* sp. from the northern part of Quang Tri Province. The formal application of the p = 3.0% threshold (Fig. 2) suggests four species in the genus *Vietnamophryne*, including *V. inexpectata*, *V. vuquangensis*, *Vietnamophryne* sp. from Quang Tri Province (with the lineages from the northern and the southern localities merged into a single "species"), and *V. orlovi* + *V. occidentalis* + *V. cuongi* + *V. aurantifusca* merged into a single "species."

Taxonomic conclusions

In addition to its distinct molecular divergence, the northern Quang Tri lineage of *Vietnamophryne* occupies distinct morphological space and is distinguishable from all currently recognized congeners (see Fig. 3). Furthermore, the northern Quang Tri lineage of *Vietnamophryne* exhibits a combination of morphological characters that differentiates it from all currently recognized congeners (see Table 3). Notably, the northern Quang Tri lineage of *Vietnamophryne* represents only the second member of the genus known to inhabit limestone karst habitats, together with *V. aurantifusca*. The concordance between molecular, morphological, and ecological evidence supports the recognition of the northern Quang Tri population as a distinct species. Although the available material is currently limited to two adult male specimens, the combination of diagnostic morphological characters and consistent molecular divergence provides sufficient evidence to recognize this lineage as a separate taxon. Therefore, this new species is formally described below.

Table 2. Uncorrected p-distance matrix showing the percentage of pairwise genetic divergence among the 16S rRNA sequences of *Vietnamophryne* species (below the diagonal). Intraspecific genetic distances for 16S rRNA sequences are shown on the diagonal and marked in bold. Notes: n/a = Not available.

	Species	1	2	3	4	5	6	7	8
1	*Vietnamophryne abscondita* sp. nov.	**0.00%**							
2	*V. aurantifusca*	3.76%	**0.00%**						
3	*V. cuongi*	3.26%	2.01%	**0.00%**					
4	*V. inexpectata*	3.77%	3.77%	3.77%	**n/a**				
5	*V. occidentalis*	4.01%	2.26%	1.75%	3.02%	**0.00%**			
6	*V. orlovi*	3.84%	1.92%	1.59%	3.52%	1.67%	**0.83%**		
7	*V. vuquangensis*	3.01%	3.80%	4.26%	4.02%	4.01%	4.18%	**0.77%**	
8	*Vietnamophryne* sp. MK239028	2.74%	4.11%	4.11%	4.12%	4.38%	4.20%	4.11%	**n/a**

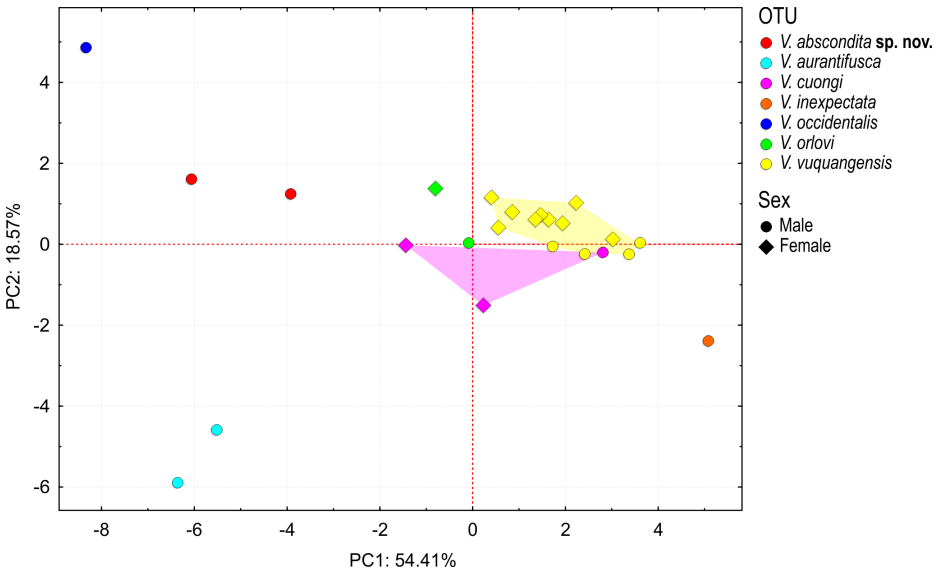

Figure 3. PCA of all OTUs (including females and males) of the genus *Vietnamophryne*. Circles indicate male specimens, and diamonds indicate female specimens. Colors correspond to those in Figs 1, 2.

