

Consequences of parallel miniaturisation in Microhylinae (Anura, Microhylidae), with the description of a new genus of diminutive South East Asian frogs

Vladislav A. Gorin¹, Mark D. Scherz^{2,3}, Dmitriy V. Korost⁴, Nikolay A. Poyarkov^{1,5}

¹ Faculty of Biology, Department of Vertebrate Zoology, Lomonosov Moscow State University, Leninskiye Gory 1/12, Moscow 119234, Russia

² Sektion Herpetologie, Zoologische Staatssammlung München (ZSM-SNSB), Münchhausenstr. 21, 81247, München, Germany

³ Institute for Biochemistry and Biology, University of Potsdam, Karl-Liebknecht-Str. 24–25, 14476 Potsdam, Germany

⁴ Geological Faculty, Petroleum Geology Department, Moscow State University, Leninskiye Gory 1, Moscow 119234, Russia

⁵ Joint Russian-Vietnamese Tropical Research and Technological Center, Nghia Do, Cau Giay, Hanoi, Vietnam

<http://zoobank.org/C6E9DCB9-E56D-48E1-A042-452A1D2043EE>

Corresponding author: Nikolay A. Poyarkov (n.poyarkov@gmail.com)

Academic editor: Rafe Brown ♦ Received 25 August 2020 ♦ Accepted 14 December 2020 ♦ Published 12 January 2021

Abstract

The genus *Microhyla* Tschudi, 1838 includes 52 species and is one of the most diverse genera of the family Microhylidae, being the most species-rich taxon of the Asian subfamily Microhylinae. The recent, rapid description of numerous new species of *Microhyla* with complex phylogenetic relationships has made the taxonomy of the group especially challenging. Several recent phylogenetic studies suggested paraphyly of *Microhyla* with respect to *Glyphoglossus* Günther, 1869, and revealed three major phylogenetic lineages of mid-Eocene origin within this assemblage. However, comprehensive works assessing morphological variation among and within these lineages are absent. In the present study we investigate the generic taxonomy of *Microhyla*–*Glyphoglossus* assemblage based on a new phylogeny including 57 species, comparative morphological analysis of skeletons from cleared-and-stained specimens for 23 species, and detailed descriptions of generalized osteology based on volume-rendered micro-CT scans for five species—altogether representing all major lineages within the group. The results confirm three highly divergent and well-supported clades that correspond with external and osteological morphological characteristics, as well as respective geographic distribution. Accordingly, acknowledging ancient divergence between these lineages and their significant morphological differentiation, we propose to consider these three lineages as distinct genera: *Microhyla sensu stricto*, *Glyphoglossus*, and a newly described genus, *Nanohyla* **gen. nov.**

Key Words

Amphibians, integrative taxonomy, narrow-mouthed frogs, micro-computed tomography, *Nanohyla* gen. nov, osteology, sexual dimorphism, taxonomic revision

Introduction

Anuran amphibians of the family Microhylidae (narrow-mouthed frogs) are globally distributed and diverse. This group currently comprises 12 subfamilies, 57 genera and over 700 recognized species, thus representing 10.3% of extant anuran diversity, making it the third largest anuran family after Hylidae and Strabomantidae (Streicher et al. 2020; Frost 2020). Microhylid frogs are

morphologically and ecologically diverse including terrestrial, arboreal and fossorial (burrowing) morphotypes (Wells 2010; Moen et al. 2015). Microhylids display extensive variation in adult external morphology, osteology, and musculature; and in many cases, parallel specializations associated with a burrowing lifestyle may have led to remarkable morphological convergence (Emerson 1971; Wu 1994; Trueb et al. 2011; Moen et al. 2015). The extensive homoplasy observed in Microhylidae hinders

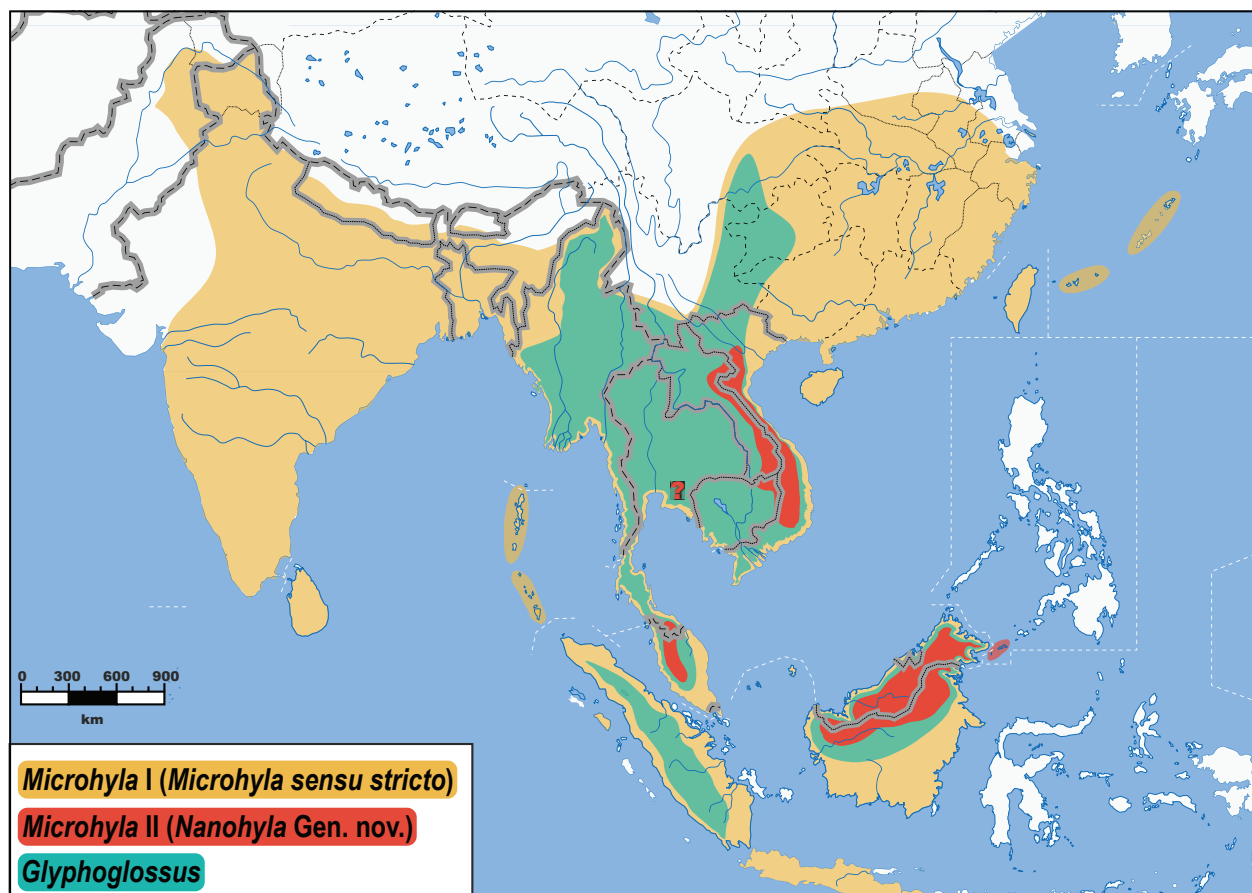


Figure 1. Distribution ranges of the three clades of the *Microhyla*–*Glyphoglossus* assemblage. Distribution area of *Microhyla* I is shown in yellow, of *Microhyla* II in red, and of *Glyphoglossus* in blue. Distributional data from Gorin et al. (2020). Question mark denotes the unconfirmed record of “*Microhyla annamensis*” from Khao Seab in eastern Thailand by Taylor (1962).

phylogenetic interpretations from morphological characters (Wu 1994); significant progress in understanding evolutionary relationships of microhylids was achieved only through molecular phylogenetic studies (de Sá et al. 2012, 2019a, 2019b; Tu et al. 2018; Streicher et al. 2020). Skeletal composition varies substantially among microhylids, showing features that are rare or absent in other anuran clades (Noble and Parker 1926; Parker 1934; Zweifel 1972; 1986; de Sá and Trueb 1991). Although osteology and morphological evolution of microhylid subfamilies from the Americas (de Sá and Trueb 1991; Lehr and Trueb 2007; Trueb et al. 2011; Zweifel 1986, and references therein), Australasia (Zweifel 1972), and Madagascar (Scherz et al. 2015, 2016, 2017, 2019) received a decent amount of researchers’ attention, studies of the Asian microhylid subfamilies, to date, have primarily focused on description of long-neglected species diversity (Matsui et al. 2011; Hasan et al. 2014; Poyarkov et al. 2014, 2018a, 2018b, 2019, 2020; Vassilieva et al. 2014; Yuan et al. 2016; Suwannapoom et al. 2018, 2020; Nguyen et al. 2019, and citations therein).

The first and only monographic revision of the family Microhylidae published over 85 years ago was largely based on osteological data (Parker 1934). In his review of Asian microhylid taxa, Parker only focused on the most

variable parts of the skeleton (such as the palatine region and pectoral girdle), but description of generalized osteology generally was not included (Parker 1934). In recent years skeletal morphology of only a few species in Microhylinae has been described in substantial detail, including the genus *Uperodon* (Chandramouli and Dutta 2015; Garg et al. 2018), *Kaloula borealis* (Boring and Liu 1937; Zhang et al. 2020), and *Glyphoglossus guttulatus* (McPartlin 2010).

The genus *Microhyla* Tschudi, 1838 currently comprises 52 nominal species (Hoang et al. 2020; Poyarkov et al. 2020a, 2020b; Frost 2020) and several undescribed candidate species (Gorin et al. 2020). It is the second largest microhylid genus after *Oreophryne* (Frost 2020) and the most species species-rich taxon of the Asian subfamily Microhylinae. Over half of *Microhyla* species diversity was described within the last 15 years (29 species, see Frost 2020), but despite substantial progress in their taxonomy, this genus remains one of the most taxonomically challenging groups of Asian frogs. The small or medium-sized terrestrial frogs of the genus *Microhyla* are distributed all over the Oriental biogeographic region (Fig. 1) and exhibit significant variation in body size (adult body size varies from 10–46 mm) and ecomorphology (e.g. body shape, finger and toe disc expansion, and limb lengths) tied to their natural history (terrestrial, semi-ar-

boreal, semi-fossorial). The smallest *Microhyla* species are amongst the smallest frogs in the world, approaching the lower body-size limit for the vertebrate bauplan (Das and Haas 2010; Kraus 2011). Phylogenetic analyses based on molecular data (Matsui et al. 2011; Garg et al. 2019; Gorin et al. 2020), provided novel insights into phylogeny of the genus and revealed significant inconsistencies with the traditional, morphology-based classifications (Parker 1934; Dubois 1987; Fei et al. 2009). The preliminary mitochondrial DNA (mtDNA) based genealogies unexpectedly suggested paraphyly of *Microhyla* with respect to the large-sized fossorial genus *Glyphoglossus* (Matsui et al. 2011; de Sá et al. 2012; Biju et al. 2019; Nguyen et al. 2019; Poyarkov et al. 2019). Additional multilocus phylogenetic (Garg and Biju 2019; Gorin et al. 2020) and phylogenomic (Tu et al. 2018; Peloso et al. 2016) studies supported monophyly of *Microhyla*, and agreed with one another in recovering the three main highly-divergent lineages within this group: the *Glyphoglossus* clade and two *Microhyla* clades (*Microhyla* I and *Microhyla* II hereafter, following Gorin et al. 2020). The three major clades of the *Microhyla*–*Glyphoglossus* assemblage were shown to have diversified in the middle Eocene (Garg and Biju 2019; Gorin et al. 2020), which makes the genus *Microhyla*, *sensu lato* (hereafter *s. lat.*) older than other microhylid genera (Feng et al. 2017; Garg and Biju 2019). The lack of information on morphological variation among and within the lineages of the *Microhyla*–*Glyphoglossus* assemblage hinders further taxonomic assessment of diversity within this group.

