Zoological Research

Supplementary Materials

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

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SUPPLEMENTARY MATERIALS AND METHODS

Specimen collection. Fieldwork was conducted in Song Hinh Protected Forest, Song Hinh District, Phu Yen Province, Vietnam by N.A. Poyarkov and Le Xuan Dac (Figure 1A) in January, 2021. Details on specimen collection and preservation presented in Supplementary Data. Specimens were deposited in the herpetological collections of Geographic coordinates were obtained using Garmin GPSMAP 60CSx and recorded in the WGS 84 datum. Specimens were collected at night by locating calling males and photographed in life *in situ* (Figure 1D) before being euthanized by 20% benzocaine. Femoral muscles and liver were sampled for genetic analyses and stored subsequently in 96% ethanol prior to preservation. Specimens were fixed in 4% formalin, transferred subsequently to 70% ethanol for preservation and deposited in herpetological collection of the Zoological Museum of Moscow State University (ZMMU) in Moscow, Russia.

External morphology. Measurements were taken using a digital caliper to the nearest 0.01 mm, subsequently rounded to 0.1 mm. We used a stereoscopic light binocular microscope when necessary. All measurements were taken on the right side of the examined specimen.

The morphometrics of adults and character terminology followed Poyarkov et al. (2014, 2018, 2019) and included the following measurements: (1) snout–vent length (SVL; measured from tip of snout to cloaca); (2) head length (HL; measured from tip of snout to hind border of jaw angle); (3) snout length (SL; measured from anterior margin of eye to tip of snout); (4) eye length (EL; measured as the distance between anterior and posterior margins of the eye); (5) nostril–eye length (N–EL; measured as the distance between the anterior margin of the eye and the nostril center); (6) head width (HW; measured as the maximum width of the head at the level of mouth angles in ventral view); (7) internarial distance (IND; measured as the distance between central points of nostrils); (8) interorbital distance (IOD; measured as the shortest distance between medial edges of eyeballs in dorsal view); (9) upper eyelid width (UEW; measured as the maximum distance between medial edge of eyeball and lateral edge of upper eyelid); (10) fore limb length (FLL; measured as the length of straightened fore limb to tip of third finger); (11) lower arm and hand length (LAL; measured as the distance between elbow and tip of third finger); (12) hand length (HAL; measured as the distance between proximal end of outer palmar (metacarpal) tubercle and tip of third finger); (13) first finger length (1FL, measured as the distance between tip and distal end of inner palmar tubercle); (14) inner palmar tubercle length (IPTL; measured as the maximum distance between proximal and distal ends of inner palmar tubercle); (15) outer palmar tubercle length (OPTL; measured as the maximum diameter of outer palmar tubercle); (16) third finger disc diameter (3FDD); (17) hind limb length (HLL; measured as the length of straightened hind limb from groin to tip of fourth toe); (18) tibia length (TL; measured as the distance between knee and tibiotarsal articulation); (19) foot length (FL; measured as the distance between distal end of tibia and tip of fourth toe); (20) inner metatarsal tubercle length (IMTL; measured as the maximum length of inner metatarsal tubercle); (21) first toe length (1TOEL), measured as the distance between distal end of inner metatarsal tubercle and tip of first toe; (22) third toe disc diameter (4TDD). Additionally, we took the following measurements for holotype description: (23–25) second to fourth finger lengths (2–3FL-O, 4FL-I; for outer side (O) of the second and third, and inner side (I) of the fourth, measured as the distance between tip and junction of the neighboring finger); (26–29) second to fifth toe lengths (measured as the outer lengths for toes II–IV, as the inner length for toe V; 2–5TOEL); (30–32) finger disc diameter for fingers I–II and IV (1–2FDD, 4FDD); (33–36) toe disc diameter for toes I–II and IV–V (1–2TDD, 4–5TDD).

Toe webbing and subarticular tubercle formulas followed Savage (1975). The sex and maturity of the specimens were checked by minor dissections and by direct observation of calling in living males prior to collection.

Acoustic analysis. Advertisement calls of *Nanohyla* sp. were taken at the type locality on 12 and 13 January 2021 at 22.45 h and at 17 °C using a portable digital audio recorder Zoom h5 (ZOOM Corporation, Tokyo, Japan) in stereo mode with 48 kHz sampling frequency and 16-bit precision. The temperature was measured at the calling site immediately after the audio recording with a digital thermometer KTJ TA218A Digital LCD Thermometer-Hydrometer.

Males were observed calling from the banks of a small temporary puddle's on the forest road, usually they were hiding under the leaves approximately in 5-10 cm from the waters' edge. When disturbed by our observations, males jumped into the puddle where they began floating on the surface of the water, usually continuing to call. We also observed floating and calling males when we carefully approached the puddle with only red head-lights turned on, therefore we assume that calling from the water surface seems to be a common habit of the males of the new species and doesn't necessary happen when males are disturbed.

Calls were analyzed using Avisoft SASLab Pro software v.5.2.14 (Avisoft Bioacoustics, Germany). Before analysis, we reduced the background noise using a low-pass filter (up to 500 Hz). All temporal parameters were analyzed with the standard marker cursor in the main window of Avisoft and frequencies of the maximum amplitude of calls and pulses were measured in the power spectrum. The spectrogram for analysis was created using a Hamming window, with FFTlength 512 points, frame 75%, and overlap 93.75%. For graphic representation of spectrograms, we lowered the sampling rate to 22.05 kHz. Figures of spectrograms were created using a Hamming window, with FFT-length 512 points, frame 50%, and overlap 93.75%. In total, we measured 99 calls from three *Nanohyla* males.

We measured seven temporal parameters: i. e., series duration, number of calls per series, call duration, intervals between successive calls within series, number of pulses per call, duration of pulses, intervals between successive pulses; and two power parameters: i.e., frequency of maximum amplitude (Fpeak) of calls and of pulses. Additionally, we calculated the pulse repetition rate (pulses/s) by counting the number of pulses within each call minus one and dividing that number by the call duration. Descriptive statistics were performed in R 4.0.3 (R Core Team, 2020) using the "descriptive" function in the "psych 2.1.6" package. Most numeral parameters are given as means±*SE* and the minimum and maximum values are given in parentheses (min-max).