Table 3. Comparison of morphological characters of *Vietnamophryne abscondita* sp. nov. with other species of the genus *Vietnamophryne*. Diagnostic differences from the new species are marked in bold. For character abbreviations, see Materials and methods. Morphological data are taken from Poyarkov et al. (2018a), Nguyen et al. (2021), Hoang et al. (2021), Ninh et al. (2023), and the present study. Notes: N/d = No data.

Species	*V. abscondita* **sp. nov.**	*V. aurantifusca*	*V. cuongi*	*V. inexpectata*	*V. occidentalis*	*V. orlovi*	*V. vuquangensis*
SVL (males)	18.1–19.0	17.6–18.2	n/d	**14.2**	**20.5**	**15.4**	**14.1–14.9**
SVL (females)	n/d	n/d	16.9–18.1	n/d	n/d	17.1	15.2–17.3
1FL/2FL	0.60–0.72	0.59–0.66	0.43–0.62	**0.30**	**0.43**	**0.50**	**0.43–0.50**
HW/HL	1.08–1.12	**1.17–1.19**	1.10–1.23	**1.01**	**0.99**	**0.87–0.98**	1.10–1.31
SL/EL	1.33	1.33–1.45	1.33–1.42	**0.95**	**0.84**	1.31–1.39	**1.03–1.30**
TYD/SVL	indistinct	**0.04–0.05**	**0.08–0.09**	**0.09**	**0.05**	**0.06–0.07**	**0.05–0.06**
Dorsolateral spots	absent	**present**	**present**	**present**	absent	**present**	**present**
Ventral coloration	yellow-orange	orange	**milky-white**	lemon-yellow	orange	lemon-yellow	lemon-yellow
Dorsal coloration	dark brown	**orange-brown**	**reddish-brown**	**reddish-brown**	**reddish-brown**	**reddish-brown**	**light brown**
Marbling on throat	pronounced, dark	**weak, grayish**	**weak, grayish**	pronounced, brown	**absent**	**weak, grayish**	**absent**
Suborbital spots	present, yellow	**absent**	**absent**	**absent**	**absent**	**absent**	**absent**
Skin on dorsum	shagreened, with pustules	**smooth, posterior pustules**	**smooth, posterior pustules**	shagreened to warty	**smooth**	**smooth, posterior pustules**	shagreened
References	*this study*	Ninh et al. (2023)	Nguyen et al. (2021)	Poyarkov et al. (2018a)	Poyarkov et al. (2018a)	Poyarkov et al. (2018a); Ninh et al. (2023)	Hoang et al. (2021)

Vietnamophryne abscondita sp. nov.

https://zoobank.org/69BDCFDE-191B-44FC-B39A-E7CE4D58F6CA

Figs 4, 5, Table 3

Holotype. VRTC NAP-12097 (field tag NAP-12097), adult male, from limestone karst forest, Kim Dien Commune, Quang Tri Province, Vietnam (17.820355°N, 105.881989°E; elevation ca. 250 m a.s.l.), collected by D.T. Nguyen, T.V. Duong, N.A. Poyarkov on 20 May 2022.

Paratype (*n* = 1). ZMMU A-8446 (field tag NAP-12098), adult male, with the same collection information as the holotype.

Diagnosis. The new species *Vietnamophryne abscondita* sp. nov. is distinguished from all of its congeners by a combination of the following morphological characters: (1) medium body size (SVL 18.1–19.0 mm in males, *n* = 2); (2) head slightly wider than long; (3) snout short, rounded in dorsal view; (4) tympanum indistinct; (5) dorsal skin finely

shagreened, lacking enlarged tubercles or a median dorsal ridge; (6) finger I longer than half of finger II (1FL/2FL ratio 59.8–71.9%); (7) toes without webbing, bearing rounded terminal discs; (8) forelimbs and hindlimbs slender; (9) dorsum dark brown to chocolate brown with irregular lighter mottling; (10) flanks and limbs densely speckled with pale spots; (11) ventral surface bright yellow-orange to orange on chest and belly, with dark marbling on throat and ventral surfaces of limbs; (12) lower jaw region with scattered pale blotches; and (13) indistinct pale yellow suborbital spotting extending from eye toward mouth corner.

Description of holotype. Adult male (Figs 4A–E, 5A), specimen in good state of preservation. Body habitus stout, miniaturized (SVL 18.14 mm). Head slightly wider than long (HL/HW 0.93); snout short and rounded in dorsal view (Fig. 4A), rounded in profile (Fig. 4C), longer than eye diameter (SL/EL 1.33). Eyes medium-sized, slightly protuberant in dorsal and lateral views (Fig. 4C); pupil round. Dorsal surface of head slightly convex; canthus rostralis rounded and indistinct; loreal region weakly concave; nostrils rounded, lateral, positioned approximately equidistant between snout tip and eye. Tympanum indistinct; supratympanic fold present, glandular. Vomerine teeth and vomerine spikes absent; a single transverse smooth palatal fold present anterior to the pharynx; tongue spatulate, free posteriorly, without papillae; vocal sac openings not discernible.

