

## A new species of *Nanohyla* (Anura: Microhylidae) from the lowland forests of southern Vietnam

### DEAR EDITOR,

We describe a new species of *Nanohyla* from the Song Hinh Protected Forest in Phu Yen Province, southern Vietnam, based on an integrative taxonomic approach. The new species represents a divergent lineage (16S rRNA gene uncorrected *P*-distance > 5.3%), which clearly differs from any other *Nanohyla* species based on a series of morphological characters, most notably the presence of white spots on the top of its head. Morphologically, *Nanohyla albopunctata* sp. nov. is characterized by small body size (male snout–vent length (SVL) 18.2–20.2 mm); moderately slender body habitus; rounded snout; distinct tympanum; rounded canthus rostralis; loreal region slightly concave; skin on dorsum tubercular, ventral surfaces smooth; mid-vertebral skin ridge and dorsomedial stripe absent; superciliary tubercles absent; supratympanic fold indistinct; finger I reduced, less than half of finger II in length; II–IV fingers bearing discs with weak terminal grooves; two distinct palmar tubercles; two metatarsal tubercles; hindlimbs long, tibiotarsal articulation of adpressed limb reaching well beyond snout; fingers free of webbing; toe webbing formula: I 1–2 II 1–2 III 1–2 IV 1½–1 V; dorsum varying from dark gray to yellowish-gray, with darker "teddy-bear"-shaped brown marking; posterior surfaces of thighs and cloacal region with several brown stripes; chin, chest, and belly with gray mottling. We also report on the male advertisement call of the new species, characterized by a series of rattling sounds, consisting of 2–6 calls lasting 0.63 s, with 1–3 initial pulses and 5–9 successive pulses at a dominant frequency of ca. 3.02 kHz. To date, *Nanohyla albopunctata* sp. nov. is known only from the monsoon lowland tropical forest at the foothills of the Ca Mountain Range in Phu Yen Province of southern Vietnam at elevations of 200–400 m a.s.l., uncommon for the generally mountain-restricted *Nanohyla* genus. Our discovery brings the total number of *Nanohyla* species to 10, seven of which occur in Vietnam. We preliminary suggest the new species be considered as Data Deficient (DD) following the IUCN Red List categories.

Frogs of the genus *Nanohyla* Poyarkov, Gorin, & Scherz (Gorin et al., 2021), until recently classified as members of the genus *Microhyla* Tschudi, are known for their small size, with some species approaching the lower body-size limit for vertebrates. Miniaturization in this group results in

considerable cryptic diversity, obscured by widespread homoplasies (Gorin et al., 2021), making taxonomic study extremely challenging. Species of *Nanohyla* are restricted to the montane forests of the Truong Son Mountains in Vietnam, Laos, and northeastern Cambodia, the Titiwangsa Mountains in Thailand and peninsular Malaysia, the lowland and montane forests of Borneo, and the Sulu Archipelago in the Philippines (Gorin et al., 2021). To date, six out of nine nominal *Nanohyla* species have been reported from Vietnam, including: *N. annamensis* (Smith), *N. arboricola* (Poyarkov, Vassilieva, Orlov, Galoyan, Tran, Le, Kretova, & Geissler), *N. hongiaoensis* (Hoang, Luong, Nguyen, Orlov, Chen, Wang, & Jiang), *N. marmorata* (Bain & Nguyen), *N. nanapollexa* (Bain & Nguyen), and *N. pulchella* (Poyarkov, Vassilieva, Orlov, Galoyan, Tran, Le, Kretova, & Geissler) (Poyarkov et al., 2021). However, recent progress in the taxonomic study of microhylids in Vietnam suggests still neglected diversity (Gorin et al., 2020; Hoang et al., 2020; Poyarkov et al., 2014, 2018a, 2018b).