Herein, we assess the status of the three lineages of the *Microhyla*–*Glyphoglossus* assemblage using an integrative taxonomic approach. We provide an updated mtDNA-based genealogy including 57 species of the group. Based on traditional (cleared-and-stained specimens and external morphology) and digital (micro-Computed Tomography, or micro-CT) methods of comparative morphology we further report on osteological variation for 23 species of the genus *Microhyla* and three species of the genus *Glyphoglossus*, thus covering all major lineages for the first time. Based on analysis of morphological, osteological, molecular, and distribution data we recognize *Glyphoglossus* and *Microhyla* I *sensu stricto* (hereafter as *s. str.*) as valid genera. Additionally, we erect a new genus for *Microhyla* II, helping to stabilize the taxonomy of this clade. We further analyze miniaturization patterns, body size, and the evolution of sexual dimorphism in the *Microhyla*–*Glyphoglossus* assemblage.

Material and methods

Taxon sampling and examined specimens

To assess the phylogenetic relationships within the *Microhyla*–*Glyphoglossus* assemblage we used the mtDNA and nuclear DNA (nuDNA) datasets from Gorin et al. (2020) with the addition of sequences of the recently

described *Mysticellus franki* (Garg and Biju 2019) and *Microhyla hongiaoensis* (Hoang et al. 2020). We used the mtDNA dataset, consisting of 12S rRNA and 16S rRNA for all examined samples, for estimation of the phylogeny (232 sequences, including 200 sequences of *Microhyla*). A combined mtDNA + nuDNA dataset, joining the long 12S rRNA–16S rRNA mtDNA fragment and *BDNF* gene sequences for a reduced set of 120 samples, equitably selected (from preliminary analysis of mtDNA; not shown) to represent all major lineages within *Microhyla*, was used to estimate a robust, multilocus, time-calibrated phylogeny. In total, we analyzed GenBank sequences from 200 specimens of 49 nominal and three candidate *Microhyla* species, five species of *Glyphoglossus*, and 32 other microhylids, including representatives of all currently recognized microhylid genera. All taxa, specimen-associated locality data, museum voucher catalog numbers, and genetic data included in our study are presented in Suppl. material 1: Table S1.

Our osteological study was based on specimens housed in herpetological collections of the Zoological Museum of Lomonosov Moscow State University (ZMMU, Moscow, Russia), the Herpetology Lab of the Vertebrate Zoology department, Faculty of Biology, Lomonosov Moscow State University (HLMU; Moscow, Russia), the Museum of Comparative Zoology, Harvard University (MCZ, Cambridge, Massachusetts, USA), and the California Academy of Sciences (CAS, San Francisco, California, USA). Altogether, for hand-preparation and histological clearing-and-staining, we used 23 specimens, which included 17 nominal species of *Microhyla* I clade, and 4 species of *Microhyla* II clade, and representing all of the currently recognized species groups, with exception of the *M. palmipes* species group, and two species of *Glyphoglossus*. All specimens were adults, fixed in either 75% ethanol or in 4% buffered formalin with subsequent storage in 70% ethanol. Additionally, for micro-computed tomography (micro-CT) study we examined the smallest representatives of *Microhyla* I and *Microhyla* II clades (*M. nepenthicola* and *M. arboricola*, respectively). We also used micro-CT scans for one *Microhyla* (*M. achatina*, the type species of the genus; MCZ-A2683, ark:/87602/m4/M79961) and two species of *Glyphoglossus*: *G. yunnanensis* (CAS-H-242243, ark:/87602/m4/M49927) and *G. molossus* (the type species of the genus; CAS-H-243121, ark:/87602/m4/M49928), downloaded from the *MorphoSource* database (www.morphosource.org) with permission. Altogether, our morphological dataset included detailed information for 23 species of *Microhyla* and 3 species of *Glyphoglossus*. Detailed information on the species and specimens included in morphological study is presented in Suppl. material 2: Table S2.

Phylogenetic inference

Nucleotide sequences were initially aligned in MAFFT v.6 (Katoh et al. 2002) with default parameters, and were

subsequently manually optimized in BioEdit 7.0.5.2 (Hall 1999). Genetic distances were calculated using MEGA 6.1 (Tamura et al. 2013). The optimal partitioning schemes for our alignment were identified with PartitionFinder 2.1.1 (Lanfear et al. 2012) using the greedy search algorithm under AICc criterion. Phylogenetic trees were reconstructed under maximum likelihood (ML) and Bayesian inference (BI). A ML analysis was implemented using the IQ-TREE webserver (Nguyen et al. 2015; Trifinopoulos et al. 2016). Clade stability was assessed by 1000 bootstrap (BS) replications and expected likelihood weights (ELW). One-thousand bootstrap pseudoreplicates (ML BS) were employed, and nodes having ML BS values of 90 and above were considered strongly supported, while nodes with values of 75–90 were regarded as significantly supported, lower values were considered to indicate lack of nodal support (Felsenstein 1985; Huelsenbeck and Hillis 1993).

Bayesian inference (BI) was performed in MrBayes v3.1.2 (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, with sampling every 100 generations. We performed five independent MCMCMC runs and the initial 10% of trees were discarded as burn-in. We checked that the effective sample sizes (ESS) were all above 200 by exploring the likelihood plots using TRACER v1.6 (Rambaut et al. 2014). We assessed the clade support with posterior probabilities (PP) (Huelsenbeck and Ronquist 2001). Nodes with PP of 0.95 and above were considered strongly supported, nodes with values of 0.90–0.94 as significantly supported, while lower values were considered as no support (Huelsenbeck and Ronquist 2001; Wilcox et al. 2002). Molecular divergence time estimation was performed in BEAST v1.8.4 (Drummond et al. 2012). Molecular clock assumptions were tested using hierarchical likelihood ratio tests in PAML v4.7 (Yang 2007), which suggested the use of uncorrelated lognormal relaxed clock for our dataset. The models and partitioning scheme from our ML analysis were also incorporated into these subsequent divergence date estimations; we set the Yule model as the tree prior, assumed a constant population size, and used default priors for all other parameters. In BEAST, we conducted two runs of 200 million generations each, sampled every 4000 steps, parameter convergence was estimated in Tracer, and the first 10% of generations discarded as burn-in. TreeAnnotator v1.8.0 (in BEAST) was used to create our maximum clade credibility tree from the remaining samples. Calibration priors and all other details of this analysis followed Gorin et al. (2020).

Osteological preparation and double staining

In order to observe both ossified and cartilaginous structures, specimens were cleared and double stained with alcian blue for cartilage and alizarin red for bone. We

used the most delicate methodology of acid-free staining (following Walker and Kimmel 2006) to preserve minute skeletal elements of the smallest species. The protocol included: (1) staining for about 24 hours in a solution of 0.05% alizarin red, 0.02% alcian blue, 45mM MgCl₂ and 70% ethanol; (2) maceration for about 24 hours at 37 °C in a saturated solution of sodium tetraborate with 1% trypsin; (3) bleaching for several hours in a solution of 1.5% H₂O₂ and 1% KOH; (4) clearing with successive changes of solutions of 25/50/75% glycerol with 0.25% KOH, for 1/3/5 days for each solution respectively; and final (5) storage in a 99% glycerol. Obtained skeletons were examined and photographed using a LEICA EZ4 dissecting stereo microscope (Leica Camera AG, Wetzlar, Germany) with a binocular-implemented ES-ESPERTS Digital camera BR-5101LC-UF.

Micro-CT scanning

We followed Micro-CT scanning of *M. nepenthicola* (ZMMU A-6028-1) and *M. arboricola* (ZMMU A-5051), using protocols of Suwannapoom et al. (2018) and Poyarkov et al. (2018). Scanning was conducted at the Petroleum Geology Department, Faculty of Geology, Lomonosov Moscow State University, using a SkyScan 1172 desktop scanner (Bruker micro-CT, Kontich, Belgium) equipped with a Hamamatsu 10 Mp digital camera. Both specimens were mounted on a polystyrene baseplate and placed inside a hermetically sealed polyethylene vessel. Scans were conducted with a resolution of 3.7 μm at 40 kV voltage and a current of 250 mA, with a rotation step of 0.3°. We used oversize mode, in which three blocks of sub-scan data were connected vertically, to obtain a general tomogram. We used 3D Slicer (Kikinis et al. 2014) for construction and processing of 3D-models. Scans were deposited in MorphoSource (http://www.morphosource.org/Detail/ProjectDetail/Show/project_id/1183).

Morphological descriptions and analyses

Osteological terminology followed Trueb (1968, 1973), Scherz et al. (2017), Suwannapoom et al. (2018), and Poyarkov et al. (2014, 2018a). Terminologies used to describe the shape of terminal phalanges (simple, knobbed, T-shaped, and Y-shaped) followed Parker (1927) and Garg et al. (2019). Comparative morphological and osteological data for other genera were taken from a number of revisions of Microhylinae (Parker 1934; Boring and Liu 1937; Duellman and Trueb 1986; Dubois 1987; Fei et al. 2009; McPartlin 2010; Chandramouli and Dutta 2015; Garg et al. 2019; Garg and Biju 2019; Poyarkov et al. 2018b; Zhang et al. 2020; Suwannapoom et al. 2020). External morphology was described following Poyarkov et al. (2014, 2019); mensural data were taken with a Mitutoyo dial caliper (Mitutoyo Corporation, Kawasaki,

Japan) to the nearest 0.1 mm. We recorded the following external morphology characters: snout-vent length (SVL, measured as distance from tip of snout to cloaca), body shape (slender, stocky, or stout, following Bain and Nguyen 2004), snout profile (in lateral and dorsal view), dorsal skin texture (smooth, shagreened, feebly granular or tuberculate), relative length of first finger (FI length: $\leq 1/2$ of FII length, $\geq 1/2$ of FII length, or reduced to a nub), widths of discs on fingers and toes, number and shape of metatarsal tubercles, the presence (vs absence) of dorsomedial grooves on fingers and toes, of a distinct dorsomedial (vertebral) line, of superciliary tubercles, and of externally visible tympanum, the level to which the tibiotarsal articulation of an addressed leg reaches (not reaching the eye, to the eye, to the snout, far beyond the snout), and the development of toe webbing (rudimentary, basal, well-developed, developed to discs; webbing and subarticular tubercle formulas follow Savage, 1975).

To assess body size and sexual dimorphism evolution in the *Microhyla–Glyphoglossus* assemblage, we compiled data on maximum snout-vent length (SVL) separately for both sexes, for each species reported in literature and/or from our own measurements of voucher specimens following Gorin et al. (2020). Size (SVL) data for all *Microhyla* and *Glyphoglossus* species are summarized in Suppl. material 3: Table S3. Comparative morphological analyses were conducted in R 3.6.3 (R Core Team 2014). Analyses of SVL measurements were carried out using their natural logarithms. Sexual dimorphism was expressed as a ratio of male to female SVL (female-biased species have > 1 , male-biased species have < 1). The tree and morphological dataset were pruned to reflect taxa represented in both, using the `treedata()` function in `geiger` (Harmon et al. 2008). Continuous trait evolution was mapped to the phylogeny using the `contMap()` function of `phytools` (Revell 2012). Phylogenetic Least Squares (PGLS) analysis of the log of male SVL against dimorphism was carried out using `caper` package (Orme et al. 2018) and plotted with `ggplot2` (Wickham 2016). Species were binned into four size categories (terminology follows Scherz et al. 2019) as follows: ≤ 13 mm (state 1: “extremely miniaturized”); (2: 13–16 mm, “highly miniaturized”); (3: 16–20 “miniaturized”); (4: 20–24 “small”).

Results

Phylogenetic relationships

Our final aligned matrix of mtDNA data contained 232 sequences (length 2478 bp), representing 49 of the 52 currently recognized species of the genus *Microhyla s. lat.*, three undescribed candidate species of *Microhyla*, and five species of *Glyphoglossus*. Our final alignment of the nuDNA *BDNF* gene was 720 bp long, and included all of the taxa sampled for the mitochondrial matrix but for six *Microhyla s. lat.* species (from clade I: *M. gadjahmadai*, *M. taraiensis*, *M. mixtura*, *M. fanjingshanensis*, and

M. beilunensis; from clade II: *M. perparva*). We here report on mitochondrial-only and nuclear-only phylogenies first, and concatenated phylogenies afterwards.