Laboratory methods. For molecular analyses, we extracted total genomic DNA from ethanol-preserved liver or femoral muscle tissue using standard phenol–chloroform–proteinase K extraction procedures with consequent isopropanol precipitation (protocols followed Hillis et al., 1996; Sambrook and Russell, 2001). We visualized the isolated total genomic DNA in agarose electrophoresis in the presence of ethidium bromide. We measured the concentration of total DNA in 1 μL using NanoDrop 2000 (Thermo Scientific), and consequently adjusted to ca. 100 ng DNA/μL. We amplified mtDNA fragments covering partial sequences of mitochondrial 12S rRNA and 16S rRNA and the complete sequence of tRNAVal to obtain a continuous fragment 2398 bp in length, and nuDNA fragment of BDNF gene 720 bp in length. These 16S rRNA mtDNA gene has been widely applied in biodiversity surveys in amphibians (Vences et al., 2005a, 2005b; Vieites et al., 2009; Matsui et al., 2011; Rakotoarison et al., 2017). We performed DNA amplification in 20-μL reactions using ca. 50 ng genomic DNA, 10 nmol of each primer, 15 nmol of each dNTP, 50 nmol additional MgCl2, Taq polymerase chain reaction (PCR) buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.1 mM MgCl2 and 0.01% gelatine) and 1 U of Taq DNA polymerase. Primers used in PCR and sequencing followed Gorin et al. (2020).

The PCR conditions followed Gorin et al. (2020) and included an initial denaturation step of 5 min at 94°C and 43 cycles of denaturation for 1 min at 94°C; primer annealing for 1 min with a touchdown programme from 65 to 55°C, reducing by 1°C every cycle; extension for 1 min at 72°C; and final extension step for 5 min at 72°C.

We loaded PCR products onto 1.0% agarose gels in the presence of ethidium bromide and visualised in agarose electrophoresis. We purified the successful PCR products using 2 μL of a 1:4 dilution of ExoSapIt (Amersham) per 5 μL of PCR product prior to cycle sequencing. A 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 version 3.1 Sequencing Standard (Applied Biosystems) and 4.2 μL of water. Successful targeted PCR products were outsourced to Evrogen® (Moscow, Russia) for PCR purification and sequencing. Sequence data collection and visualization were carried out on an ABI 3730xl Automated Sequencer (Applied Biosystems). The obtained sequences were deposited in GenBank under the accession numbers MZ702077–MZ702079 (12S rRNA), MZ702092–MZ702094 (16S rRNA) and MZ708796–MZ708798 (BDNF) (Supplementary Table S1).

Phylogenetic analyses. To estimate phylogenetic relationships, we used the concatenated mt- and nuDNA dataset of Gorin et al. (2021) with the addition of the sequences of *Glyphoglossus huadianensis* (Zhang et al., 2021) and our newly obtained sequences (see Supplementary Table S1), thus covering all major lineages within the *Microhyla-Nanohyla-Glyphoglossus* assemblage. The initial dataset was cut to one sequence per species, *Kaloula baleata* was used as an outgroup to root the tree. In total, concatenated mt- and nuDNA data for 65 specimens were included in the final analysis, including all nine recognized species of the genus *Nanohyla*.

Nucleotide sequences were initially aligned in MAFFT v. 6 (Katoh et al., 2002) with default parameters, and subsequently checked by eye in BioEdit v. 7.0.5.2 (Hall, 1999) and slightly adjusted. We determined mean uncorrected genetic distances (p-distances) between sequences with MEGA 6.0 (Tamura et al., 2013). MODELTEST v. 3.6 (Posada and Crandall, 1998) was applied to estimate the optimal evolutionary models for the subsequent analyses.

We reconstructed phylogeny using Bayesian Inference (BI) and Maximum Likelihood (ML) approaches. We conducted BI in MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003); Metropolis-coupled Markov chain Monte Carlo (MCMCMC) analyses were run with one cold chain and three heated chains for one million generations and sampled every 1000 generations. We performed two independent MCMCMC runs and the initial 100 trees were discarded as burn-in. We assessed confidence in tree topology by the frequency of nodal resolution (posterior probability; BI PP) (Huelsenbeck and Ronquist, 2001). We used IQ-TREE (Nguyen et al., 2015) to reconstruct ML phylogenies. Confidence in tree topology for ML analysis was assessed by 10,000 ultrafast bootstrap replications for ML analysis (UFB) (Minh et al., 2013). In both datasets, we regarded tree nodes with BI PP and UFB values over 0.95 to be sufficiently resolved a priori. BI PP and UFB values between 0.95 and 0.90 were regarded as tendencies. Lower values were considered to indicate unresolved nodes (Huelsenbeck and Hillis, 1993; Minh et al. 2013).

NOMENCLATURAL ACTS REGISTRATION

The electronic version of this article in portable document format represents a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new names contained in the electronic version are effectively published under that Code from the electronic edition alone (see Articles 8.5–8.6 of the Code). This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information can be viewed through any standard web browser by appending the LSID to the prefix http://zoobank.org/.

Publication LSID: urn:lsid:zoobank.org:pub:7384225D-095A-4671-8247-80B5C5904EC1

Nanohyla albopunctata, LSID: urn:lsid:zoobank.org:act:86E8EE57-DC21-4584-935D-C5FAC6B7F095

SUPPLEMENTARY RESULTS

Measurements of holotype (in mm): SVL 18.2; HL 5.6; SL 2.0; EL 1.9; N-EL 1.1; HW 8.2; IND 1.8; IOD 2.5; UEW 1.4; FLL 11.4; LAL 8.7; HAL 3.7; IPTL 0.6; OPTL 0.9; HLL 36.1; TL 12.5; FL 10.0; IMTL 0.9; 1FL 0.9; 2FL 1.2; 3FL 3.4; 4FL 1.5; 1TOEL 1.9; 2TOEL 2.1; 3TOEL 6.1; 4TOEL 7.7; 5TOEL 4.5; 1FDD 0.3; 2FDD 0.4; 3FDD 0.6; 4FDD 0.4; 1TDD 0.5; 2TDD 0.9; 3TDD 0.9; 4TDD 0.6; 5TDD 0.6.