During field surveys in the Song Hinh Protected Forest (hereafter, SHPF) in Phu Yen Province, Vietnam, we collected three adult microhylid frogs, which were tentatively identified as *Nanohyla* sp. These specimens differed from all other described *Nanohyla* species by having characteristic white spots between the eyes. Subsequent integrative analysis based on morphological, acoustic, and molecular data demonstrated that the SHPF population represents a distinct evolutionary lineage, which could not be assigned to any known *Nanohyla* species. Therefore, we herein describe this population as a new species.

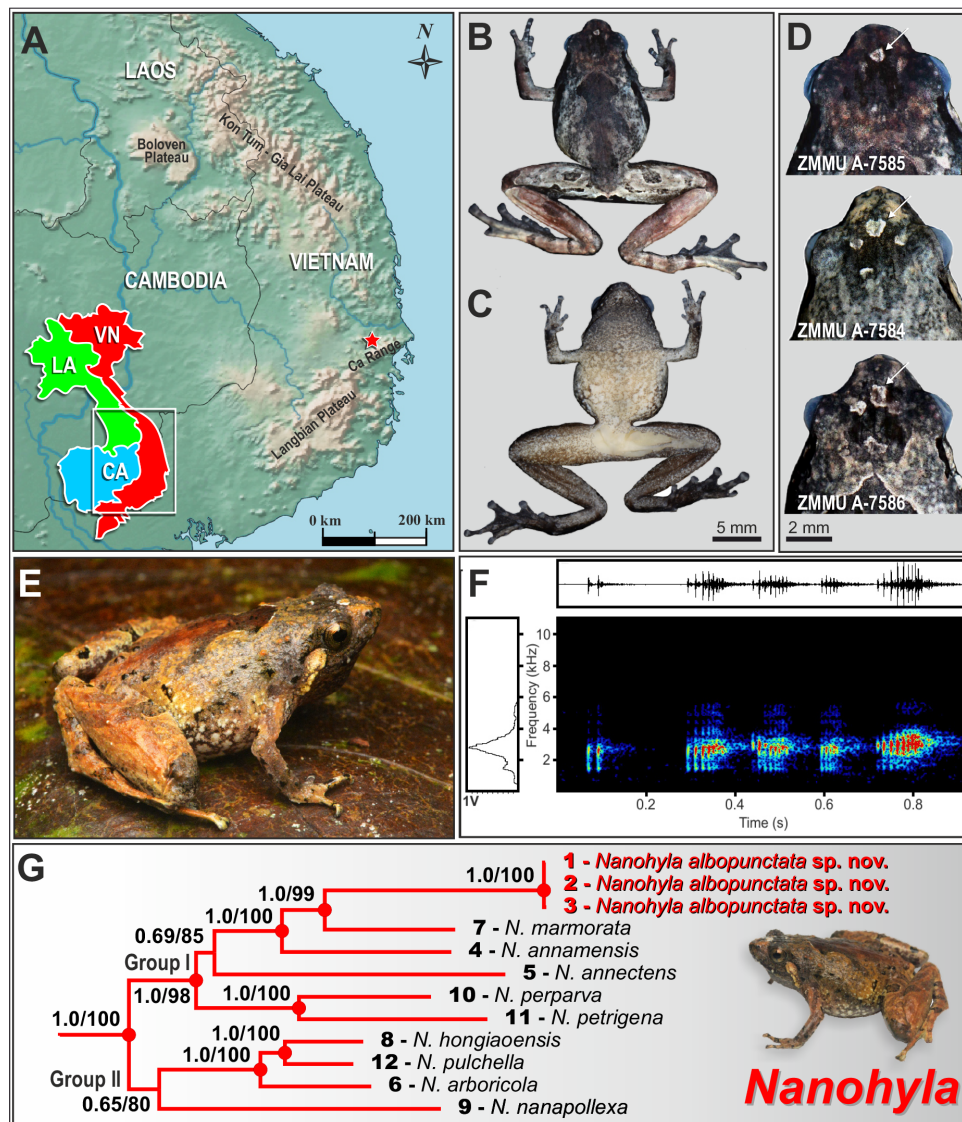
Fieldwork was conducted in SHPF, Song Hinh District, Phu Yen Province, Vietnam (Figure 1A), by N.A. Poyarkov and Le Xuan Dac in January 2021. Details on specimen collection and preservation are presented in the Supplementary Materials. Specimens were deposited in the herpetological collection of the Zoological Museum of Moscow State University (ZMMU) in Moscow, Russia. Descriptions of morphological characteristics followed Poyarkov et al. (2014, 2019) (see Supplementary Materials). Advertisement calls were recorded at the breeding site. Analyses of acoustic data generally followed Poyarkov et al. (2019) and are detailed in the Supplementary Materials.