Both BI and ML phylogenetic methods resulted in identical topology of mtDNA-based genealogical relationships for the *Microhyla–Glyphoglossus* assemblage (Fig. 2). All analyses concordantly resolved three strongly supported major clades within the group: *Microhyla* I, *Microhyla* II, and *Glyphoglossus*, as indicated by Bayesian posterior probabilities of 1.0 and ML bootstrap node support of 100% (node support values are hereafter provided as PP/BS); the majority of ingroup nodes also received strong support (PP/BS $\geq 0.95/95\%$). Although the *Microhyla–Glyphoglossus* assemblage was recovered to be a monophyletic group with strong support (1.0/100), the relationships among the three main clades within it remained essentially unresolved according to the mtDNA dataset, and the grouping of *Microhyla* I + *Glyphoglossus* received no nodal support (-/70) (Fig. 2; Suppl. material 6: Figure S1A and Suppl. material 6: Figure S2). Phylogenetic analyses of the nuDNA *BDNF* gene suggested monophyly of *Microhyla* I + *Microhyla* II grouping with moderate to strong node support (0.90/97; Suppl. material 6: Figure S1B), despite the short length of this marker. Relationships at shallower nodes within the respective clades were less strongly resolved than in the mtDNA phylogeny. The combined mtDNA + nuDNA analyses (3207 bp) yielded a topology largely congruent with that of the nuDNA alone, but with lower node support values for the *Microhyla* I + *Microhyla* II clade (0.46/90; Fig. 3; detailed in Suppl. material 6: Figure S1C). Thus, while the three major clades in the *Microhyla–Glyphoglossus* assemblage are strongly and consistently recovered as monophyletic, the monophyly of *Microhyla s. lat.* remains tentative, with practically no signal in the mitochondrial dataset but some signal in *BDNF*. As the combined dataset yielded a better resolved phylogeny that is also more consistent with previous work (e.g. Tu et al. 2018), we use that tree for further analyses (time tree, ancestral state reconstruction) and discussion below.

The observed topological patterns within the *Microhyla–Glyphoglossus* assemblage were congruent with earlier results of Gorin et al. (2020) in recovering eight major species groups within *Microhyla s. lat.* (clades A–H, see Figs 2–3), and the genus *Glyphoglossus* (clade I, see Figs 2–3). The only difference with results of Gorin et al. (2020) is the phylogenetic placement of the recently described *M. hongjiaoensis* as sister species to *M. pulchella* (Figs 2–3). Since a detailed description of phylogenetic relationships within the genus *Microhyla* was provided by Gorin et al. (2020), we only focus here on a general description of the most important basal nodes, crucial for discussion in the present study.

The most species-rich clade, *Microhyla* I, is widely distributed from mainland southern China, Hainan and Taiwan, and the Ryukyu Archipelago of Japan in the north, through the Indochina Peninsula, to India, and Sri Lanka in the west, and through the Malayan Peninsula to Borneo,

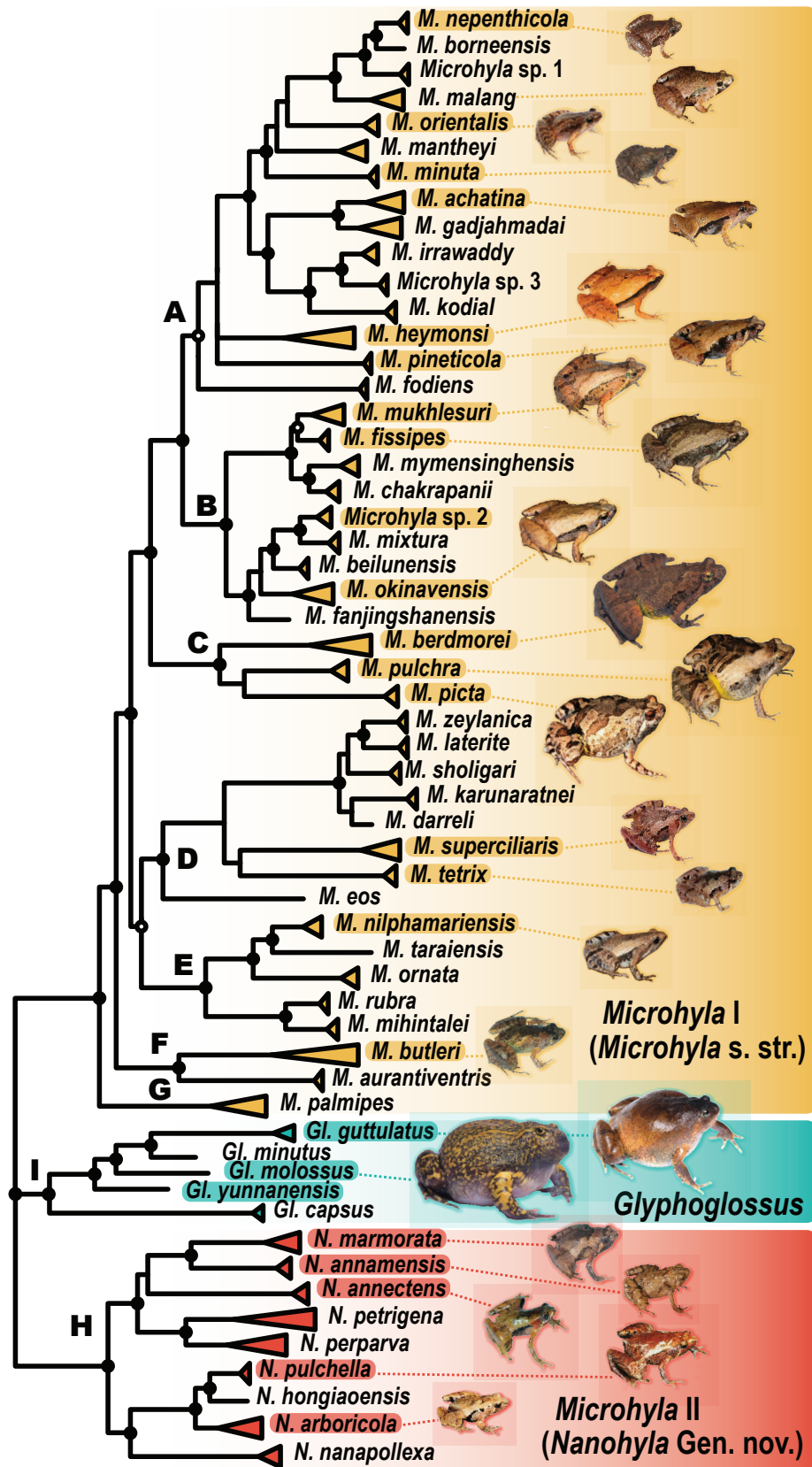


Figure 2. Diversity of the *Microhyla*–*Glyphoglossus* assemblage based on an updated mtDNA-genealogy derived from the analysis of 2478 bp of alignment including 12S rRNA, tRNA^{Val}, 16S rRNA gene fragments. Black circles correspond to well-supported (PP \geq 0.95; BS \geq 90) and white circles to moderately supported (0.95 > PP \geq 0.90; 90 > BS \geq 75) nodes; no circles indicate unsupported nodes. Letters A–I denote the species groups of Gorin et al. (2020). Photos by Nikolay A. Poyarkov, Indraneil Das, Vladislav A. Gorin, Parinya Pawangkhanant, Luan Thanh Nguyen, and Evgeniya N. Solovyeva. For full version of this tree showing the outgroups and node support values see Suppl. material 7: Figure S2.

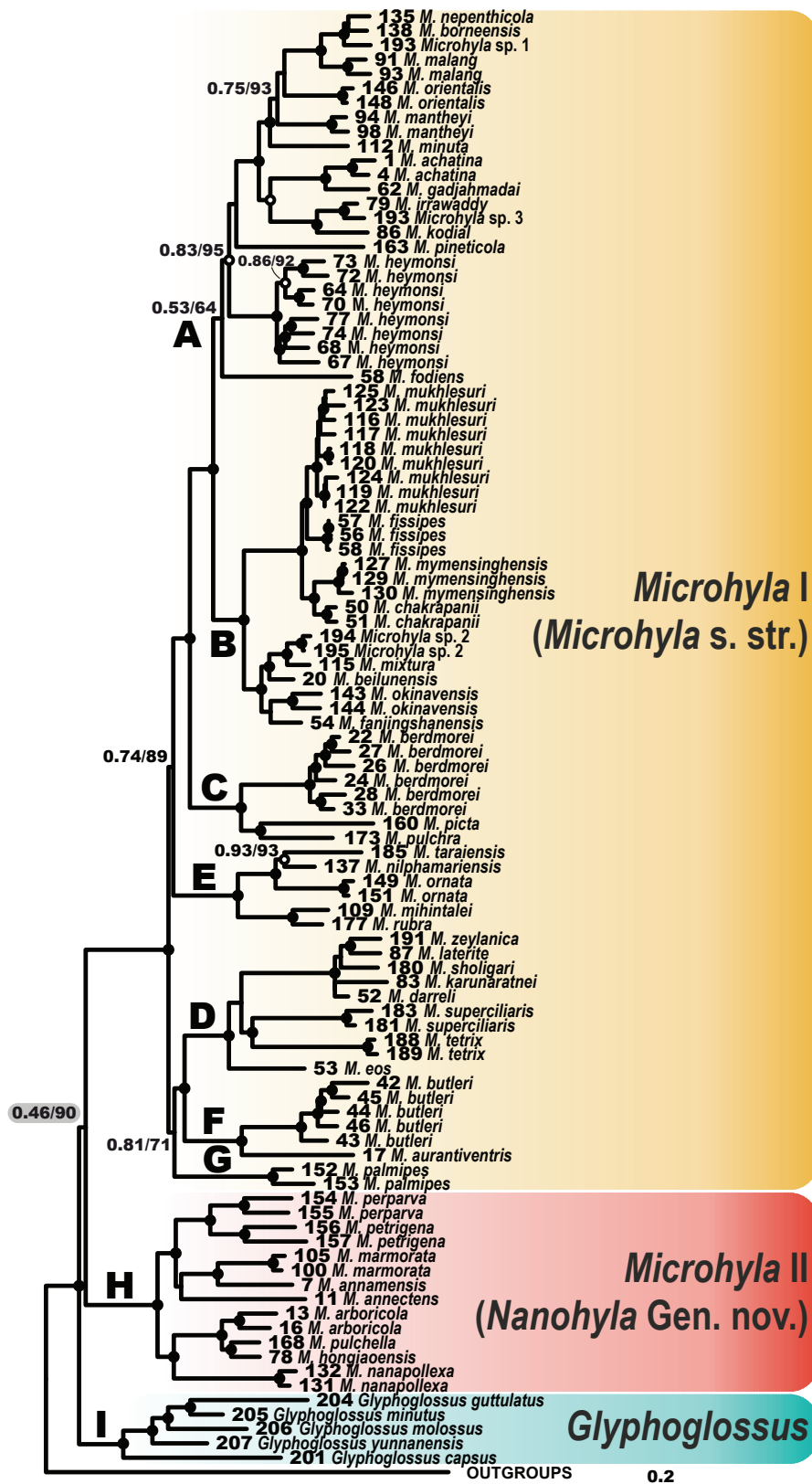


Figure 3. Bayesian inference tree of the *Microhyla*–*Glyphoglossus* assemblage derived from the combined mtDNA + nuDNA analysis of 3207 bp of alignment including 12S rRNA, tRNAVal, 16S rRNA and *BDNF* gene fragments. Black circles correspond to well-supported (PP ≥ 0.95; BS ≥ 90) and white circles to moderately supported (0.95 > PP ≥ 0.90; 90 > BS ≥ 75) nodes; no circles indicate unsupported nodes. Letters A–I denote the species groups of Gorin et al. (2020). For voucher specimen information and GenBank accession numbers see Suppl. material 1: Table S1. Yellow, red, and blue color denotes *Microhyla* I, *Microhyla* II, and *Glyphoglossus*, respectively. Numbers at tree nodes correspond to PP/BS support values, respectively (shown only for moderately supported nodes). For full version of this tree showing the outgroups and node support values see Suppl. material 6: Figure S1.