Conservation status: At present, the new species is known only from its type locality in southern Vietnam. Given the lack of information, we suggest *Nanohyla albopunctata* **sp. nov.** to be considered a Data Deficient (DD) following the International Union for Conservation of Nature's Red List categories (IUCN Standards and Petitions Subcommittee, 2022).

Comparisons. Morphological comparisons of the nominal *Nanohyla* species are summarized in Supplementary Table S4. Clearly, the most distinguishable feature of *Nanohyla albopunctata* **sp. nov.** is presence of characteristic white spots on top of head, which were not reported for 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 having smaller head size (HL/SVL 29.2–20.8% vs. 34.1–37.2% in *N. marmorata*); by having comparatively wider head (HW/SVL 40.6–44.0% vs. 36.7–37.5% in *N. marmorata*); by having head wider than long (HW/HL 139.0–142.9%) vs subequal in *N. marmorata* (HW/HL 98.6–110.1%); by having shorter foot length (FL/SVL 54.9–56.4% vs. 77.2–77.7% in *N. marmorata*); by moderately slender body habitus (vs. moderately stocky in *N. marmorata*); rounded snout profile (vs. bluntly rounded in *N. marmorata*); by tubercular dorsum skin (vs. smooth or feebly pustular in *N. marmorata*); and by 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** in *N. marmorata*). *Nanohyla albopunctata* **sp. nov.** can be differentiated from *N. annamensis* by its generally larger male SVL 18.2-20.2 (vs. 12.2- 19.8); moderately slender body habit (vs. moderately stocky); rounded snout profile (vs. bluntly rounded); slightly tubercular skin on dorsum (vs. warty or strongly tubercular); OMT present (vs absent, see Gorin et al., 2021). The new species can be differentiated from *N. annectens* by its generally larger male SVL 18.2-20.2 (vs. 14.6-18.4); moderately slender body habit (vs. slender); tubercular skin (vs. smooth); OMT present (vs absent); distribution in southern Vietnam (vs. peninsular Thailand and Malaysia). *Nanohyla albopunctata* **sp. nov.** is clearly different from *N. arboricola* by notably larger SVL 18.2-20.2 (vs. 15.9-17.0); rounded snout profile (vs. pointed); tubercular dorsum skin (vs. feebly granular); OMT present (vs absent); well-developed web (vs. basal). The new species is different from *N. hongiaoensis* in its larger SVL 18.2-20.2 (vs. 13.5-14.6); moderately slender body habit (vs. slender); rounded snout profile (vs. bluntly rounded); tubercular dorsum skin (vs. scattered by small tubercles); FMG present (vs. absent). The new species can be differentiated from *N. nanapollexa* by its larger SVL 18.2-20.2 (vs. 13.5-16.6); moderately slender body habit (vs. slender); tubercular dorsum skin (vs. smooth); F1<1/2 F2 (vs. F1 reduced to nub or bulge); OMT present (vs absent); distribution in southern Vietnam (vs. central parts of Vietnam). *Nanohyla albopunctata* **sp. nov.** can be differentiated from *N. perparva* by its larger SVL 18.2-20.2 (vs. 10.9-14.5); moderately slender body habitat (vs. moderate); rounded snout profile (vs. obtusely pointed); tubercular dorsum skin (vs. smooth); F1<1/2 F2 (vs. F1 reduced to nub or bulge); OMT present (vs absent); distribution in southern Vietnam (vs Borneo). Finally, the new species is different from *N. petrigena* by larger F1<1/2 F2 (vs. F1 reduced to nub or bulge); OMT present (vs absent); distribution in southern Vietnam (vs 18.2-20.2); moderately slender (vs. moderately stout); rounded snout profile (vs. obtusely pointed); tubercular skin (vs. smooth); F1<1/2 F2 (vs. F1 reduced to nub or bulge); OMT present (vs absent); distribution in southern Vietnam (vs Borneo and Sulu Archipelago of Philippines).