For phylogenetic analyses, we sequenced a fragment spanning the 12S–16S region of mtDNA and the *BDNF* gene of nuDNA. We combined these data with a dataset obtained from a previous study on the group (Gorin et al., 2021) (see Supplementary Materials). GenBank accession numbers,

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**Figure 1** Phylogenetic relationships, acoustics and external morphology of *Nanohyla albopunctata* sp. nov.

A: Type locality of *Nanohyla albopunctata* sp. nov. in Song Hinh Protected Forest, Phu Yen Province, Vietnam. B: Holotype of *Nanohyla albopunctata* sp. nov. (ZMMU A-7585) in dorsal view. C: Holotype of *Nanohyla albopunctata* sp. nov. (A-7585) in ventral view. D: Dorsal view of heads of the type series of *Nanohyla albopunctata* sp. nov., showing characteristic white blotches. E: Holotype of *Nanohyla albopunctata* sp. nov. (A-7585) in life. F: Sonogram of a male advertisement call of *Nanohyla albopunctata* sp. nov. G: Phylogenetic relationships of *Nanohyla* based on concatenated 12S-16S rRNA and *BDNF* gene sequences. For a full version of a tree see Supplementary Figure S3. Values at the nodes correspond to BIPP/UBF support values, respectively; filled circles represent strongly supported nodes with BIPP and UFB support >0.95 and 95%, respectively; nodes lacking circles are not supported. For specimen and sequence data see Supplementary Table S1. Photos by N. A. Poyarkov.

museum vouchers, and localities of specimens used in this study are summarized in Supplementary Table S1. DNA extraction, amplification, and sequencing protocols followed Poyarkov et al. (2018a) and are detailed in the Supplementary Materials. We reconstructed the phylogeny using Bayesian inference (BI) and maximum-likelihood (ML) approaches (see Supplementary Materials). The concatenated mtDNA+nuDNA dataset comprised 65 specimens, including all nine currently recognized species in the genus *Nanohyla*.

The BI and ML methods yielded highly similar topologies and phylogenetic relationships for the *Microhyla-Nanohyla-Glyphoglossus* assemblage (Supplementary Figure S1). Although the assemblage itself received strong nodal support (1.0/100; hereafter nodal support is given as Bayesian inference posterior probabilities (BIPP)/ ultrafast bootstraps (UBS), respectively), the relationships among the three genera

remained essentially unresolved (0.64/54). As detailed descriptions of the phylogenetic relationships within the assemblage have already been provided by Gorin et al. (2020, 2021), we only focused on the relationships within the genus *Nanohyla*. Our results showed that *Nanohyla* is monophyletic (1.0/100), composed of two major groups (Figure 1G). The first group (Group I; 1.0/98) contained *N. annamensis*, *N. annectens*, *N. marmorata*, *N. perparva*, *N. petrigena*, and *Nanohyla* sp. from SHPF. Within this clade, two species from Borneo and adjacent islands (*N. perparva* and *N. petrigena*) were recovered as sister species (1.0/100). Three species from Indochina (*N. annamensis*, *N. marmorata*, and *Nanohyla* sp. from SHPF) formed a strongly supported clade (1.0/100). Among them *N. marmorata* was recovered as a sister species (1.0/99) to *Nanohyla* sp. from SHPF. The second group of *Nanohyla* received poor nodal support (Group II, 0.65/80) and

contained *N. arboricola*, *N. hongiaoensis*, *N. nanapollexa*, and *N. pulchella*. Within this clade, *N. nanapollexa* formed a sister lineage to the remaining taxa, while *N. pulchella* and *N. hongiaoensis* were recovered as sister species (1.0/100) (Figure 1G).