Sumatra, Java and Bali in the south (Fig. 1). The *Microhyla* I clade contained the following species, clustered in seven species groups (Fig. 2): The *Microhyla achatina* group (clade A), including *M. achatina* Tschudi, 1838; *M. borneensis* Parker, 1928; *M. fodiens* Poyarkov, Gorin, Zaw, Kretova, Gogoleva, Pawangkhanant & Che, 2019; *M. gadjohmadai* Atmaja, Hamidy, Arisuryanti, Matsui & Smith, 2018; *M. heymonsi* Vogt, 1911; *M. irrawaddy* Poyarkov, Gorin, Zaw, Kretova, Gogoleva, Pawangkhanant & Che, 2019; *M. kodial* Vineeth, Radhakrishna, Godwin, Anwasha, Rajashekhar & Aravind, 2018; *M. malang* Matsui, 2011; *M. mantheyi* Das, Yaakob & Sukumaran, 2007; *M. minuta* Poyarkov, Vassilieva, Orlov, Galoyan, Tran, Le, Kretova & Geissler, 2014; *M. nepenthicola* Das & Haas, 2010; *M. orientalis* Matsui, Hamidy & Eto, 2013; *M. pineticola* Poyarkov, Vassilieva, Orlov, Galoyan, Tran, Le, Kretova & Geissler, 2014; and two undescribed candidate species, *Microhyla* sp. 1 and *Microhyla* sp. 3.

The *Microhyla fissipes* group (clade B), including *M. beilunensis* Zhang, Fei, Ye, Wang, Wang & Jiang, 2018; *M. chakrapanii* Pillai, 1977; *M. fanjingshanensis* Li, Zhang, Xu, Lv & Jiang, 2019; *M. fissipes* Boulenger, 1884; *M. mixtura* Liu & Hu, 1966 in Hu et al. (1966); *M. mukhlesuri* Hasan, Islam, Kuramoto, Kurabayashi & Sumida, 2014; *M. mymensinghensis* Hasan, Islam, Kuramoto, Kurabayashi & Sumida, 2014; *M. okinavensis* Stejneger, 1901; and an undescribed candidate species, *Microhyla* sp. 2.

The *Microhyla bermorei* group (clade C), including *M. bermorei* (Blyth, 1856); *M. picta* Schenkel, 1901; and *M. pulchra* (Hallowell, 1861).

The *Microhyla superciliaris* group (clade D), including *M. darreli* Garg, Suyesh, Das, Jiang, Wijayathilaka, Amarsinghe, Alhadi, Vineeth, Aravind, Senevirathne, Mee-gaskumbura & Biju, 2019; *M. eos* Biju, Garg, Kamei & Maheswaran, 2019; *M. karunaratnei* Fernando & Siriwardhane, 1996; *M. laterite* Seshadri, Singal, Priti, Ravikanth, Vidisha, Saurabh, Pratik & Gururaja, 2016; *M. sholigari* Dutta & Ray, 2000; *M. superciliaris* Parker, 1928; *M. tatrix* Suwannapoom, Pawangkhanant, Gorin, Juthong & Poyarkov, 2020; and *M. zeylanica* Parker & Osman-Hill, 1949.

The *Microhyla ornata* group (clade E), including *M. mihintalei* Wijayathilaka, Garg, Senevirathne, Karunaratna, Biju & Mee-gaskumbura, 2016; *M. nilphamariensis* Howlader, Nair, Gopalan & Merilä, 2015; *M. ornata* (Duméril & Bibron, 1841); *M. rubra* (Jerdon, 1854); and *M. taraiensis* Khatiwada, Shu, Wang, Thapa, Wang & Jiang, 2017.

The *Microhyla butleri* group (clade F), including *M. aurantiventris* Nguyen, Poyarkov, Nguyen, Nguyen, Tran, Gorin, Murphy & Nguyen, 2019; and *M. butleri* Boulenger, 1900.

The *Microhyla palmipes* group (clade G), including *M. palmipes* Boulenger, 1897. The distribution area of the *Microhyla* II clade is restricted to the montane forest areas in the Annamite (Truong Son) Mountains in East Indochina (Vietnam, eastern Laos, northeastern Cambodia), Malay- an Peninsula (Titivangsa Mountain Range), mountains of Borneo (Sarawak, Sabah of Malaysia, Brunei and northern Kalimantan, Indonesia), and the southwestern-most islands of the Sulu Archipelago of the Philippines (Fig. 1).

It contains the following nine species (clade H, Fig. 2) of the *M. annectens* group: *M. annamensis* Smith, 1923; *M. annectens* Boulenger, 1900; *M. arboricola* Poyarkov, Vassilieva, Orlov, Galoyan, Tran, Le, Kretova & Geissler, 2014; *M. hongiaoensis* Hoang, Nguyen, Luong, Nguyen, Orlov, Chen, Wang & Jiang, 2020; *M. marmorata* Bain & Nguyen, 2004; *M. nanapollexa* Bain & Nguyen, 2004; *M. perparva* Inger & Frogner, 1979; *M. petrigena* Inger & Frogner, 1979; and *M. pulchella* Poyarkov, Vassilieva, Orlov, Galoyan, Tran, Le, Kretova & Geissler, 2014.

Finally, the *Glyphoglossus* clade (clade I, Fig. 2) covers the whole Thai-Malaysian Peninsula, parts of Indochina, including Myanmar, and also penetrates northward, as far as southern mainland China; and southward as far as Sumatra and Borneo (Fig. 1). It contains the following five species: *G. capsus* (Das, Min, Hsu, Hertwig & Haas, 2014); *G. guttulatus* (Blyth, 1856); *G. minutus* (Das, Yaakob & Lim, 2004); *G. molossus* Günther, 1869; and *G. yunnanensis* (Boulenger, 1919).

Divergence times

Estimated node-ages (mean age estimate \pm 95% highest posterior density interval [95% HPD]) for main nodes are detailed in Suppl. material 4: Table S4 and Suppl. material 8: Figure S3. The results of the divergence time estimation fully agree with Gorin et al. (2020), suggesting that the most recent common ancestor (MRCA) of *Microhyla* and *Glyphoglossus* originated around the early Eocene ca. 50.9 million years ago (hereafter Ma, 95% HPD 44.2–58.7) (Suppl. material 8: Figure S3). This estimate coincides with some previous estimates (48.8 Ma; 45.9–53.2, Feng et al. 2017), but is significantly younger than other reports (61.5, 56.6–66.5, Garg and Biju 2019). The *Microhyla*–*Glyphoglossus* assemblage radiated into the three major clades in the middle Eocene (44.1, 38.5–49.6), notably later than other estimates (48.7, 44.1–53.2, Garg and Biju 2019). Subsequent diversification of each genus-level radiations of *Microhyla* I, *Microhyla* II, and *Glyphoglossus* clades initiated much later in the early to middle Oligocene (ca. 35–25 Ma, Gorin et al. 2020).

Comparative osteology

A total of 26 species examined for osteological variation allows us to clarify similarities and variation in skeletal morphology among and within the three clades of the *Microhyla*–*Glyphoglossus* assemblage. Detailed information on species' characters' states is presented in Suppl. material 5: Table S5. Overall skeletal morphology and the main osteological features for representatives of each clade are illustrated in Figures 4–7. Skull and hand morphology for cleared and stained representatives of these three clades is provided in Suppl. material 8, 9.

Below, we provide comparative osteological descriptions for the three clades of the *Microhyla*–*Glyphoglossus* assemblage: *Microhyla* I, *Microhyla* II, and *Glyphoglossus*.

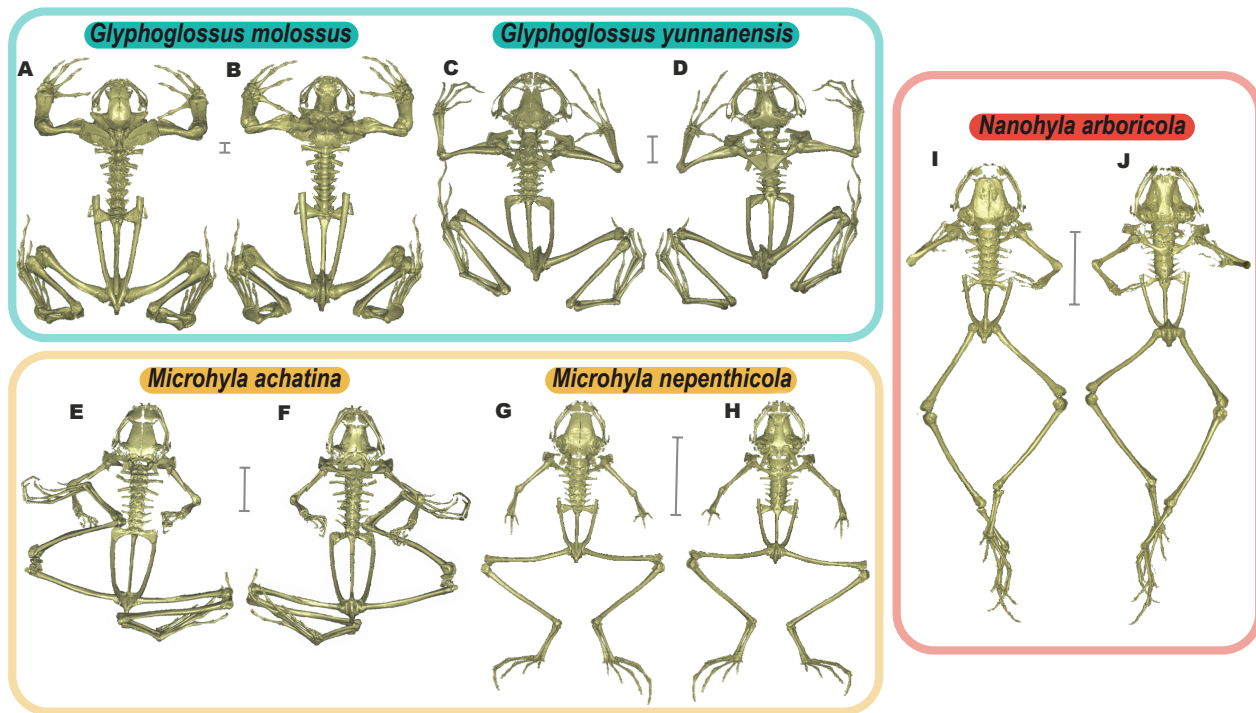


Figure 4. General osteology of the *Microhyla–Glyphoglossus* assemblage representatives. The full skeletons are shown for *Glyphoglossus molossus* (A – dorsal, B – ventral views), *Glyphoglossus yunnanensis* (C – dorsal, D – ventral views), *Microhyla achatina* (E – dorsal, F – ventral views), *Microhyla nepenthicola* (G – dorsal, H – ventral views), and *Nanohyla arboricola* (I – dorsal, J – ventral views). Note: figures display only calcified structures; cartilages are omitted due to limitations of micro-CT scanning. Scale bar equals 5 mm.

(A) *Microhyla* I clade

This clade includes *M. achatina*, the type species of the genus *Microhyla*, and is the most widely distributed, species rich, and ecologically and morphologically diverse group of the *Microhyla–Glyphoglossus* assemblage Clade I includes most small- to medium-sized terrestrial species, along with several large species; they are adapted to fossorial (*M. picta*), or semi-fossorial (*M. fodiens*, *M. rubra*, *M. mihintalei*) lifestyles (Fig. 2). This diversity is also reflected in osteological features, which demonstrate conspicuous variation among species (Fig. 4; Suppl. material 5: Table S5). Due to marked morphological variation, providing a comprehensive morphological diagnosis of this speciose group remains a challenging task; below we summarize available information on skeletal traits.