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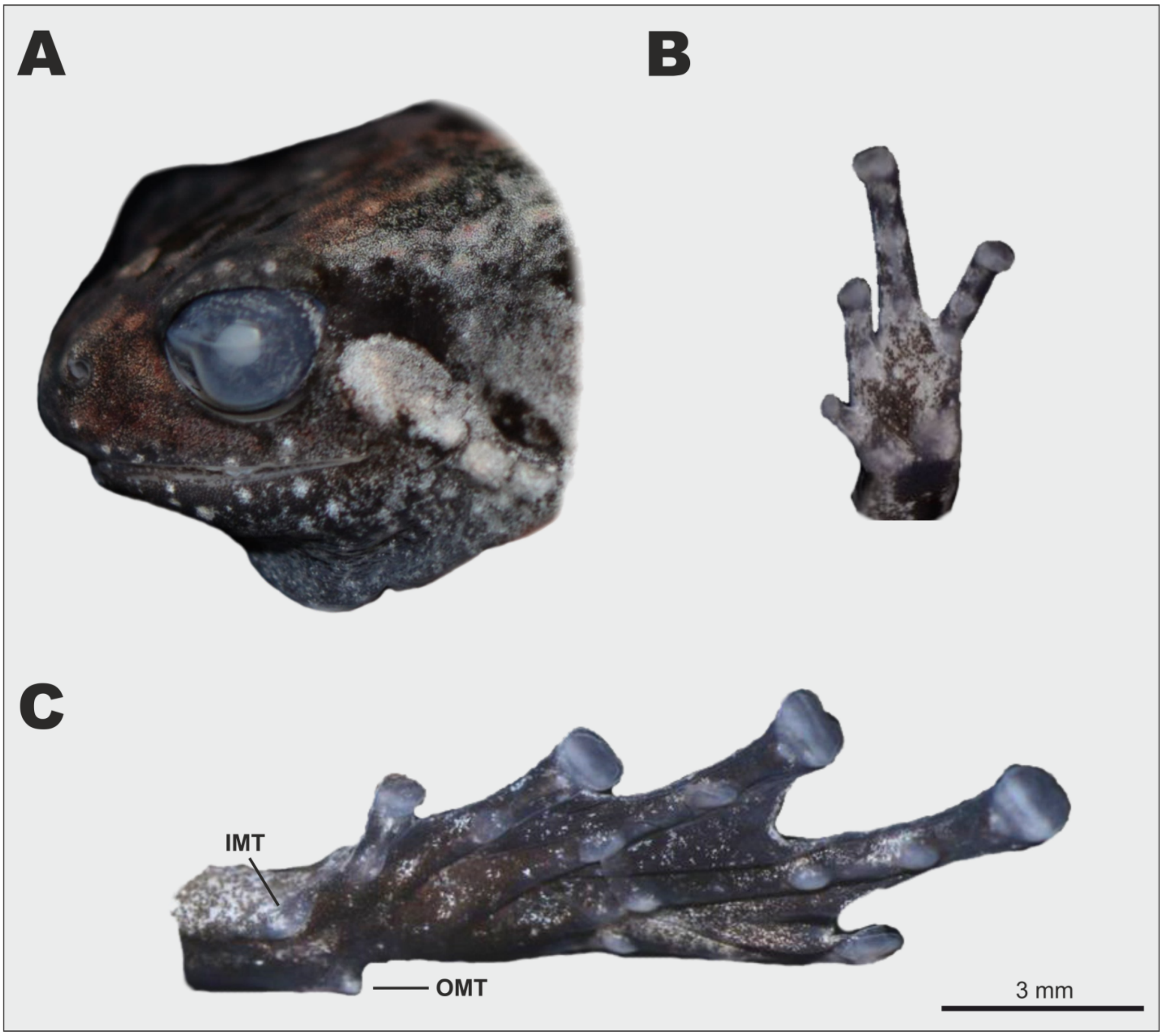
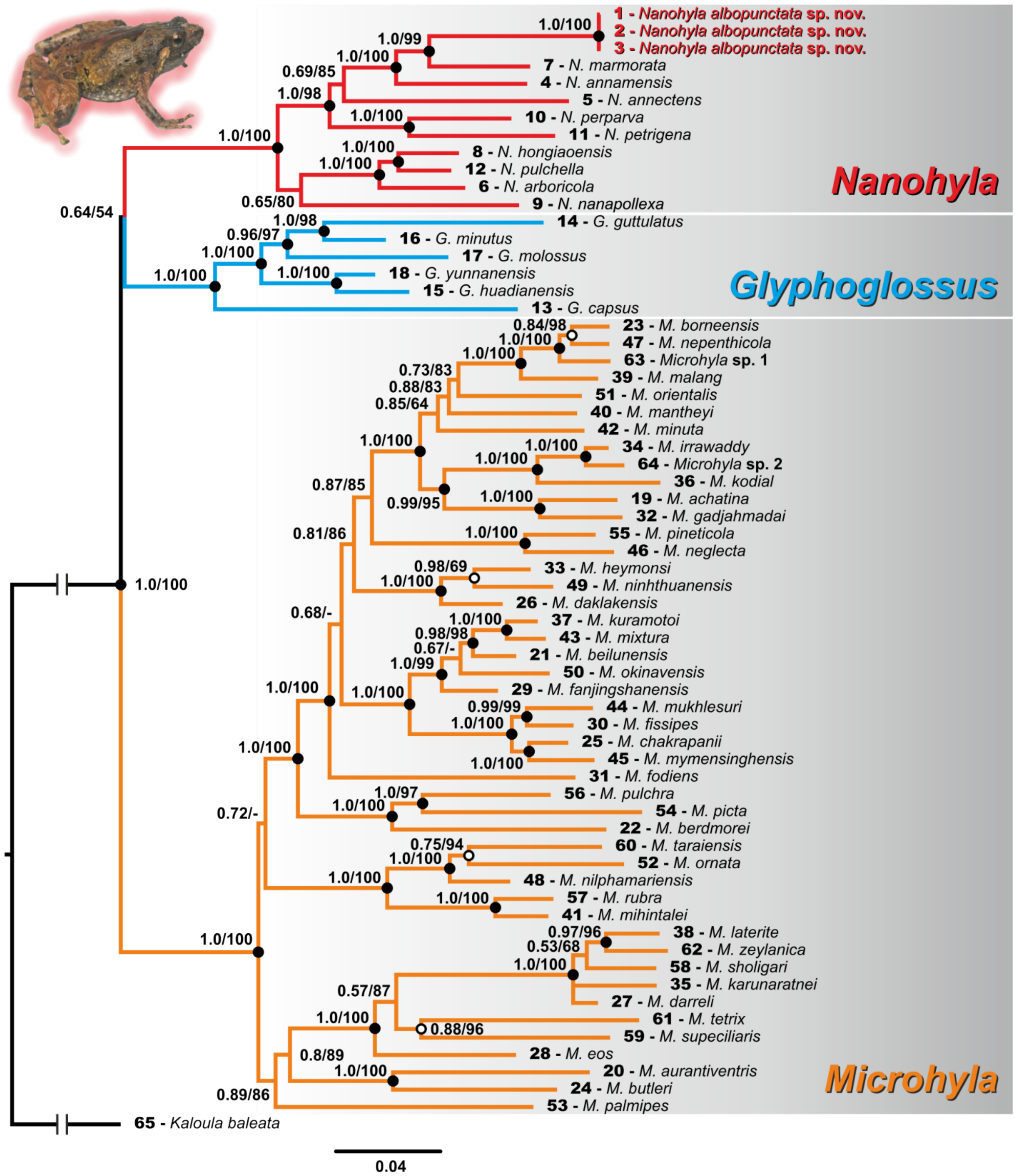


Supplementary Figure S1 Holotype of *Nanohyla albopunctata* **sp. nov.** (ZMMU A-7587). (A) Head in ventral view; (B) plantar view of left hand; (C) plantar view of left foot. IMT – inner metatarsal tubercle, OMT – outer metatarsal tubercle. Photographs by P. V. Yushchenko.

Supplementary Figure S2 Paratype males of *Nanohyla albopunctata* **sp. nov.** (ZMMU A-7584, left; and ZMMU A-7586, right), in dorsal view in preservative. Photographs by P. V. Yushchenko.