Forelimbs comparatively short, approximately one-third of hindlimb length (FLL/HLL 0.53); hand shorter than lower arm, approximately half of forelimb length (HAL/FLL 0.43). Fingers short, slender, rounded in cross-section; Finger I well developed, exceeding half the length of Finger II (1FL/2FL 0.60); relative finger lengths I < II < IV < III (Fig. 4D). Finger webbing and dermal fringes absent. First fingertip slightly protuberant, forming a small nub; tips of Fingers II–IV rounded, not dilated; finger discs absent; terminal grooves absent. Subarticular tubercles under fingers indistinct; nuptial pads absent. Two palmar tubercles present: inner palmar tubercle small and rounded; outer palmar tubercle rounded with indistinct margins, slightly shorter than inner palmar tubercle (IPTL/OPTL 0.87); palmar surface smooth; supernumerary palmar tubercles absent.

Hindlimbs comparatively short and robust; tibia length approximately half of snout–vent length (TL/SVL 0.51); tibiotarsal articulation of adpressed limb reaching eye level. Foot slightly shorter than tibia (FL/TL 1.39). Relative toe lengths I < II < V < III < IV (Fig. 4E). Toes rounded in cross-section; toe webbing absent; dermal fringes absent; tips of Toes III and IV slightly dilated, terminal grooves absent. Subarticular tubercles under toes indistinct. Single metatarsal tubercle present: inner metatarsal tubercle rounded and flattened (IMTL/1TOEL 0.52); outer metatarsal tubercle absent.

Skin on dorsum finely shagreened, covered with dense, low, rounded granules giving a slightly roughened but not warty appearance. No enlarged tubercles, dorsolateral ridges, or median dorsal ridge present. Dorsal granulation more evident under oblique light, forming a uniform, finely textured surface across head, body, and dorsal surfaces of limbs. Posterior dorsum without clustered pustules or enlarged nodules. Skin on flanks similarly shagreened, with granules slightly more pronounced than on mid-dorsum; no glandular ridges observed. Dorsolateral white spots irregular in shape and size, scattered diffusely along flanks and lateral surfaces of head and body; spots not arranged in a distinct linear series. Head skin finely granular; loreal and temporal regions shagreened. Upper eyelid smooth to very finely granular. Skin on dorsal surfaces of forelimbs and hindlimbs finely shagreened, matching dorsal trunk texture. Ventral surfaces of limbs smooth, lacking granules. Skin on throat, chest, and belly smooth, glossy in life, without visible granulation or folds except for a single transverse palatal fold internally. No ventral dermal tubercles or asperities observed. Ventral skin appears thinner and more translucent than dorsal skin.

Coloration in life (Fig. 5A). Dorsal surfaces dark brown to chocolate brown, densely mottled with irregular lighter brown and yellowish speckles, producing a marbled appearance; head slightly darker than dorsum, with diffuse pale yellow spotting on loreal, suborbital, and temporal regions, extending from eye toward mouth corner. Flanks and dorsal surfaces of limbs heavily speckled with small pale yellow to cream spots. A faint pale supralabial stripe present. Ventral surface bright yellow-orange to orange on chest and belly, gradually fading to paler yellow toward the groin; throat and ventral surfaces of limbs with irregular dark brown marbling. Lower jaw region with scattered pale blotches; palms and soles greyish with diffuse pale mottling.

Variation. The paratype ZMMU A-8446 (Figs 4F, G, 5B) generally agrees with the holotype in overall morphology and coloration but is slightly larger in body size (SVL 19.04 mm vs. 18.14 mm in the holotype). Minor variation is observed in coloration, with the paratype exhibiting a darker dorsal background and less contrasting pale mottling. Ventral yellow-orange coloration is slightly paler, and dark marbling on the throat and ventral surfaces of limbs is less pronounced. Dorsal skin texture in the paratype is similarly finely shagreened, without enlarged tubercles or dorsal ridges.