Skull

Skull longer than wide, wider than long, or almost in equal proportions among species of *Microhyla* I (Fig. 5; Suppl. material 5: Table S5). Widest part of skull located posteriorly and giving head a triangular or trapezoid shape (Fig. 5). Frontoparietals longer than broad, narrowing anteriorly, in contact along medial border but not fused, lacking any dorsal crests; partially fused with or separated from exoccipital posteriorly and prootic posterolaterally. Exoccipitals always separate, in contact medially. Nasals large and widely separated, chondrified peripherally; processus paraorbitalis broad, in some species

blunt, posterior edge concave, anterior edge convex (Fig. 5). Sphenethmoid well-ossified, always clearly separated from parasphenoid, with a concave posterior edge. Prootics ossified anteromedially; crista parotica cartilaginous with posterior margin mineralized. Squamosal ossified, with a well-developed ventral ramus and poorly developed otic and zygomatic rami. Operculum slightly mineralized. Majority of columella (stapes) mineralized, with only pars externa plectra cartilaginous; tympanic annulus completely chondrified (Suppl. material 10: Figure S5H). Premaxilla well-ossified, its alary process oriented slightly anteriorly, distal part bending laterally. Maxilla well-ossified; anteriorly in contact with labial portion of premaxilla (eleutherognathine condition); edentate; pars facialis moderately high in lateral view. Quadratojugal reduced, with a chondrified posterior articulation with angulosplenic; not in anterior contact with maxilla. Support of upper jaw taken over by pterygoid, with long anterior ramus, broad posterior ramus, and short medial ramus. Vomers small, widely separated, triangular in shape. Neopalatines present or absent. Nasal capsules mineralized posteriorly or entirely cartilaginous. Mentomeckelians ossified, connected to dentaries and to each other through Meckel’s cartilage. Dentary fused with angulosplenic. Parasphenoid smooth; cultriform process of parasphenoid narrowing anteriorly, terminating at level of sphenethmoid with a chondrified notch (Fig. 5). Hyoid plate completely cartilaginous, anterolateral (alary) processes of hyoid plate present, recurved, posterolateral processes slender, posteromedial processes strongly ossified,

elongated, straight, wider at proximal ends, chondrified at distal ends, separated by a chondrified parahyoid (Suppl. material 10: Figure S5M).

Vertebral column

Vertebral column is diplasiocoelus, typically comprising eight presacral vertebrae (PSV) (Fig. 6C), with the exception of extremely miniaturized species *M. nepenthicola*, which has PSV I and II fused (Fig. 6D). PSV II–VII procoelous and VIII amphicoelous. Transverse processes of PSV II–IV longer and wider than V–VIII, transverse processes of PSV VI–VIII oriented anterolaterally; orientation of transverse processes to other vertebrae varies (Suppl. material 5: Table S5). Transverse processes of sacrum moderately expanded, with distal end about twice as wide as proximal end. Urostyle shorter than trunk vertebrae, bearing a weak dorsal crest that tapers posteriorly and vanishes at two-thirds of urostyle length (Fig. 6); its articulation with sacrum is bicondylar.

Appendicular skeleton

Pectoral girdle with a firmisternal arrangement. Coracoids, scapulae, and suprascapulae present; first two fully ossified; suprascapula largely chondrified. Coracoids robust with wide proximal end. Omosternum generally absent, except for *M. puchra*, where a tiny cartilaginous omosternum is present (Suppl. material 5: Table S5). Procoracoids indistinct. Clavicles absent. Cartilaginous sternum large, partially mineralized, fan-shaped or bifurcate (Suppl. material 10: Figure S5D, E); xiphisternum completely cartilaginous.

Hand skeleton including seven largely calcified carpal elements: carpale distale II, carpale distale III–V fused into a single large element, prepollex (consisting of two elements), Element Y, radiale, and ulnare (Fig. 7C–D). Metacarpals long and fully ossified; hand phalangeal formula: 2-2-3-3; all phalanges ossified; distal phalanx of finger III simple, conical-, bobbin- or T-shaped. Foot skeleton with four tarsal elements, including ossified tarsale distale II–III, centrale and a prehallux; prehallux mineralized in all species examined (Suppl. material 10: Figure S5A). Metatarsals fully ossified, long and relatively more massive than metacarpals; foot phalangeal formula: 2-2-3-4-3; all phalanges ossified. Terminal phalanges of toe III T-shaped or simple.

(B) *Microhyla* II clade

This is a compact clade of nine species belonging to the *M. annectens* group previously recovered by Gorin et al. (2020), encompassing minute- or small-sized terrestrial or semi-arboreal species with short triangular-shaped body habitus, inhabiting montane forests in Indochina and Sundaland (Fig. 2). The clade *Microhyla* II is rather uniform in skeletal composition, and examined species

share a set of osteological characters that clearly separate this group from the two other clades of the *Microhyla*–*Glyphoglossus* assemblage (Suppl. material 5: Table S5).

Skull

Skull longer than wide or almost equal (Figs 4, 5); widest portion posterior, giving head triangular shape (Fig. 5). Frontoparietals longer than broad, narrowing anteriorly, in contact along medial border and fused posteriorly, lacking any dorsal crests; posteriorly fused with exoccipitals. Exoccipitals completely fused with each other (Fig. 5), except *M. pulchella*, (partial; Suppl. material 5: Table S5). Nasals large, broadly separated, chondrified peripherally, processus paraorbitalis narrow and cultriform, posterior edge concave, anterior edge oblique (Fig. 5). Sphenethmoids ossified, completely fused with parasphenoid (in *M. pulchella* an indistinct suture remains laterally, so the fusion is partial), extending posteroventrally nearly to level of prootics along parasphenoid. Prootics ossified anteromedially, crista parotica entirely cartilaginous. Squamosal ossified, with well-developed long ventral and otic rami and poorly developed zygomatic ramus (Fig. 5O; Suppl. material 5: Table S5). Operculum mineralized. Columella largely mineralized with only pars externa plectra cartilaginous, tympanic annulus completely chondrified (Suppl. material 10: Figure S5G). Premaxilla well-ossified, alary process oriented slightly anteriorly, distal part bending laterally. Maxilla well-ossified; anteriorly in contact with labial portion of premaxilla; edentate; pars facialis moderately high in lateral view. Quadratojugal reduced further than *Microhyla* I clade, not in anterior contact with maxilla; support of upper jaw taken over by pterygoid. Pterygoid with long anterior ramus (pronounced concavity along ramus that is much more laterally oriented than in *Microhyla* I clade), broad posterior ramus, and short medial ramus. Vomers small, widely separated, triangular in shape. Neopalatines present as very thin elements. Mentomeckelians ossified, connected to dentaries and to each other through Meckel's cartilage. Dentary fused with angulosplenial. Parasphenoid smooth; cultriform process of parasphenoid broad, completely fused with sphenethmoid laterally (Fig. 5O), terminating at level of neopalatines with a chondrified notch. Hyoid plate completely cartilaginous, anterolateral processes of hyoid plate present, recurved, posterolateral processes slender, posteromedial processes strongly ossified, elongated, straight, chondrified at distal ends, wider at proximal ends, separated by a chondrified parahyoid.

Vertebral column

Vertebral column diplasiocoelus, including eight presacral vertebrae, with the exception of one of the smallest species of the group, *M. arboricola*, which has PSV I and II fused (Fig. 6E). PSV II–VII procoelous and VIII amphicoelous. Transverse processes of PSV II–IV longer and wider than in PSV V–VIII; transverse processes of PSV II, VII and

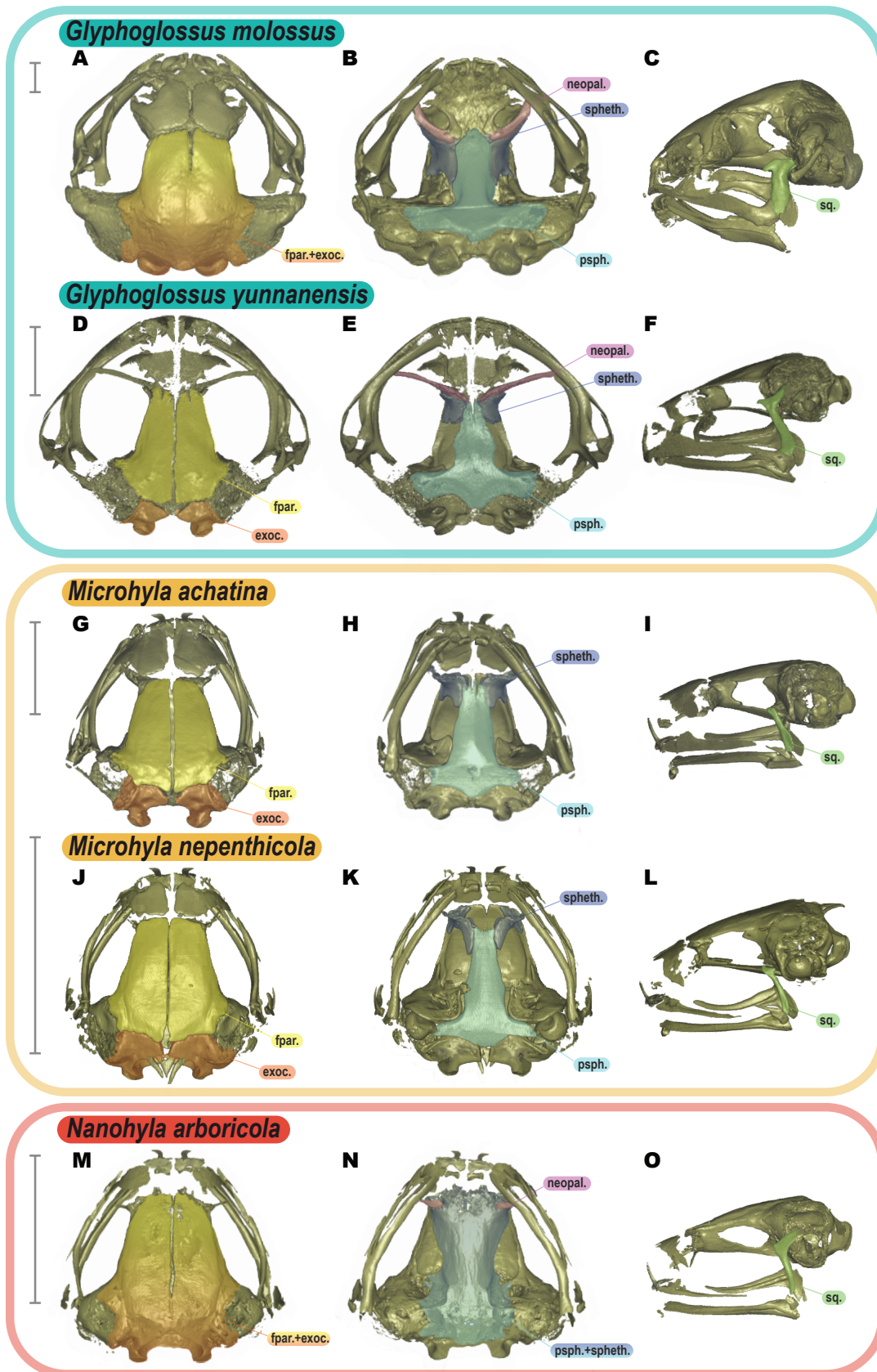


Figure 5. Cranial osteology of the *Microhyla*–*Glyphoglossus* assemblage representatives. The skulls are shown in dorsal / ventral / lateral views for *Glyphoglossus molossus* (A / B / C, respectively), *Glyphoglossus yunnanensis* (D / E / F, respectively), *Microhyla achatina* (G / H / I, respectively), *Microhyla nepenthicola* (J / K / L, respectively), and *Nanohyla arboricola* (M / N / O, respectively). Note: figures display only calcified structures; cartilages are omitted due to limitations of micro-CT scanning. Scale bar equals 3 mm.