Supplementary Figure S3 Phylogenetic relationships of the *Microhyla-Nanohyla-Glyphoglossus* assemblage. Bayesian inference tree derived from the analysis of 3118 bp including long 12S—16S rRNA mtDNA and BDNF nuDNA gene fragments. For voucher specimen information and GenBank accession numbers see Supplementary Table S1. Colors denote genera of *Microhyla-Nanohyla-Glyphoglossus* assemblage: *Microhyla* (orange), *Nanohyla* (red) and *Glyphoglossus* (blue). Numbers at tree nodes correspond to the posterior probability (BI PP) and ultrafast bootstrap support (UFB) values, respectively. Circles filled with black represent strongly supported nodes with BPP and UFB support >0.95 and 95%, respectively; nodes lacking circles are not supported. *Kaloula baleata* sequence was used as an outgroup. Photograph of the new species by Nikolay A. Poyarkov.

Supplementary Table S1 Museum voucher information, geographic localities, and GenBank accession numbers of specimens and sequences used in this study.

No.	Species	Locality	Museum / Sample ID	Accession numbers			Reference
	Ingroup			**12S rRNA**	**16S rRNA**	**BDNF**	
1	*Nanohyla albopunctata* **sp. nov.**	Vietnam, Phu Yen, Song Hinh	ZMMU A-7584	MZ702077	MZ702092	MZ708796	*this paper*
2	*Nanohyla albopunctata* **sp. nov.**	Vietnam, Phu Yen, Song Hinh	ZMMU A-7585	MZ702078	MZ702093	MZ708797	*this paper*
3	*Nanohyla albopunctata* **sp. nov.**	Vietnam, Phu Yen, Song Hinh	ZMMU A-7586	MZ702079	MZ702094	MZ708798	*this paper*
4	*Nanohyla annamensis*	Vietnam, Lam Dong, Bidoup-Nui Ba NP	ZMMU A-5075-06	MN534748	MN534533, MN534639	MN534443	Gorin *et al.* 2020
5	*Nanohyla annectens*	Malaysia, Selangor, Genting	ZMMU A-6042-1	MN534746	MN534531, MN534637	MN534442	Gorin *et al.* 2020
6	*Nanohyla arboricola*	Vietnam, Dak Lak, Chu Yang Sin NP	ZMMU A-4845-60	MN534759	MN534543, MN534650	MN534447	Gorin *et al.* 2020
7	*Nanohyla marmorata*	Vietnam, Kon Tum, Kon Plong	ZPMSU 04854	MN534750	MN534535, MN534641	MN534445	Gorin *et al.* 2020
8	*Nanohyla hongiaoensis*	Vietnam, Lam Dong, Bidoup-Nui Ba N.P.	CIB-VNMN 07617	–	MN475176	–	Hoang *et al.* 2020
9	*Nanohyla nanapollexa*	Vietnam, Kon Tum, Kon Plong	ZMMU A-5635	MN534757	MN534541, MN534648	MN534444	Gorin *et al.* 2020
10	*Nanohyla perparva*	Indonesia, Kalimantan, Balikpapan	KUHE UN	AB634614	AB634672	–	Matsui *et al.* 2011
11	*Nanohyla petrigena*	Malaysia, Sabah, Maliau Basin	BORN 22412	AB634616	AB634674	KM509302	Matsui *et al.* 2011
12	*Nanohyla pulchella*	Vietnam, Lam Dong, Bidoup–Nui Ba NP, Ca Hoi	ZMMU A-5045	MN534765	MN534549, MN534656	MN534448	Gorin *et al.* 2020
13	*Glyphoglossus capsus*	Malaysia, Sarawak, Padawan, Gunung Penrissen mt.	UNIMAS MYS:9389	–	KJ488544	–	Das *et al.* 2014
14	*Glyphoglossus guttulatus*	Thailand, Kanchanaburi, Pilok	KUHE 35163	AB634627	AB634685	AB611864	Matsui *et al.* 2011
15	*Glyphoglossus huadianensis*	China, Yunnan, Lijiang	2014005781	–	MN860396	–	Zhang *et al.* 2021

16	*Glyphoglossus minutus*	Malaysia, Pahang, Temerloh	KUHE 52463	AB598316	AB598340	–	Matsui 2011
17	*Glyphoglossus molossus*	Thailand, Tak, Barrntak	KUHE 35182	AB201182	AB201193	EF396009	Matsui *et al.* 2005
18	*Glyphoglossus yunnanensis*	China, pet trade	KUHE 44148	AB634626	AB634684	KM509234	Matsui *et al.* 2011
19	*Microhyla achatina*	Indonesia, Java, Ujung Kulong	ZMMU A-5070	MN534670	MN534462, MN534563	MN534402	Gorin *et al.* 2020
20	*Microhyla aurantiventris*	Vietnam, Gia Lai, Kon Ka Kinh NP	ITBCZ-4360	MN534727	MH286427	MN534431	Nguyen *et al.* 2019; Gorin *et al.* 2020
21	*Microhyla beilunensis*	China, Sichuan	CIB 20070248	AB634611	AB634669	–	Matsui *et al.* 2011
22	*Microhyla berdmorei*	Thailand, Suratthani, Khao Sok NP	ZMMU NAP-04133	MN534711	MN534503, MN534604	KC180094	Gorin *et al.* 2020
23	*Microhyla borneensis*	Malaysia, Sarawak, Kidi (Bidi)	UNIMAS FN 1874ZAC600	–	MN534550, MN534657	MN534394	Gorin *et al.* 2020
24	*Microhyla butleri*	Malaysia, Tasik Pedu Lake, Kedah	ZMMU NAP-06827	MN534734	MN534521, MN534625	MN534434	Gorin *et al.* 2020
25	*Microhyla chakrapanii*	India, Andaman Island, Havelock	ZISP 13874	MN534698	MN534490, MN534591	MN534422	Gorin *et al.* 2020
26	*Microhyla daklakensis*	Vietnam: Dak Lak, Nam Kar	VNMN06818	–	MT808945	–	Hoang *et al.* 2021
27	*Microhyla darreli*	India, Kerala, Thiruvanan Thapuram, Karamana	ZSI/WGRC/V/A/962	–	MH807390	MH807429	Garg *et al.* 2018
28	*Microhyla eos*	India, Arunachal Pradesh, Changlang, Namdapha N.P.	ZSIC 14312	–	MN160599	MN167548	Biju *et al.* 2019
29	*Microhyla fanjingshanensis*	China, Guizhou	–	MF538787		–	Zhao *et al.* 2018
30	*Microhyla fissipes*	China, Taiwan, Kaohsiung, Zhongliao-shan mt.	ZMMU A-5333	MN534695	MN534487, MN534588	MN534419	Gorin *et al.* 2020
31	*Microhyla fodiens*	Myanmar, Magway, Kan Pauk	ZMMU A-5960	MK208926		MN534401	Gorin *et al.* 2020
32	*Microhyla gadjahmadai*	Indonesia, Sumatra, Lampung	MZB Amp 15291	AB634622	AB634680	–	Matsui *et al.* 2011
33	*Microhyla heymonsi*	China, Taiwan, Pingtong, Yongchin, Qi Kong	ZMMU A-4975	MN534679	MN534471, MN534572	MN534407	Gorin *et al.* 2020
34	*Microhyla irrawaddy*	Myanmar, Magway, Pakkoku	ZMMU A-5966	MK208928		MN534403	Gorin *et al.* 2020
35	*Microhyla karunaratnei*	Sri Lanka, Sinharaja FR	released	MN534738	MN534524, MN534629	MN534438	Gorin *et al.* 2020