Uncorrected *P*-distances for the 16S rRNA gene fragment between *Nanohyla* species are shown in Supplementary Table S2. The interspecific distances within *Nanohyla* varied from *P*=2.5% (between *N. hongiaoensis* and *N. pulchella*) to *P*=9.4% (between *N. nanapollexa* and *Nanohyla* sp. from SHPF). The newly discovered population of *Nanohyla* from SHPF was most closely related to *N. marmorata*, showing a significant level of genetic distinctiveness (*P*=5.3%), sufficiently higher than the formal threshold of *P*=3.0% used to indicate species-level status in anuran biodiversity surveys (Vieites et al., 2009).

Our phylogenetic results demonstrated that the *Nanohyla* sp. from SHPF differs from all other congeners and likely represents an independent evolutionary lineage. These findings are consistent with the observed morphological and acoustic differences (see below in “Comparisons”). Thus, based on our integrative taxonomic approach, we formally describe the SHPF population of *Nanohyla* as a new species below.

#### Taxonomic account

##### *Nanohyla albopunctata* Poyarkov, Gorin, & Trofimets sp. nov.

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(Figure 1; Supplementary Figures S1–S4 and Tables S3, S4) Song Hinh pygmy narrow-mouthed frog (English); Nhái bầu đốm trắng (Vietnamese)

**Holotype:** ZMMU A-7585, adult male (Figure 1B–E; Supplementary Figure S1), collected in Song Hinh Protected Forest, Song Hinh District, Phu Yen Province, Vietnam (N12.77522°, E109.04606°; 310 m a.s.l.) by N.A. Poyarkov and Le Xuan Dac on 15 January 2021.

**Paratypes:** Two adult males ZMMU A-7584 and ZMMU A-7586 (Supplementary Figure S2), with the same collection information as the holotype.

**Diagnosis:** *Nanohyla albopunctata* sp. nov. is characterized by a combination of the following characters: small body size (male SVL 18.2–20.2 mm); body moderately slender; snout rounded in lateral view; tympanum distinct; canthus rostralis rounded, distinct; loreal region slightly concave; nares round, oriented anterolaterally; distinct white spots on top of head; skin on dorsum tubercular, ventral surfaces smooth; dorsolateral row of glandular tubercles; mid-vertebral skin ridge and dorsomedial stripe absent; superciliary tubercles absent; supratympanic fold indistinct; finger I small, less than half of finger II in length; II–IV fingers bearing discs with weak terminal grooves; all toes bearing prominent discs with dorso-terminal grooves; two distinct palmar tubercles (inner palmar tubercle ovoid, outer palmar tubercle rounded); two metatarsal tubercles (inner elongated, prominent; outer rounded, protruding); hindlimbs long, tibiotarsal articulation of adpressed limb reaching well beyond snout; fingers free of webbing, dorsolaterally flattened; toe webbing well-developed, webbing formula: I 1–2 II 1–2 III 1–2 IV 1½–1 V; dorsum varying from dark gray to yellowish-gray, with darker “teddy-bear”-shaped brown marking; posterior surfaces of thighs and cloacal region with several brown stripes; chin, chest, and

belly with gray mottling.