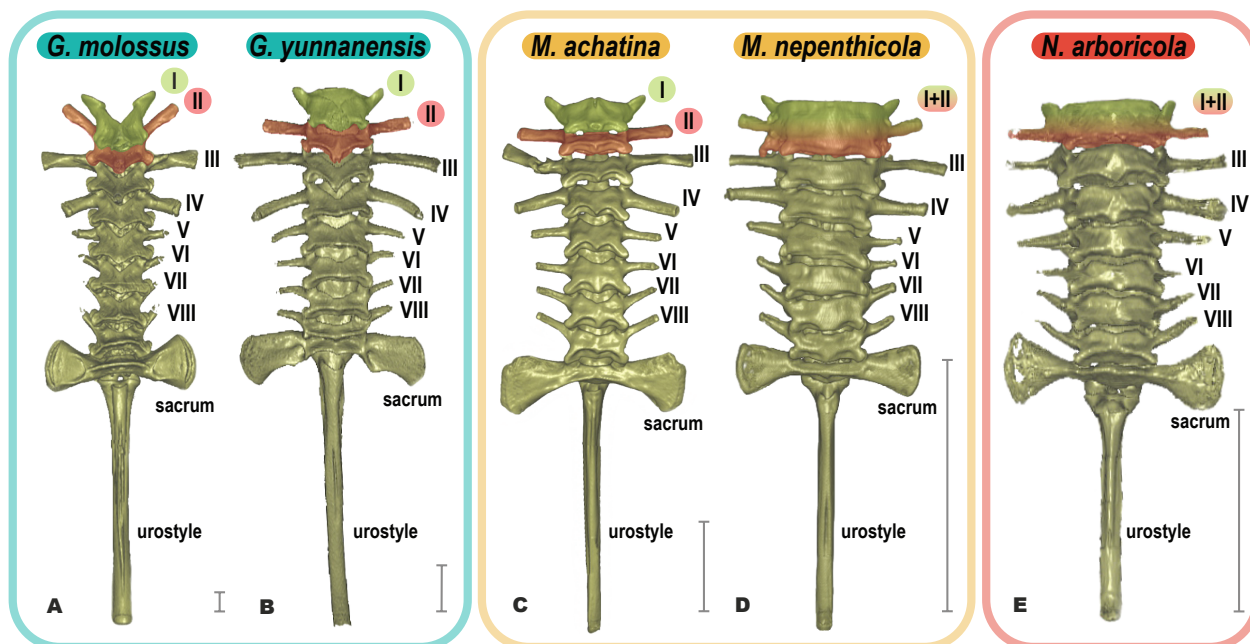


Figure 6. Axial skeleton composition in the *Microhyla*–*Glyphoglossus* assemblage representatives. The vertebral columns are shown in dorsal view for *Glyphoglossus molossus* (A), *Glyphoglossus yunnanensis* (B), *Microhyla achatina* (C), *Microhyla nepenthicola* (D) and *Nanohyla arboreicola* (E). Numerals (I–VIII) correspond to the numbers of presacral vertebrae (PSV); I+II denotes fusion of the two first PSV. Note: figures display only calcified structures; cartilages are omitted due to limitations of micro-CT scanning. Scale bar equals 3 mm.

Table 1. Summary of osteological differences between *Glyphoglossus*, *Microhyla s. str.* and *Nanohyla* gen. nov. Diagnostic features of the new genus that are subjectively considered by us to be most reliable are highlighted in bold. Asterisk (*) denotes states observed in *G. molossus* exclusively. For species-specific data, see Suppl. material 5: Table S5.

Character	<i>Glyphoglossus</i>	<i>Microhyla s. str.</i>	<i>Nanohyla</i> gen. nov.
Skull shape	Wider than long	Subequal, longer than wide, or wider than long	Subequal or longer than wide
Frontoparietal–exoccipital junction	Separated	Separated	Fused
Exoccipitals	Separated	Separated	Fused or incompletely fused
Nasal capsules	Ossified	Ossified, partly mineralized, or cartilaginous	Ossified, partly mineralized, or cartilaginous
Neopalatines	Obscured	Present or absent	Present
Maxillary teeth	Present or absent*	Absent	Absent
Vomers	Large	Small	Small
Vomerine teeth	Present or absent*	Absent	Absent
Anterior ramus of pterygoid	Thin and blunt or massive and blunt*	Thin, blunt	Thin, tapered
Sphenethmoid and parasphenoid	Separated	Separated	Fused or incompletely fused
Otic ramus of squamosal	Long	Short	Long
Tympanic annulus	Present	Present or reduced	Present
Columella	Fully ossified	Poorly mineralised	Poorly mineralised
Crista parotica	Fully ossified	Posteriorly ossified	Cartilaginous
Clavicles	Present or absent*	Absent	Absent
Omosternum	Absent	Usually absent	Present
Prehallux	Ossified	Mineralized	Cartilaginous
Terminal phalanges of finger III	Simple	T-shaped, knobbed, or simple	T-shaped
Distance between vomers	Narrow	Wide	Wide

VIII oriented anterolaterally, IV and V posterolaterally, III and VI perpendicular to vertebral column axis, with exception of *M. marmorata*, which has transverse processes of PSV VI oriented anterolaterally. Transverse processes of sacrum notably expanded, with distal end more than twice as wide as proximal. Urostyle shorter than trunk vertebrae, bearing a weak dorsal crest, tapering posteriorly; vanishes completely at 2/3 urostyle length (Fig. 6E).

Appendicular skeleton

Pectoral girdle firmisternal. Coracoids, scapulae, and suprascapulae present; coracoid and scapula fully ossified; suprascapula largely chondrified. Coracoids robust with wide proximal end. Cartilaginous omosternum present (Suppl. material 10: Figure S5F). Procoracoids indistinct. Clavicles absent. Sternum large, cartilaginous, partially

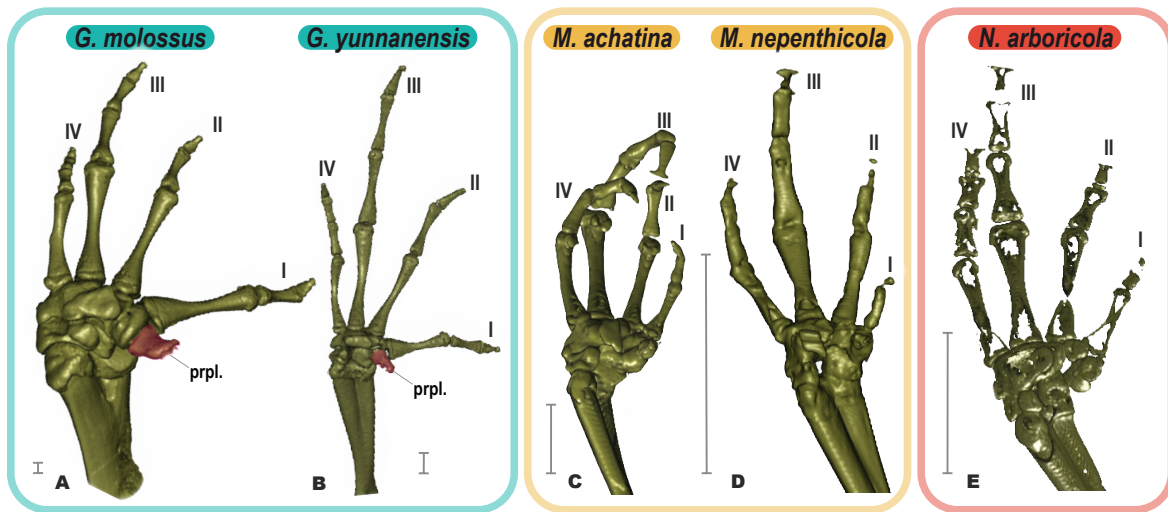


Figure 7. Hand skeleton composition in the *Microhyala–Glyphoglossus* assemblage representatives. The hands are shown in ventral view for *Glyphoglossus molossus* (A), *Glyphoglossus yunnanensis* (B), *Microhyala achatina* (C), *Microhyala nepenthicola* (D) and *Nanohyla arboricola* (E). Note: figures display only calcified structures; cartilages are omitted due to limitations of micro-CT scanning. Scale bar equals 1 mm.

mineralized, bifurcate or fan-shaped; xiphisternum completely cartilaginous.

Manus skeleton with seven largely calcified carpal elements, including carpale distale II, carpale distale III–V (fused into a single large element), prepollex (consisting of two separate elements), Element Y, radiale, and ulnare (Fig. 7E). Metacarpals long and fully ossified; phalangeal formula: 2-2-3-3; all phalanges ossified, with exception of *M. arboricola*, in which metacarpals and phalanges ossified only peripherally (Fig. 7E); distal phalanx of finger III T-shaped. Foot skeleton with four tarsal elements, including ossified tarsale distale II–III, centrale and a prehallux; prehallux cartilaginous in all species examined (Suppl. material 10: Figure S5B). Metatarsals fully ossified, elongated and much more massive than metacarpals; phalangeal formula: 2-2-3-4-3; all phalanges ossified. Terminal phalanges of toe III T-shaped.

(C) *Glyphoglossus* clade

In our analysis, three species of *Glyphoglossus* (of nine recognized) were examined, so the variation of skeletal characters in this genus might be underestimated. All *Glyphoglossus* species are adapted to fossorial lifestyle, and are easily distinguished from all other members of the group by their large body size, stocky and globular habitus, and enlarged inner metacarpal tubercle used for burrowing. Species of *Glyphoglossus* inhabit lowland areas of southern mainland China, Indochina, and Sundaland (Fig. 2). A broad range of morphological variation has been documented: *G. molossus* is notably different from *G. yunnanensis* and *G. guttulatus* (until recently, both were classified as members of the genus *Calluella* Stoliczka, 1872, now considered a junior synonym of *Glyphoglossus* based on its phylogenetic placement;

Peloso et al. 2016). Owing to the morphological uniqueness of *G. molossus*, morphological features of this species are marked with an asterisk (*).

Skull

Skull notably wider than long (Fig. 4). Skull widest at mid-length, giving head a widened, rounded shape. Frontoparietals longer than broad, narrowing anteriorly, connecting medially with a suture along whole length, or anteriorly, separated or fused* (Fig. 5A) medially, lacking dorsal crests, separated or fused* with exoccipitals (separate) posteriorly. Nasals large, separated, chondrified peripherally; processus paraorbitalis well-developed, pointed laterally or anteriorly* (Fig. 5). Sphenethmoid separate, well ossified, restricted to anterior third of brain case or nearly closing lateral wall of brain case* (Fig. 5C). Prootics ossified anteromedially or completely*, crista parotica mineralized medially or completely*. Squamosal ossified, with well-developed ventral ramus and less developed, but distinct otic and zygomatic rami. Operculum cartilaginous or ossified*. Columella largely ossified, with only pars externa plectra cartilaginous; tympanic annulus completely chondrified. Premaxilla well-ossified, alary process oriented slightly posteriorly, distal portion straight or bending laterally*. Maxilla well-ossified, anteriorly contacting labial portion of premaxilla; teeth present or absent*; pars facialis moderately to notably high, and oriented towards processus paraorbitalis of nasal*. Quadratojugal robust, with rounded cartilaginous articulation with angulosplenial, anteriorly articulating with or fused* to maxilla. Pterygoid massive, with a long anterior ramus, broad posterior ramus, and short medial ramus. Vomers large, shape either complex or U-shaped*, defining lower floor of nasal capsule. Neopalatines obscured by postchoanal vomerine

processes, fused or replaced completely*. Nasal capsules mineralized posteriorly or obscured by postchoanal vomerine processes*. Mentomeckelians ossified, connected to dentaries, and to each other through Meckel's cartilage. In *G. molossus*, ventral portion of mentomeckelian cartilage protruding and greatly mineralized, forming a unique beard-like structure, shaping the characteristically flattened snout profile* (Fig. 2). Dentary fused with angulosplenic. Parasphenoid smooth, its cultriform process broad, tapering anteriorly or not*, terminating at level of sphenethmoid or nasal capsules*, with a chondrified notch. Hyoid plate completely cartilaginous, its anterolateral processes well-developed, recurved, posterolateral processes slender, posteromedial processes strongly ossified, elongated, straight, chondrified at distal ends, wider at proximal ends, separated by a chondrified parathyoid. Each posteromedial process bears two bony flanges; one oriented laterally, another medially.