36	*Microhyla kodial*	India, Karnataka, Mangaluru	–	–	MF919454	MH807431	Vineeth *et al.* 2018
37	*Microhyla kuramotoi*	Japan, Okinawa, Ishigaki Isl.	released	MN534700	MN534492, MN534593	MN534424	Gorin *et al.* 2020
38	*Microhyla laterite*	India, Karnataka, Udupi, Manipal	BNHS 5965	KT600670	KT600663	MH807432	Seshadri *et al.* 2016
39	*Microhyla malang*	Malaysia, Sarawak, Kubah NP	ZMMU A-6043	MN534662	MN534454, MN534555	MN534396	Gorin *et al.* 2020
40	*Microhyla mantheyi*	Malaysia, Taman Negara NP	ZMMU NAP-6745	MN534665	MN534457, MN534558	KM509300	Gorin *et al.* 2020
41	*Microhyla mihintalei*	Sri Lanka, Rathambaldama	released	MN534726	MN534515, MN534619	MN534430	Gorin *et al.* 2020
42	*Microhyla minuta*	Vietnam, Dong Nai, Cat Tien NP	ZMMU A-5048-91	MN534667	MN534459, MN534560	MN534400	Gorin *et al.* 2020
43	*Microhyla mixtura*	China, Sichuan, Wanyuan, Hua'e-shan mt.	CIB 20170526001	MH234529	MH234540	–	Zhang *et al.* 2018
44	*Microhyla mukhlesuri*	Bangladesh, Chittagong	IABHU-3959	MN534692	MN534484, MN534585	MN534416	Gorin *et al.* 2020
45	*Microhyla mymensinghensis*	Bangladesh, Mymensingh	IABHU-4129	MN534699	MN534491, MN534592	MN534423	Gorin *et al.* 2020
46	*Microhyla neglecta*	Vietnam, Lam Dong, Bidoup–Nui Ba NP, Giang Ly	ZMMU A-7303	MW147168	MW147155	–	Poyarkov *et al.* 2020
47	*Microhyla nepenthicola*	Malaysia, Borneo, Sarawak, Kubah NP	ZMMU A-6028-1	MN534658	MN534450, MN534551	MN534393	Gorin *et al.* 2020
48	*Microhyla nilphamariensis*	Bangladesh, Nilphamari	IABHU-4212	MN534721	MN534614	MH807435	Gorin *et al.* 2020
49	*Microhyla ninhthuanensis*	Vietnam: Ninh Thuan, Phuoc Binh	HAO185	–	MT808934	–	Hoang *et al.* 2021
50	*Microhyla okinavensis*	Japan, Okinawa island, Yomitan son, Kina	ZMMU A-6027-1	MN534704	MN534496, MN534597	MN534426	Gorin *et al.* 2020
51	*Microhyla orientalis*	Indonesia, Java, Yogyakarta	ZMMU A-5067-2	MN534663	MN534455, MN534556	MN534397	Gorin *et al.* 2020
52	*Microhyla ornata*	Sri Lanka, Rathambaldama	released	MN534723	MN534512, MN534616	MN534428	Gorin *et al.* 2020
53	*Microhyla palmipes*	Indonesia, Bali, Bedegul	MZB Amp 16255	AB634612	AB634670	MN539668	Matsui *et al.* 2011
54	*Microhyla picta*	Vietnam, Ba Ria-Vung Tau, Binh Chau, Phuok Buu NP	ZMMU A-4918-45	MN534719	MN534510, MN534612	MN534427	Gorin *et al.* 2020
55	*Microhyla pineticola*	Vietnam, Lam Dong, Bidoup–Nui Ba NP, Giang Ly	ZMMU A-5043	MW147172	MW147166	MN534399	Poyarkov *et al.* 2020

56	*Microhyla pulchra*	Laos, Khammouan, Nakai-Nam Theun	ZISP FN-00154	MN534716	MN534507, MN534609	EF396021	Gorin *et al.* 2020
57	*Microhyla rubra*	India, Andhra Pradesh, Bapatla	ZMMU A-5006-19	MK208936		MN534429	Poyarkov *et al.* 2019; Gorin *et al.* 2020
58	*Microhyla sholigari*	India, Karnataka, Udupi District, Manipal	ATREE MISH 3	KT600669	KT600676	MH807438	Seshadri *et al.* 2016
59	*Microhyla superciliaris*	Thailand, Songkhla	ZMMU A6024-1	MN534744	MN534530, MN534635	MN534441	Matsui *et al.* 2011
60	*Microhyla taraiensis*	Nepal, Mechi, Jamun Khadi, Jhapa	–	MF496241		–	Khatiwada *et al.* 2018
61	*Microhyla tetrix*	Thailand, Suratthani, Khao Sok NP	ZMMU A-6032	MN534740	MN534526, MN534631	–	Gorin *et al.* 2020
62	*Microhyla zeylanica*	Sri Lanka, Central Province, Nuwara Eliya	released	MN534737	MN534523, MN534628	MN534437	Gorin *et al.* 2020
63	*Microhyla* sp. 1	Malaysia, Borneo, Sabah, Danum Valley	RMBR 2171	MN534660	MN534452, MN534553	–	Gorin *et al.* 2020
64	*Microhyla* sp. 2	Myanmar, Sagaing	USNM 523975	–	MG935884	–	Mulcahy *et al.* 2018
	Outgroup						
65	*Kaloula baleata*	Indonesia, Sumba	KUHE 32313	AB634629	AB634687	KM509289	Matsui *et al.* 2011

Supplementary Table S2 Uncorrected interspecific (below diagonal) and intraspecific (on the diagonal) genetic *p*-distances for 16S rRNA mtDNA gene fragment (in percentage) for species on the genus *Nanohyla*.