**Description of holotype:** Adult male (Figure 1B–E; Supplementary Figure S1), good state of preservation, SVL 17.6 mm; body slender, head wider than long (HL/HW 0.74); snout nearly rounded in dorsal view, rounded in profile, slightly protruding above lower jaw, less than eye diameter (EL/SL 0.86); eye rounded, protuberant both in lateral and dorsal views, pupil circular; dorsal surface of head flat, canthus rostralis rounded, distinct; loreal region slightly concave; nostrils rounded, placed more towards lateral sides of snout, located closer to tip of snout than to eye (N-EL/SL 0.55); tympanum externally distinct; supratympanic fold indistinct; maxillary and vomerine teeth absent; vocal sac single, subgular; tongue elliptical, entire and free at base, lacking papillae.

Forelimbs short, one-third of hindlimb length (FLL/HLL 31.6%); hand short, comprising 42.5% of lower arm length (HAL/LAL) and 32.4% of forelimb length (HAL/FLL); fingers thin, rounded in cross-section, finger I reduced, less than half of finger II in length; relative length of fingers: I<II<IV<III. Finger webbing absent; dermal fringes on fingers absent; tips of all fingers flattened, tip of finger I not enlarged, tips of fingers II–IV notably widened, forming discs, lacking median longitudinal grooves, with weak dorso-terminal grooves; third finger disc largest; subarticular tubercles on volar surface of fingers barely distinct, flattened; finger subarticular tubercle formula: 1:1:2:2; nuptial pad absent; two palmar tubercles: inner palmar tubercle rounded; outer palmar tubercle ovoid-shaped, longer than inner (IPTL/OPTL 66.6%); inner and outer palmar tubercles separated by narrow space lacking medial or supernumerary palmar tubercles.

Hindlimbs comparatively long, tibia much longer than half of snout-vent length (TL/SVL 68.7%), hindlimb almost two times longer than snout-vent length (HLL/SVL 198.3%); tibiotarsal articulation of adpressed limb reaching well beyond snout (checked prior to preservation); tibia longer than foot (FL/TL 80.0%); relative toe lengths: I<II<V<III<IV; tarsal fold on inner surface of tarsus absent; tips of all toes widened, forming broad and flattened terminal discs; all toe discs with dorso-terminal grooves; discs on toes III and IV largest, distally with tiny median dorso-longitudinal notches not reaching one-third of disc length; toes long, thin, slightly flattened in cross-section; toe webbing well developed between all toes, reaching discs at all toes except toe IV, webbing formula: I 1–2 II 1–2 III 1–2 IV 1½–1 V; toe subarticular tubercles distinct, rounded, slightly protruding; toe subarticular tubercle formula: 1:1:2:3:2; two metatarsal tubercles: inner metatarsal tubercle elongated, oval, prominent; outer metatarsal tubercle small, rounded, protruding.

Skin on dorsum tubercular; tympanum distinct; several large glandular tubercles forming row in supratympanic area running from posterior eye corner dorsolaterally to groin; upper eyelids covered with several tiny tubercles along outer edge of eyelids; large superciliary tubercles or projections absent; mid-vertebral dermal ridge absent; skin on dorsolateral surfaces shagreened; dorsal surfaces of limbs covered with numerous small flattened tubercles, ventral surfaces smooth.

**Coloration of holotype** (Figure 1B–E): In life, dorsal surfaces of head and body brownish-gray; dorsal surface of snout light gray; distinctly darker reddish-brown “teddy-bear”-shaped dorsal marking at starting scapular region, running posteriorly; pair of rounded light beige spots on dorsal surface in sacral area; azygous light brown blotch above cloaca. Head dorsally