Vertebral column

Vertebral column diplasiocoelus, with eight presacral vertebrae. PSV II–VII procoelous, PSV VIII amphicoelous. PSV I very unusual in shape in *G. molossus*, with highly extended condylar arms. Transverse processes of PSV II–IV longer and wider than V–VIII, transverse processes of PSV II, VII and VIII oriented anterolaterally, IV and V posterolaterally, III and VI at right angle to vertebral column axis. In *G. molossus* transverse processes of PSV greatly shortened, II and VI–VIII oriented anterolaterally, IV oriented posterolaterally, III and V at the right angle to the body axis* (Fig. 6A). Sacral transverse processes moderately expanded, with the distal end about twice as wide as the proximal end. The urostyle notably shorter than the trunk vertebrae (Fig. 6), bearing a dorsal crest that tapers posteriorly and vanishes at about one third of the urostyle length (Fig. 6B), or continues almost to the end of the urostyle* (Fig. 6A).

Appendicular skeleton

Pectoral girdle firmisternal. Coracoids, scapulae, and suprascapulae present; first two fully ossified; suprascapulae largely chondrified. Coracoids robust, with proximal end, or both ends widened*. Omosternum absent. Procoracoids present or absent*. Clavicles present or absent*. Cartilaginous sternum large, partially mineralized, fan-shaped; xiphisternum completely cartilaginous.

Hand skeleton with six largely calcified carpal elements: carpale distale II, carpale distale III–V fused into a single large element, prepollex (consisting of two elements), element Y, radiale and ulnare (Fig. 7A–B). Metacarpals long and fully ossified; phalangeal formula: 2-2-3-3; all phalanges ossified, notably shortened in *G. molossus**; distal phalanx of finger III simple. Foot with four tarsal elements, including ossified tarsale distale II–III, centrale, and prehallux; prehallux enlarged and ossified (Suppl. material 10: Figure S5C). Metatarsals fully

ossified, long, more massive than metacarpals; phalangeal formula: 2-2-3-4-3; all phalanges ossified. Terminal phalanx of toe III simple, conical.

Body size and sexual dimorphism evolution

Clades I and II of *Microhyla* are inferred to have independently reduced in body size from a moderately small common ancestor (males estimated at 25.3 mm, 95% CI 18.8–34.2; Fig. 8). Within *Microhyla* I, two clades arose from miniaturized common ancestors, the *Microhyla superciliaris* species group (common ancestor estimated at 17.7 mm), and the *M. achatina* species group (common ancestor estimated at 19.6 mm; a second clade, composed of *Microhyla* sp. 3 and *M. kodial*, likely independently reduced in size with a common ancestor of 18.3 mm). A few lineages have also reduced in body size below 20 mm independently (Fig. 8), giving a total of eight transitions to SVL < 20 mm. The common ancestor of all *Microhyla* II species was apparently miniaturized (male SVL estimated at 18.1 mm), and most lineages reduced further. Two lineages, *M. annamensis* + *M. marmorata* and *M. pulchella*, have increased in body size independently and repeatedly from miniaturized ancestors, to their modern body sizes. In *Microhyla* I, the *M. bermorei* species group substantially increased in body size. Among *Glyphoglossus*, *G. molossus* is an extreme outlier in body size, and is substantially larger than other equivalent-level clades. Across the entire assemblage, male SVL exhibits substantial phylogenetic signal (Pagel's $\lambda = 1.00$).

Sexual size dimorphism exhibits no phylogenetic signal (Pagel's $\lambda = 7.2 \times 10^{-5}$), changing sporadically across the tree, and is weakly positively correlated with log(male SVL) (PGLS, $F_{1,51} = 5.478$, adjusted $R^2 = 0.07928$, $P = 0.02321$; Fig. 9B). Most species of *Microhyla* I and II exhibit female-biased size dimorphism (above the $y = x$ line in Fig. 9A), and among these, species with the smallest males exhibit the greatest degree of size dimorphism (Fig. 9B). Only six species are male-biased (*Microhyla* sp. 1, *M. mantheyi*, *M. mukhlesuri*, *M. superciliaris*, *M. mihintalei*, and *G. molossus*), including both the largest (*G. molossus*) and the smallest (*Microhyla* sp. 1) species in our dataset.

Discussion

A fully resolved taxonomic framework should approximate the phylogenetic relationships of its members, allowing the user to roughly infer basic information from the framework itself (Wake 2013). This information includes monophyly of the recognized taxonomic groupings, and their differences in sets of biologically significant traits. A taxonomic framework that allows such information to be accurately inferred maximizes its utility. Additionally, the taxonomic framework should, ideally, be optimized for stability, reducing the need for additional taxonomic changes in future (Vences et al. 2013). All recent

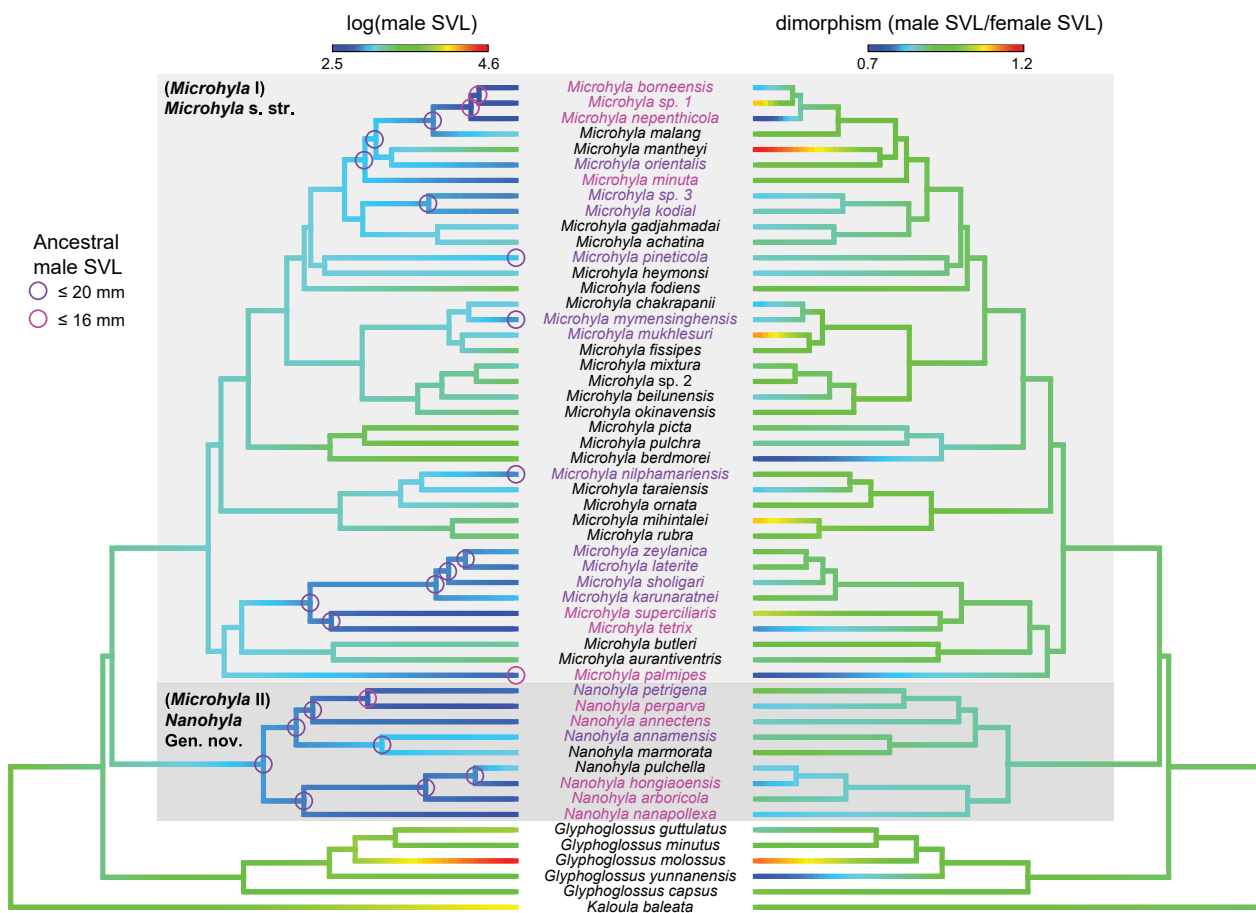


Figure 8. Continuous ancestral state reconstruction of male body size (left) and sexual size dimorphism (right) in the *Microhyla*–*Glyphoglossus* assemblage. Species names in purple have at least one sex with maximum SVL ≤ 20 mm, in fuchsia at least one sex with maximum SVL ≤ 16 mm. Circles at nodes are based on inferred ancestral male SVL.

phylogenetic studies of the subfamily Microhylinae agree that (i) *Glyphoglossus* and *Microhyla s. lat.* are closely related, and (ii) *Microhyla s. lat.* consists of two deeply-divergent lineages (*Microhyla* I and II of Gorin et al. [2020]). The relationship between these two *Microhyla* clades and *Glyphoglossus* evidently cannot be resolved with mitochondrial DNA alone (e.g., Matsui et al. 2011; Poyarkov et al. 2018b, 2019; Nguyen et al. 2019; Li et al. 2019; Gorin et al. 2020), likely due to a combination of the considerable age of these splits (>40 Ma), resulting in saturation and loss of phylogenetic signal, and the moderately rapid succession in which they apparently occurred. Nuclear data, especially multilocus datasets, do, however, support the monophyly of *Microhyla s. lat.* (Peloso et al. 2016; Tu et al. 2018; Garg and Biju 2019; Gorin et al. 2020; and the present paper). However, as will become evident in the following, we find there to be substantial evidence supporting the treatment of the two major clades within *Microhyla s. lat.* as separate genera.

Although present evidence indicates that we can be moderately confident in the respective monophyly of *Microhyla s. lat.* and *Glyphoglossus*, it is also worth noting that the two lineages within *Microhyla s. lat.* are very old. The *Microhyla*–*Glyphoglossus* assemblage radiated within a narrow period in the middle Eocene, with the

origin of *Glyphoglossus* dating to 44.1 Ma (38.5–49.6), while the basal split within *Microhyla s. lat.* is estimated at 43.9 Ma (37.8–48.2) (Suppl. material 4: Table S4, Suppl. material 8: Figure S3). These two estimates are very close and their 95% credibility intervals overlap, suggesting near-simultaneous origin of *Glyphoglossus*, *Microhyla* I, and *Microhyla* II. These estimates are notably older than the ages of all other microhyline genera (except *Chaperina*), which may have diverged in the late Eocene (the split between *Micryletta* and *Mysticellus* [40.9 Ma, 33.3–47.7]) or Oligocene (the split between *Kaloula* and *Uperodon* 27.4 Ma [19.4–34.9], and the split between *Phrynella* and *Metaphrynella* is estimated at 23.0 Ma [16.2–29.1]; Suppl. material 4: Table S4, Suppl. material 8: Figure S3). Similar results were also reported by Garg and Biju (2019), who provided even older estimates for all microhyline genera. Thus, the two clades within *Microhyla s. lat.* are of equal or greater age than other genera in this subfamily. While age has not historically been taken into account in most higher taxonomy, it is nonetheless desirable for taxa of equal rank to be of generally comparable age (Hennig 1966; Vences et al. 2013).