Measurements (in mm): Holotype (VRTC NAP-12097; adult male): SVL 18.14; HL 6.36; HW 6.85; SL 2.19; EL 1.65; N–EL 1.47; IND 1.98; IOD 2.23; UEW 1.01; FLL 9.61; LAL 7.91; HAL 4.11; 1FL 0.70; 2FL 1.17; 3FL 2.28; 4FL 1.70; IMTL 0.48; 1TOEL 0.93; 2TOEL 1.90; 3TOEL 3.15; 4TOEL 4.89; 5TOEL 2.15; IPTL 0.48; OPTL 0.55. Paratype (ZMMU A-8446; adult male): SVL 19.04; HL 6.53; HW 7.32; SL 2.48; EL 1.87; N–EL 1.38; IND 2.39; IOD 2.58; UEW 1.20; FLL 10.49; LAL 8.46; HAL 4.34; 1FL 0.87; 2FL 1.21; 3FL 2.38; 4FL 1.84; IMTL 0.54; 1TOEL 0.93; 2TOEL 2.15; 3TOEL 3.20; 4TOEL 5.07; 5TOEL 2.52; IPTL 0.49; OPTL 0.57.

Etymology. The specific name is a Latin adjective in the nominative singular, derived from *absconditus*, meaning “hidden,” “secret,” or “concealed.” The name refers to the secretive semifossorial lifestyle of the species, which inhabits leaf litter and soil within limestone crevices and grottos in karst formations of Quang Tri Province, Vietnam. The following common names are