with five distinct white spots, larger spot triangular, located medially between anterior corners of eyes, two smaller spots located at medial edges of upper eyelids of both eyes, two less distinct white spots located posteriorly on head midline at level of posterior corners of eyes. Body flanks dull gray with numerous tiny white speckles; eyelids brownish gray; lateral sides of head gray with dark mottling. Dorsal surfaces of forelimbs brown; black spots on dorsal surface of each forearm located closer to distal end. Dorsal surfaces of thighs and shanks reddish-brown with two thin dark brown lines alternating on each hindlimb, forming continuous pattern when leg folded; cloacal area with large black blotch dorsally edged with beige and with small lighter brown medial streak. Fingers and toes dorsally reddish to light brown. Ventral surfaces with very thin gray mottling, becoming denser towards throat; ventral surfaces of limbs gray; hands and feet ventrally with dark gray mottling. Pupil black, circular, edged with narrow copper line, dense copper reticulations throughout iris; sclera dark. After preservation in ethanol for two years, coloration faded and turned dark gray, ventral surfaces faded to light gray. Light markings on dorsum and dark stripes on limbs still distinct, and coloration pattern generally remained unchanged.

**Variation:** Morphometric variation of the type series is presented in Supplementary Table S3. In general, all paratypes agreed well with the description of the holotype. Males (adult male SVL 18.2–20.2 mm, mean  $19.4 \pm 0.86$  mm;  $n=3$ ) of the new species are larger than most other *Nanohyla* species (Supplementary Table S4); females of the new species were not recorded. Coloration of the paratypes matched that of the holotype; all specimens exhibited distinctive white spots on the dorsal surface of the head (Figure 1D).

**Advertisement calls:** Males were observed calling from the banks of a small (ca. 2 m in diameter) temporary puddle on a forest road within the polydominant evergreen tropical forest. Calling males concealed themselves in leaf litter on the water's edge and when disturbed, jumped into the water and floated on the surface, emitting a few calls. The male advertisement call of the new species consisted of a characteristic series of rattling sounds, resembling the sound of a ratchet to the human ear, similar to "krrrrr... krrrrrr... krrrrrr...". The series contained 2–6 calls with pulses (Supplementary Table S5; Figure 1F). Usually, the first call of the series included 1–3 pulses ( $2.62 \pm 0.52$ ), while successive calls consisted of  $7.49 \pm 0.23$  pulses (1–13). The intervals between successive pulses within a call varied slightly, usually decreasing from the beginning to the end of the call. Males called in small groups of two to three individuals, with the vocalizations of one stimulating the rest to call. This resulted in overlapping series of calls and reduced distances between series, which led to increased pulse repetition rates and reduced intervals between successive calls and pulses, likely due to increased arousal levels during chorusing.

At present, the advertisement calls for only two *Nanohyla* species have been described: i.e., *N. petrigena* and *N. marmorata* (see Poyarkov et al., 2019, and references therein). The advertisement call of the new species differs from that of *N. marmorata* by lower number of pulses per call (2–6 vs. 9–13) and from that of *N. petrigena* by lower call peak frequency ( $F_{\text{peak}} 3\,020 \pm 27.6$  vs.  $4\,430 \pm 32.2$ ) (Supplementary Table S5; Poyarkov et al., 2019).

**Distribution and Natural history:** *Nanohyla albopunctata* sp. nov. is currently known exclusively from its type locality in

SHPF in Phu Yen Province, Vietnam. The new species was recorded at comparatively low elevations (ca. 200–400 m a.s.l.) within the low- to mid-elevation tropical forests of the Ca Mountain Range. The actual extent of its distribution is unknown. Males were recorded at night (after 1900h) calling from the banks of a small temporary pool on the forest road. Syntopic species of amphibians included *Occidozyga martensii*, *Theloderma truongsongense*, *Rhacophorus annamensis*, *Polypedates megacephalus*, and *Leptobrachium pullum*. Breeding of the new species was observed in January. However, further information on its ecology, including diet and potential predators, is lacking.

**Etymology:** The specific name "*albopunctata*" is a Latin adjective in the nominative singular, meaning "white-spotted". The name is given in reference to the distinctive white spots found on the top of the head of the new species.