	Species	1	2	3	4	5	6	7	8	9	10
1	*Nanohyla albopunctata* **sp. nov.**	**0.0**									
2	*N. annamensis*	7.4	-								
3	*N. annectens*	6.0	7.4	-							
4	*N. arboricola*	8.8	8.8	6.4	-						
5	*N. hongiaoensis*	7.8	6.6	6.6	4.1	-					
6	*N. marmorata*	5.3	4.9	5.7	7.8	6.0	-				
7	*N. nanapollexa*	9.4	9.2	8.4	7.4	7.0	8.6	-			
8	*N. perparva*	6.4	7.2	7.4	7.4	6.0	6.2	9.0	-		
9	*N. petrigena*	6.8	7.4	7.0	7.2	6.0	7.0	7.0	6.0	-	
10	*N. pulchella*	8.4	8.2	6.0	2.5	3.1	7.4	7.2	7.0	7.0	**-**

Supplementary Table S3 Measurements of type series of *Nanohyla albopunctata* **sp. nov.** (in mm).

Museum ID	**ZMMU A-7585**	**ZMMU A-7584**	**ZMMU A-7586**		
Sex	male	male	male		
Type status	holotype	paratype	paratype	**Mean**	***SD***
SVL	18.2	20.2	19.8	19.4	*0.86*
HL	5.6	5.9	5.8	5.8	*0.12*
SL	2.0	2.8	2.5	2.4	*0.33*
EL	1.9	2.4	2.7	2.3	*0.33*
N-EL	1.1	1.8	1.5	1.5	*0.29*
HW	8.2	8.0	8.2	8.1	*0.09*
IND	1.8	2.9	2.6	2.4	*0.46*
IOD	2.5	2.8	2.7	2.7	*0.12*
UEW	1.4	1.7	1.3	1.5	*0.17*
FLL	11.4	11.8	11.8	11.7	*0.19*
LAL	8.7	9.6	9.1	9.1	*0.37*
HAL	3.7	3.8	4.4	4.0	*0.31*
1FL	0.9	0.9	1.1	1.0	*0.09*
IPTL	0.6	0.7	0.6	0.6	*0.05*
OPTL	0.9	0.8	1.0	0.9	*0.08*
3FDD	0.6	0.6	0.7	0.6	*0.05*
HLL	36.1	37.6	37.9	37.2	*0.79*
TL	12.5	12.6	12.8	12.6	*0.12*
FL	10.0	10.9	11.4	10.8	*0.58*
IMTL	0.9	0.9	0.5	0.8	*0.19*
1TOEL	2.1	2.0	2.4	2.2	*0.17*
4TDD	0.6	1.1	1.1	0.9	*0.24*
2FL	1.2	1.5	2.2	1.6	*0.42*
3FL	3.4	3.1	3.1	3.2	*0.14*
4FL	1.5	1.9	1.6	1.7	*0.17*
2TOEL	3.4	3.4	4.1	3.6	*0.33*
3TOEL	6.1	6.1	6.1	6.1	*0.00*
4TOEL	7.7	7.6	8.6	8.0	*0.45*
5TOEL	4.5	3.9	4.8	4.4	*0.37*
1FDD	0.3	0.3	0.3	0.3	*0.00*
2FDD	0.4	0.5	0.5	0.5	*0.05*
4FDD	0.4	0.6	0.6	0.5	*0.09*
1TDD	0.5	0.5	0.8	0.6	*0.14*
2TDD	0.9	0.9	1.0	0.9	*0.05*
3TDD	0.9	1.3	1.1	1.1	*0.16*
5TDD	0.6	1.1	0.8	0.8	*0.21*

Supplementary Table S4 Morphological comparisons of *Nanohyla* species. For character abbreviations see Materials and methods. Asterisk (*) indicates coloration in preservative. (Continues on next page).

Species ¥ Character	**SVL**	**HL**	**SL**	**EL**	**HW**	**IOD**	**TL**	**FL**	**Body habitus**	**Snout profile**	**Dorsum skin**
Nanohyla albofrontalis **sp. nov.**	18.2–20.2	5.6–5.9	2.0–2.8	1.9–2.7	8.0–8.2	2.5–2.8	12.5–12.8	10.0–11.4	Moderately slender	Rounded	Tubercular
N. annamensis	12.2–19.8	4.5–8.0	2.0–2.9	1.7–2.4	4.9–7.7	2.0–2.7	8.1–13.0	6.9–11.5	Moderately stocky	Bluntly rounded	Strongly tubercular
N. annectens	14.6-18.4	—	—	—	—	—	—	—	Slender	Rounded	Smooth
N. arboricola	15.9–17.0	5.5–5.7	2.1–2.5	1.9–2.0	5.5	1.9–2.2	8.4–8.8	7.0–7.7	Moderately slender	Pointed	Feebly granular
N. hongiaoensis	13.5–14.6	3.9–4.7	1.8–2.0	1.3–1.6	3.8–4.9	2.1–2.6	8.5–8.8	11.5–12.1	Slender	Bluntly rounded	Slightly tubercular
N. marmorata	18.8-23.2	7.0–7.9	2.6–3.3	1.9–2.9	6.9–8.7	2.2–3.0	12.1–14.6	14.6–17.9	Moderately stocky	Bluntly rounded	Smooth or feebly pustular
N. nanapollexa	13.5-16.6	7.4	2.3	1.9	6.1	1.9	11.1	13.6	Slender	Rounded	Smooth
N. perparva	10.9-14.5	—	—	—	—	—	—	—	Moderate	Obtusely pointed	Smooth
N. petrigena	13.9-17.8	—	—	—	—	—	—	—	Moderately stout	Obtusely pointed	Smooth, posteriorly with tubercles
N. pulchella	14.7–21.6	4.3–7.1	2.1–3.0	1.7–2.9	5.4–7.8	2.1–2.8	9.3–13.2	8.1–12.5	Moderately stocky	Bluntly rounded	Smooth

Supplementary Table S4 (Continued).