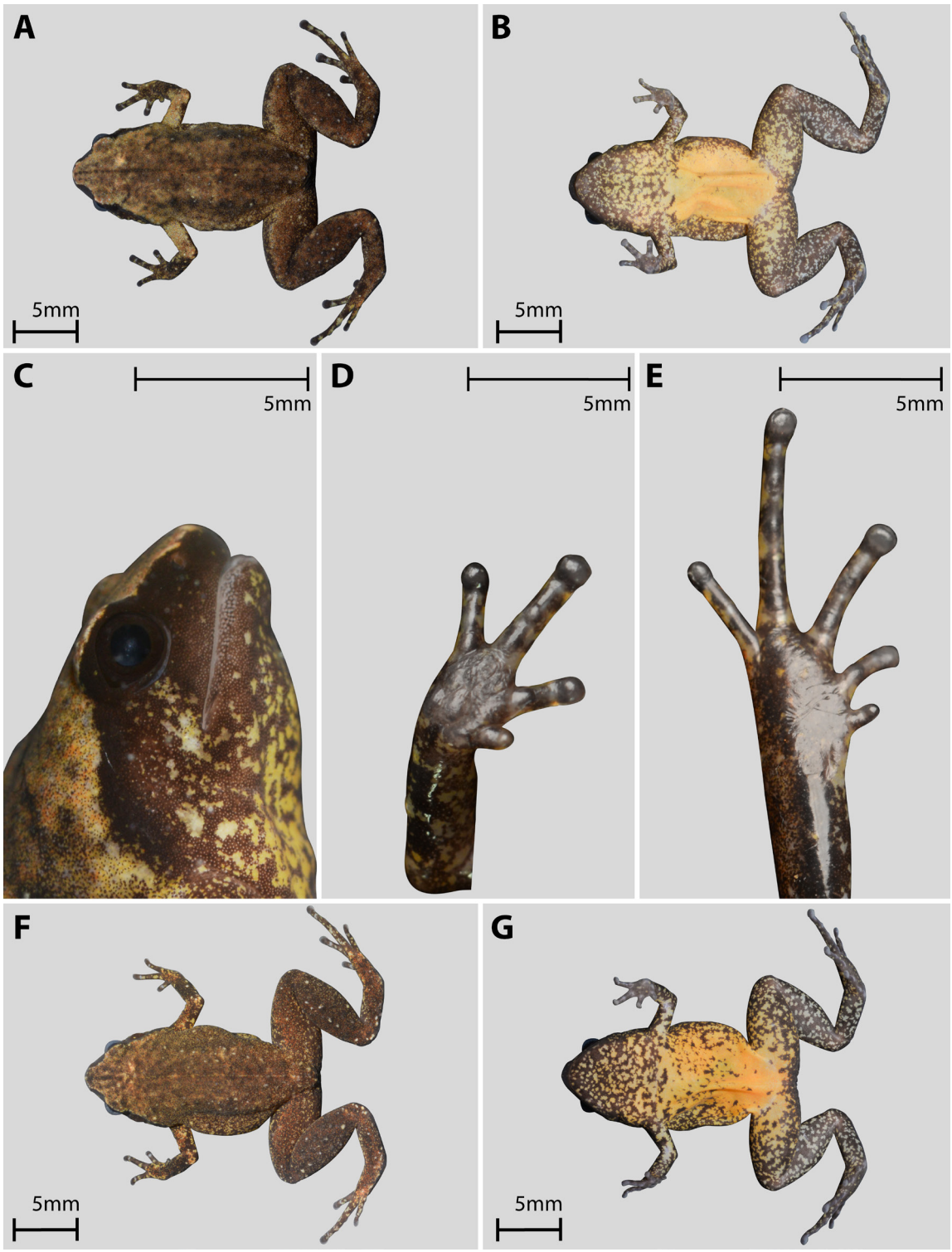

Figure 4. Morphology of the type series of *Vietnamophryne abscondita* sp. nov. **A–E.** Holotype VRTC NAP-12097 (adult male); **A.** Dorsal view; **B.** Ventral view; **C.** Lateral view of head; **D.** Palmar view of right hand; **E.** Thenar view of right foot. **F, G.** Paratype ZMMU A-8446 (adult male); **F.** Dorsal view; **G.** Ventral view. Photographs by N.A. Poyarkov. Scale bar: 5 mm.

recommended: "Karstic Dwarf Frog" (English), "Nhái lùn đá vôi" (Vietnamese), and "Карстовый узкоротик" (*Karstovyi uzkorotik*, Russian).

Comparisons. *Vietnamophryne abscondita* sp. nov. steadily differs from all currently recognized species of *Vietnamophryne* in having tympanum completely hidden by the skin of glandular supratympanic fold (vs. tympanum externally visible in all other congeners). Other diagnostic characters distinguishing the new species from all currently recognized congeners are summarized in Table 3. Below, individual comparisons of *Vietnamophryne abscondita* sp. nov. with other *Vietnamophryne* species are provided.

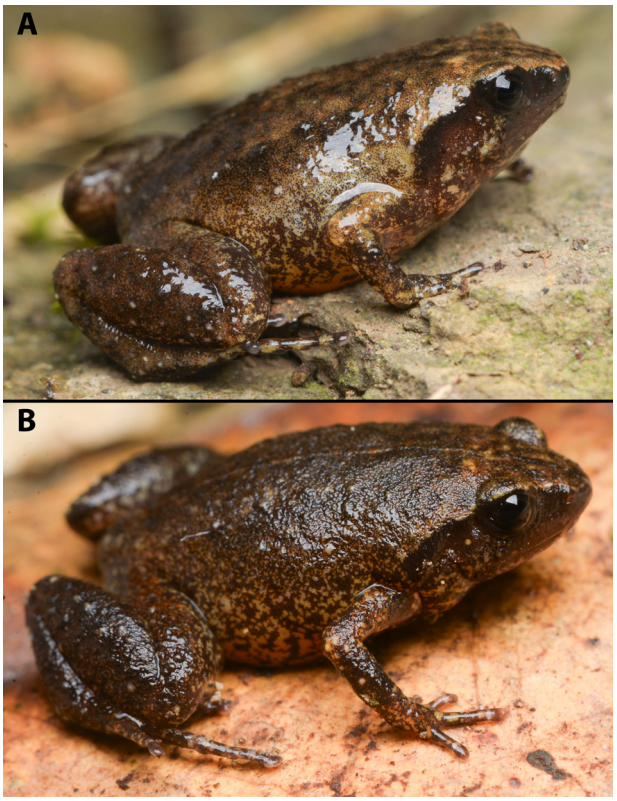

Figure 5. Type specimens of *Vietnamophryne abscondita* sp. nov. in life. **A.** Holotype VRTC NAP-12097 (adult male); **B.** Paratype ZMMU A-8446 (adult male). Photographs by N.A. Poyarkov.

Vietnamophryne abscondita sp. nov. further differs from *V. aurantifusca* by slightly larger male body size (SVL 18.1–19.0 mm vs. 17.6–18.2 mm), brighter ventral coloration (bright yellow-orange vs. orange), darker dorsal background (dark brown to chocolate brown vs. reddish- to orange-brown), dense pale speckling on dorsum, flanks, and limbs (vs. few dorsal pustules posteriorly), more conspicuous dark marbling on throat and ventral surfaces of limbs (vs. weak gray marbling), and presence of indistinct pale yellow suborbital spotting extending from eye toward mouth corner (vs. absent).

Vietnamophryne abscondita sp. nov. further differs from *V. cuongi* by slightly larger male body size (SVL 18.1–19.0 mm vs. female SVL 16.9–18.1 mm), although this comparison should be interpreted with caution due to the absence of male specimens for *V. cuongi*; darker dorsal background (dark brown to chocolate brown vs. brown to reddish-brown); dense pale speckling on dorsum and limbs (vs. few dorsal pustules posteriorly); absence of a distinct whitish canthal stripe and brick-red supratympanic tubercles (vs. present); brighter ventral coloration (bright yellow-orange with conspicuous dark marbling vs. milky white with gray marbling); and presence of indistinct pale yellow suborbital spotting extending from eye toward mouth corner (vs. absent).