**Comparisons:** Morphological comparisons of the new species with other congeners are summarized in Supplementary Table S4. The most distinctive feature of *Nanohyla albopunctata* sp. nov. is the presence of characteristic white spots on the top of its head, which are not reported in any other *Nanohyla* species. Specifically, *Nanohyla albopunctata* sp. nov. can be differentiated from its sister species *N. marmorata*, distributed in central and northern Vietnam, by smaller head size (HL/SVL 29.2%–20.8% vs. 34.1%–37.2%); comparatively wider head (HW/SVL 40.6%–44.0% vs. 36.7%–37.5%); head wider than long HW/HL 139.0%–142.9% (vs. subequal HW/HL 98.6%–110.1%); shorter foot length (FL/SVL 54.9%–56.4% vs. 77.2%–77.7%); body habit moderately slender (vs. moderately stocky); snout profile rounded (vs. bluntly rounded); dorsum skin tubercular (vs. smooth or feebly pustular); and foot webbing formula (I 1–2 II 1–2½ III 1–2 IV 2–1 V vs. I 1–2 II 1–1¾ III 1½–2¾ IV 2¾–1 V).

The SHPF is located on the northern slopes of the Ca Mountain Range, the northeastern outcrop of the Langbian Plateau, closely approaching the sea in Phu Yen Province. The area is characterized by high annual rainfall (ca. 3 500 mm/year), with over 180 rainy days a year on average. The Ca Mountain Range climate likely provides suitable ecological niches for typical montane amphibians, such as *Nanohyla* species, even at relatively low elevations. During our SHPF survey, we recorded several other montane species, including megophryids (*Ophryophryne elfina* and *Leptobrachella macrops*) and rhacophorids (*Theloderma truongsongense*), at relatively low elevations (200–400 m a.s.l.). Nguyen et al. (2020) also recorded *Gonyosoma boulengeri* (Colubridae) in SHPF, which represents the southernmost known locality of this species, typically distributed in southern China and northern Vietnam. The unique environmental conditions of SHPF, along with the currently revealed herpetofaunal endemism (Duong et al., 2018), calls for additional conservation of this territory. Today, habitat loss is considered one of the greatest threats to amphibians in Southeast Asia, particularly Vietnam. Of all Indochinese countries, Vietnam has the highest relative number of threatened amphibian species, along with the lowest proportion of species of least concern. The need for biological exploration of this region is urgent due to the intensification of logging, road construction, agricultural pressure, and other human activities (Poyarkov et al., 2021). Further studies on the herpetofauna of SHPF are required for urgent and adequate evaluation of local diversity and respective conservation measures.

## NOMENCLATURAL ACTS REGISTRATION

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## SCIENTIFIC FIELD SURVEY PERMISSION INFORMATION

Permission for field surveys in Song Chinh Protected Forest was granted by the People's Committee of Phu Yen Province, Vietnam (#05/UBND-KT of 04.01.2021).

## SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

## COMPETING INTERESTS

The authors declare that they have no competing interests.

## AUTHORS' CONTRIBUTIONS

N.A.P. and V.A.G. designed the study. N.A.P. and L.X.D. collected specimens in the field. A.V.T. and V.A.G. performed molecular experiments. V.G., S.S.G., and N.A.P. performed data analyses. V.A.G. and N.A.P. wrote the manuscript. A.V.T., L.X.D., and N.A.P. revised the manuscript. All authors read and approved the final version of the manuscript.

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Vladislav A. Gorin<sup>1</sup>, Alexey V. Trofimets<sup>1</sup>, Svetlana S. Gogoleva<sup>2,3</sup>, Le Xuan Dac<sup>2</sup>, Nikolay A. Poyarkov<sup>1,2,\*</sup>

<sup>1</sup> Department of Vertebrate Zoology, Lomonosov Moscow State University, Moscow 119991, Russia

<sup>2</sup> Joint Vietnam-Russia Tropical Science and Technology Research Centre, Cau Giay, Hanoi 122000, Vietnam

<sup>3</sup> A.N. Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow 119071, Russia

\*Corresponding author, E-mail: n.poyarkov@gmail.com

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