Species ¥ Character	F1**	FD	FMG	TD	TMG	MTT	DML	SCT	Tibtars	Foot webbing
Nanohyla albofrontalis **sp. nov.**	F1 < 1/2 F2	+	+, weak	+	+, weak	2	-	-	Well beyond snout	I 1 – 2 II 1– 2½ III 1–2 IV 2 –1 V
N. annamensis	F1 < 1/2 F2	+	+	+	+	2 (1)	-	-	Well beyond snout	I 1–2¼ II 1–2½ III 1½–2¾ IV 3–1 V
N. annectens	F1 < 1/2 F2	+	+	+	+	1	-	-	Well beyond snout	I 1–1 II 1–1 III 1–3 IV 3–1 V
N. arboricola	F1 < 1/2 F2	+, on F2-F4	+, weak	+	+	1	-	-	Well beyond snout	I 1⅔–2¼ II 2–3 III 2½–3½ IV 3–1½ V
N. hongiaoensis	F1 < 1/2 F2	+, weak on F2-F4	-	+	+, weak T2-T5	2	-	-	Well beyond snout	I1 – 2II1– 2½ III1–2½IV2½ –1V
N. marmorata	F1 < 1/2 F2	+	+	+	+	2 (1)	-	-	Well beyond snout	I 1–2 II 1–1¾ III 1½–2¾ IV 2¾–1 V
N. nanapollexa	nub or bulge	+	+	+	+	1	-	-	Well beyond snout	I 1–2 II 1–2½ III 2½–2½ IV 2½–1 V
N. perparva	nub or bulge	+	-	+	+	1	-	-	Well beyond snout	I 1–1 II 1–1 III 1–2 IV 2–1 V
N. petrigena	nub or bulge	+	+, weak	+	+	1	-	-	Well beyond snout	I 1–1 II 1–1 III 1–2 IV 2–1 V
N. pulchella	F1 < 1/2 F2	+, on F2-F4	+, weak	+	+, weak	1	-	-	Well beyond snout	I 1½–2 II 1–2 III 1–2½ IV 2¼–1 V

Supplementary Table S4 (Continued).

Species ¥ Character	**Dorsal colour**	**Dorsal pattern**	**Ventral colour**	**Ventral pattern**	**Distribution**	**References**
Nanohyla albofrontalis **sp. nov.**	Brownish-grey	'Teddy-bear' dorsal marking, two light beige spots on sacrum	Grey	Grey mottling	Ca Range, Phu Yen (Vietnam)	this paper
N. annamensis	Grey-brown	Black streaks above shoulders, dark V-shaped marking	Brownish	Orange or beige mottling	Lam Dong, Dak Lak, Khanh Hoa (Vietnam)	Poyarkov et al. 2014
N. annectens	Dark brown	Dark interorbital bar	Brown	Brown marbling	Peninsular Thailand and Malaysia	Poyarkov et al. 2014; Parker , 1934
N. arboricola	Pinkish-beige to light ochre	Brownish interorbital bar, V-shaped or 'teddy-bear' marking edged with beige	Greyish-beige on belly to reddish-brown on throat	Cream-yellow or whitish mottling	Dak Lak, Khanh Hoa (Vietnam)	Poyarkov et al. 2014
N. hongiaoensis	Greyish brown to light-brown	Dark-brown interorbital bar; dark-brown 'teddy-bear' marking	White-grey	Dark-grey mottling	Lam Dong (Vietnam)	Hoang et al. 2020
N. marmorata	* Grey to dark brown	* Black 'teddy-bear' pattern	* Grey-brown	* Brown mottling, large white marbling posteriorly	Nge An, Ha Tinh, Quang Binh, Quang Tri, Quang Nam, Thua Thien-Hue, Gia Lai, Kon Tum (Vietnam); Khammouane, Bolikhamxai (Lam Dong)	Poyarkov et al. 2014; Bain and Nguyen, 2004
N. nanapollexa	* Tan, head grey	* Dark-grey 'teddy-bear' marking	-	* Brown mottling	Quang Nam (Vietnam)	Poyarkov et al. 2014
N. perparva	-	-	-	No dark marking	Sabah and Sarawak, Malaysia; Kalimantan (Indonesia)	Poyarkov et al. 2014

N. petrigena	* Grey, head darker	* Dark-grey 'teddy-bear' marking	* Dark grey	* Irregular white markings	Sabah, Sarawak (Malaysia), Brunei, Kalimantan (Indonesia), Tawitawi Isl. (Philippines)	Poyarkov et al. 2014
N. pulchella	Orange-red to reddish-brown	Brownish interorbital bar, dark ‘teddy-bear’ marking	Pinkish to reddish-brown	Cream to pink spots or reticulations	Lam Dong, Dak Lak (Vietnam)	Poyarkov et al. 2014, Hoang et al. 2020

Supplementary Table S5 Measurements of advertisement call parameters for *Nanohyla albopunctata* **sp. nov.** Abbreviations: *N* — number of series/calls/pulses, s — seconds, ms — milliseconds, Hz — hertz.

Parameters	***N***	**Mean**	**SE**	**Median**	**Min**	**Max**
Series duration, s	21	0.78	0.05	0.77	0.34	1.22
Number calls per series	21	4.71	0.21	5	2	6
Call duration, ms	99	63.1	3.1	73	2.7	119.3
Intercall duration, ms	77	130.72	6.8	112.9	61.3	276
Number of pulses per call	99	6.45	0.29	7	1	13
Pulse duration, ms	639	3.21	0.03	3.2	1.5	5.6
Interpulse duration, ms	541	7.9	0.23	7	1.5	90
Pulse repetition rate	92	86.45	2.66	87.87	37.31	138.89
Call Fpeak, Hz	99	3020	27.6	3180	2530	3460
Pulse Fpeak, Hz	639	3040	11.17	3180	1400	3750