Vietnamophryne abscondita sp. nov. further differs from *V. inexpectata* by larger male body size (SVL 18.1–19.0 mm vs. 14.2 mm); more developed first finger in males (1FL/2FL 0.60–0.72 vs. 0.30); finely shagreened dorsal skin lacking enlarged posterior pustules (vs. dorsum mostly smooth anteriorly, becoming shagreened posteriorly with conspicuous large flat pustules); darker dorsal background (dark brown to chocolate brown vs. reddish-brown); brighter ventral coloration (bright yellow-orange with conspicuous dark marbling vs. lemon-yellow with fine brown marbling); and presence of indistinct pale yellow suborbital spotting extending from eye toward mouth corner (vs. absent).

Vietnamophryne abscondita sp. nov. further differs from *V. occidentalis* by smaller male body size (SVL 18.1–19.0 mm vs. 20.5 mm); darker dorsal background (dark brown to chocolate brown vs. reddish-brown); dense pale speckling on dorsum and limbs (vs. more uniform reddish dorsum with sparse speckling); absence of enlarged posterior dorsal pustules (vs. present); brighter ventral coloration (bright yellow-orange with conspicuous dark marbling vs. orange with weak or absent marbling); and presence of indistinct pale yellow suborbital spotting extending from eye toward mouth corner (vs. absent).

Vietnamophryne abscondita sp. nov. further differs from *V. orlovi* by larger male body size (SVL 18.1–19.0 mm vs. 15.4 mm); more developed first finger in males (1FL/2FL 0.60–0.72 vs. 0.50); darker dorsal background (dark brown to chocolate brown vs. reddish-brown); denser pale speckling on dorsum and limbs (vs. more uniform reddish dorsum with sparse speckling); absence of enlarged posterior dorsal pustules (vs. present in *V. orlovi*); brighter ventral coloration (bright yellow-orange with conspicuous dark marbling vs. lemon-yellow with weaker marbling); and presence of indistinct pale yellow suborbital spotting extending from eye toward mouth corner (vs. absent).

Finally, *Vietnamophryne abscondita* sp. nov. further differs from *V. vuquangensis* by larger male body size (SVL 18.1–19.0 mm vs. 14.1–14.9 mm); more developed first finger in males (1FL/2FL 0.60–0.72 vs. 0.43–0.49); darker dorsal background (dark brown to chocolate brown vs. light to medium brown); denser pale speckling on dorsum and limbs (vs. more uniform dorsum with sparse speckling); finely shagreened dorsal skin (vs. mostly smooth dorsum); brighter ventral coloration (bright yellow-orange with conspicuous dark marbling vs. ivory-lemon to lemon-yellow); and presence of indistinct pale yellow suborbital spotting extending from eye toward mouth corner (vs. absent).

Distribution, natural history, and conservation status. *Vietnamophryne abscondita* sp. nov. is currently known only from its type locality in limestone karst forests of Quang Tri Province, central Vietnam. Specimens were found in leaf litter and soil microhabitats at the entrance to a small cave within an evergreen karst forest, suggesting a semi-fossorial lifestyle similar to that reported for congeners. The new species was recorded in syntopy with a rhacophorid frog *Gracixalus quyeti* (Nguyen, Hendrix, Böhme, Vu & Ziegler, 2008), which was found in the same limestone cave.

Although the species is presently documented from a single locality, extensive limestone karst formations oc-

cur throughout Quang Tri Province and adjacent areas, indicating that it may have a broader distribution than currently recognized. Forest habitats in these karst regions are subject to anthropogenic pressures, including deforestation, habitat fragmentation, land-use conversion associated with *Acacia* plantations, infrastructure development, and livestock grazing. However, there is currently no evidence of population decline, severe fragmentation, or extreme fluctuations. Given the presumed wider distribution and lack of direct information indicating significant threats at the population level, it is provisionally suggested that *Vietnamophryne abscondita* sp. nov. be classified as Least Concern (LC) under the IUCN Red List Categories and Criteria, pending further field surveys to clarify its true range and conservation status.

Discussion

The asterophryine phylogeny reconstructed in the current work provides a few curious observations. Recognizing the limitations of mtDNA markers in the reconstruction of phylogenetic relationships (see, e.g., Hillis et al. 2021; Dufresnes et al. 2023; Gallego-Garcia et al. 2023), it is still impossible to ignore how little resolution at the genus level was obtained using a single mtDNA marker in this study. Not only did the Austro-Papuan radiation of the subfamily Asterophryinae receive low or insignificant levels of support for major nodes, but even the Asian radiation was essentially resolved as a trichotomy between *Gastrophrynoides*, *Siamophryne*, and *Vietnamophryne*, unlike in previous works (Poyarkov et al. 2018a; Suwannapoom et al. 2018). The poor state of genus-level resolution in this group is highlighted by multiple incongruences shown in previous studies with larger datasets (Feng et al. 2017; Tu et al. 2018; Streicher et al. 2020). As noted by Frost (2026) in the commentary accompanying the Amphibian Species of the World database:

"Interesting to see how anuran classification has evolved over the years, particularly with the application of lots of molecular data. Two taxa remain remarkably unstable with respect to their internal structure: Asterophryinae *(*Microhylidae*) and* Brachycephaloidea*. Just amazing to me."*

This amazement is shared, and the results further corroborate it, highlighting the importance of using nuDNA and other lines of evidence in future phylogenetic studies on this group.

The findings provide new insights into species-level divergence in the genus *Vietnamophryne*. Both molecular and morphological evidence support the recognition of *Vietnamophryne abscondita* sp. nov. as a distinct evolutionary lineage. Phylogenetic analyses of mitochondrial 16S rRNA sequences consistently recover the Quang Tri population as a well-supported independent clade, with uncorrected *p*-distances exceeding 3% from all currently recognized congeners. However, the estimations of interspecific *p*-distances from previous studies (Poyarkov et al. 2018a; Hoang et al. 2021; Nguyen et al. 2021; Ninh et al. 2023) could not be matched. As stated in Poyarkov et al. (2018a), a divergence from the common practice of studying the particular end-fragment of the 16S rRNA mtDNA gene was made, and a much longer fragment of 12S–16S was used instead, which explains this discrepancy. Hoang et al. (2021), Nguyen et al. (2021), and Ninh et al. (2023) do not state explicitly which datasets were used for *p*-distance calculation, but based on the sequences uploaded to GenBank, it is suspected that their methodology may also have differed, using 707 bp, 1,068 bp, and 810 bp fragments of the 16S rRNA mtDNA gene, respectively. As different parts of the genome may have different mutation rates, direct comparison between the estimations of *p*-distances among these studies is essentially unreliable. The results of species delimitation analyses further complicate this matter, as some analyses either indicate undescribed diversity (PTP, bPTP, ABGD) or over-splitting (ASAP, $p = 3\%$ threshold) and are overall incongruent with the current understanding of species diversity in this genus. In light of these results, evidence for the genetic distinctiveness of *V. orlovi*, *V. occidentalis*, *V. cuongi*, and *V. aurantifusca* may appear less apparent, suggesting that further integrative studies are needed to test whether they represent independent biological species.

Morphologically, *Vietnamophryne abscondita* sp. nov. is diagnosed by a unique combination of characters, including medium body size, finely shagreened dorsal skin lacking enlarged tubercles or a median vertebral ridge, relatively well-developed Finger I, absence of toe webbing, and distinctive dorsal and ventral coloration. The concordance between molecular divergence and morphological distinctiveness provides integrative support for species recognition. At the same time, the results underscore persistent taxonomic challenges within the *Vietnamophryne orlovi* species complex, including *V. aurantifusca*, *V. cuongi*, *V. occidentalis*, and *V. orlovi*. As demonstrated above, available molecular datasets indicate relatively shallow mitochondrial divergences among some of these taxa, while many diagnostic differences proposed in original descriptions rely heavily on morphometric ratios and subtle external characters. In particular, several species have been differentiated primarily on the basis of ratios such as HW/HL, SL/EL, and TYD/SVL (e.g., Ninh et al. 2023). Ratio-based characters are inherently sensitive to limited sample sizes, ontogenetic variation, preservation artifacts, measurement protocols, and inter-observer bias. Small absolute differences in linear measurements can produce proportionally larger differences in ratios, especially in miniaturized taxa such as *Vietnamophryne*, potentially exaggerating perceived interspecific divergence (Gorin et al. 2021). This issue is especially pronounced for tympanum diameter (TYD). In life, the tympanum in *Vietnamophryne* is frequently indistinct and may be partially or completely concealed beneath surrounding skin, rendering its margins difficult to delimit with precision. Furthermore, the apparent visibility and size of the tympanum may change after fixation or dehydration—the tympanic membrane can become more exposed or appear retracted in preserved specimens or in individuals experiencing temporary water loss (T.V. Nguyen,

pers. obs.). As a consequence, TYD and derived ratios (e.g., TYD/SVL) may be affected by artefactual variation and should not be treated as reliable standalone diagnostic characters without corroboration from additional independent evidence. Given these considerations, minor differences in morphometric ratios should be interpreted cautiously when delimiting species boundaries in *Vietnamophryne*. Species limits within the *V. orlovi* complex should be regarded as provisional until tested with broader geographic sampling, larger specimen series, standardized morphometric protocols, and multilocus genomic data. Although no taxonomic changes are proposed here, a comprehensive integrative revision of the genus is clearly warranted.

The discovery of *Vietnamophryne abscondita* sp. nov. from limestone karst forests of Quang Tri Province, Vietnam, substantially expands current knowledge of the geographic distribution and ecological diversity of the genus. Whereas most previously described congeners were reported from northern Vietnam and adjacent regions of northern Indochina, records from central Vietnam have remained comparatively scarce. The discovery of the new species therefore fills an important distributional gap in the Central Annamites (Truong Son Mountains) and provides further evidence that species diversity of *Vietnamophryne* in this region remains underestimated.

Ecologically, *Vietnamophryne abscondita* sp. nov. represents only the second member of the genus known to inhabit limestone karst habitats, together with *V. aurantifusca*. Limestone systems are characterized by pronounced environmental heterogeneity, habitat fragmentation, and geographic isolation, all of which promote localized diversification and micro-endemism. The occurrence of two karst-associated species within the genus suggests that these habitats may represent an important, yet still insufficiently explored, component of *Vietnamophryne* diversity. The results provide further evidence that limestone areas represent hotspots of herpetofaunal diversity in Indochina (Idiiatullina et al. 2023; Sitthivong et al. 2023; Grassby-Lewis et al. 2025; Vogel et al. 2025), including narrow-ranged and point-endemic microhylid frog species (e.g., Suwannapoom et al. 2018; Poyarkov et al. 2018b; Nguyen et al. 2024). Further surveys across limestone landscapes of central Vietnam are therefore likely to reveal additional undocumented lineages of *Vietnamophryne*.

Finally, the apparent rarity of *Vietnamophryne* species in collections is likely at least partly an artifact of their cryptic, semi-fossorial lifestyle. Members of the genus are typically encountered only sporadically in leaf litter or shallow soil layers and are rarely detected by conventional visual encounter surveys (Poyarkov et al. 2018a). This low detectability, combined with their small body size and patchy distributions, suggests that current knowledge of *Vietnamophryne* diversity remains severely incomplete. Targeted microhabitat-focused surveys, including intensive leaf-litter searches and shallow soil excavation in both evergreen forests and limestone karst systems, are therefore essential to better document species richness and distribution patterns in this genus. Such efforts will be particularly important in the Central Annamites, where extensive karst formations remain poorly explored and are likely to harbor additional undescribed lineages. For example, mitochondrial data indicate the presence of a genetically distinct lineage of *Vietnamophryne* sp. from Bac Huong Hoa Nature Reserve (ITBCZ 5406; southern part of Quang Tri Province, Vietnam), which is closely related to *Vietnamophryne abscondita* sp. nov., but may represent an additional undescribed taxon. However, without detailed morphological comparisons and examination of voucher material, its taxonomic status cannot be evaluated. Future integrative assessment of this lineage should therefore be prioritized.

Overall, the discovery of *Vietnamophryne abscondita* sp. nov. reinforces the Central Annamites as a major center of amphibian diversity and highlights limestone karst ecosystems as important reservoirs of micro-endemic lineages. Continued field exploration combined with integrative taxonomic approaches will be essential for resolving species boundaries within *Vietnamophryne*, documenting cryptic diversity, and improving understanding of diversification processes in one of Southeast Asia's most biogeographically complex regions.

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number UP-AE64-02-04-005, issued to C. Suwannapoom), were strictly compliant with the ethical conditions of the Thailand Animal Welfare Act, and were approved by the Institutional Ethical Committee of the Joint Vietnam-Russia Tropical Science and Technology Research Center (VRTC). Fieldwork, including the collection of animals in the field, was authorized by the Institute of Animals for Scientific Purpose Development (IAD), Bangkok, Thailand (permit numbers U1-01205-2558 and UPAE59-01-04-0022, issued to C. Suwannapoom).

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Supplementary material 1

Morphometric data on the genus *Vietnamophryne* used in the PCA-analysis

Authors: Nikolay A. Poyarkov, Vladislav A. Gorin, Son Xuan Le, Alexei V. Trofimets, Tang Van Duong, Duc Trong Nguyen, Parinya Pawangkhanant, Chatmongkon Suwannapoom, Tan Van Nguyen

Data type: docx

Explanation note: For character abbreviations see Materials and methods. An asterisk (*) denotes the holotype of a species. (Continues on the next page).

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Supplementary material 2

The contribution of morphometric characters to the PCA-analysis

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