In addition to their substantial age, we have identified a number of important osteological and external

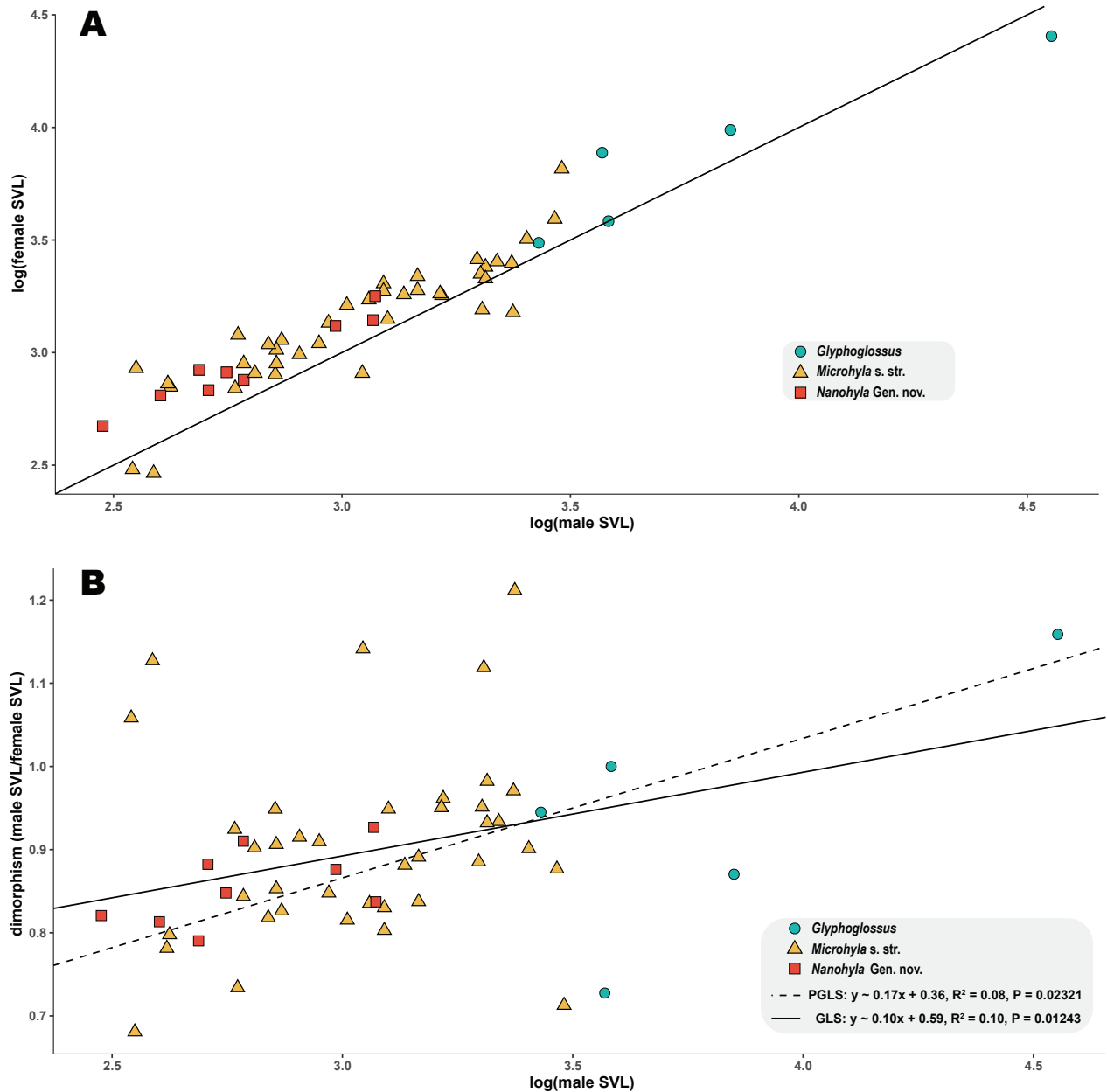


Figure 9. Relationships between body size among sexes (A), and between male body size and sexual size dimorphism (B) in *Microhyla s. str.*, *Nanohyla* gen. nov., and *Glyphoglossus*. The line in (A) represents $x=y$.

morphological differences that distinguish the three clades within this assemblage, including the two clades within *Microhyla s. lat.* These include body size and shape, number and shape of metatarsal tubercles, adaptation to burrowing lifestyle, extent of toe webbing, relative size of the first finger (F1) (Fig. 10), and the presence of an external tympanum (Fig. 11). The absence of an externally visible tympanum traditionally was regarded as one of the key diagnostic characters of the genus *Microhyla* (Boulenger, 1882; Parker 1934; Garg et al. 2019). In all species of *Microhyla* I, the tympanum is hidden under the skin of the supratympanic fold. However, a closer examination of all species of *Microhyla* II demonstrates that six (of nine) taxa actually have an external tympanum that is discernable in breeding males (Fig. 11). The presence of an externally visible tympanum in the majority of the *Microhyla* II species

suggests it may be an important character for diagnosing this clade from *Microhyla* I (Suppl. material 5: Table S5).

Furthermore, there are pronounced differences in the patterns of geographical distribution among the three clades of the *Microhyla–Glyphoglossus* assemblage (Fig. 1) which, along with their ecological differences, suggest that they may warrant recognition as separate genera of Microhylinae. The available hypothesis of the biogeographic history of this assemblage (Gorin et al. 2020) demonstrated that the group originated in Southeast Asia. The smaller members of *Microhyla* II clade are closely associated with perhumid montane forests, and their distribution is limited by mountain ridges among Borneo, the Thai-Malay Peninsula and Indochina (Fig. 1). At the same time, large-sized burrowing species of *Glyphoglossus* can aestivate during the dry season, and have become more widely distributed

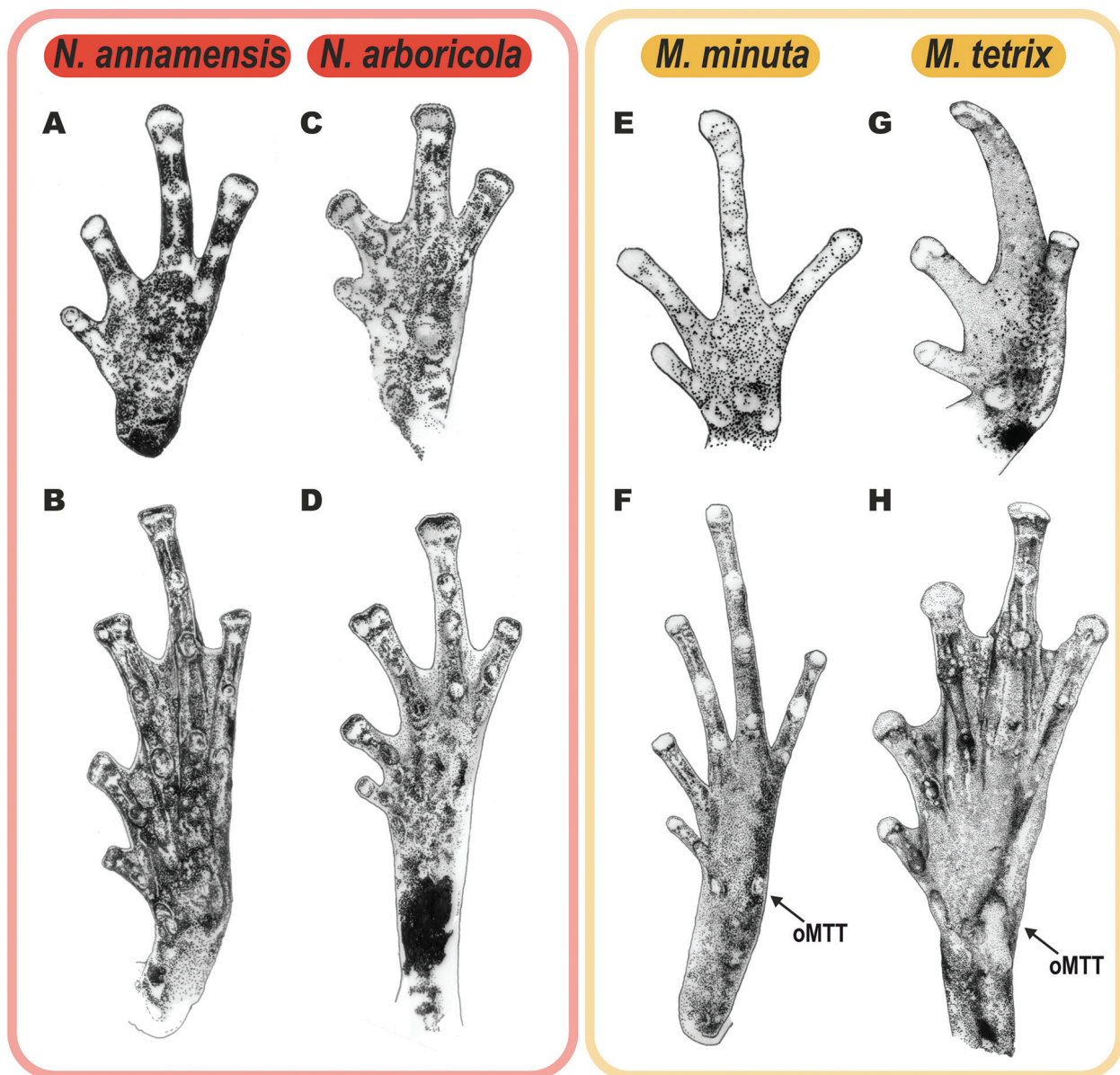


Figure 10. Palmar views of hands (above) and thenar views of feet (below) of the representative *Microhyla s. str.* and *Nanohyla* gen. nov. species: *N. annamensis* (A, B), *N. arboricola* (C, D), *M. minuta* (E, F), and *M. tetrix* (G, H). Arrow indicates outer metatarsal tubercle. Not to scale. Line drawings by Valentina D. Kretova.

across Southeast Asia and seasonally dry plains of Central Indochina and Myanmar (Fig. 1; Gorin et al. 2020). *Microhyla* I is the most diverse clade in terms of morphological and ecological adaptations, and species of this group have colonized almost the entire Asian Realm, including southern and eastern China (Fig. 1).

The cumulative evidence suggests to us that continuing to recognize the superficially similar *Microhyla* I and II clades as members of a single genus would conceal information on the ancient divergence between these lineages, as well as the differences between them in a number of biologically relevant organismal traits. Put another way, recognizing the two clades as separate genera would enhance the diagnosability of the respective genera, make them more comparable units to other genera, and fully stabilize the taxonomy of the *Microhyla*–*Glyphoglossus* assemblage (if coalescent phylogenomic reconstructions were to reveal

the clades to be paraphyletic with respect to *Glyphoglossus*, no taxonomic changes would be necessary). Splitting them would therefore be in accordance with all three of the Priority Taxon Naming Criteria (TNCs) of Vences et al. (2013): Monophyly, Clade Stability, and Diagnosability, as well as the secondary TNCs Time Banding and Biogeography. We also contend that this solution is superior to the obvious alternatives, which are (i) sinking all three clades into a single genus, or (ii) recognizing the two clades within *Microhyla s. lat.* as subgenera. The former would maximize monophyly and clade stability, but would seriously compromise the diagnosability of the genus, whereas the latter would continue to satisfy the three priority TNCs but would not optimize under the Time Banding TNC. In the following, we therefore divide *Microhyla s. lat.* (hitherto containing 52 species), into two genera consisting of 43 (*Microhyla* I) and nine (*Microhyla* II) species each. As there are no available

names for *Microhyla* II, we formally describe it as a new genus, and provide revised taxonomic accounts of *Microhyla s. lat.* and *Glyphoglossus*.

Taxonomic accounts

Nanohyla Poyarkov, Gorin & Scherz, gen. nov.

<http://zoobank.org/0624CCB0-DC63-40F9-A7B7-7F8627B491BB>

Figs 10, 11; Suppl. material 5: Table S5

Chresonymy. *Microhyla* (partim)—Boulenger 1900; Smith 1923; Inger and Frogner 1979; Inger 1989; Bain and Nguyen 2004; Poyarkov et al. 2014; Hoang et al. 2020.

Microhyla (*Microhyla*) (partim)—Dubois 1987 (as a part of the subgenus *Microhyla*).

Type species. *Microhyla annectens* Boulenger, 1900.

Etymology. The genus name is derived from the Greek *vāvoς* (*nanos*), meaning “dwarf”, “pygmy”, and the mythological figure, Hylas (Ancient Greek: *Ἵλας*), which is probably derived from the Ancient Greek verb *ὕλαω* meaning “to bark” (Bourret 1942). In classical mythology, Hylas, son of King Theiodamas, was a youth who served as Heracles’ companion, lover, and servant. Heracles took Hylas with him on the Argonauts’ expedition, during which Hylas was kidnapped by nymphs of the spring in Pegae, Mysia, and turned into an echo. Heracles left the ship and was searching for Hylas for a great length of time, calling his name: “*His adjunxit Hylan nautae quo fonte relictum / Clamassent ut littus Hyla! Hyla! omne sonaret*” (“The mariners cried on Hylas till the shore / Then Re-echoed Hylas! Hylas! soothed...”; Virgil 1916, Ecl. 6, 43). The genus name refers to the small body size (< 25 mm) of all known *Nanohyla* species, while maintaining resemblance to its sister genus *Microhyla*, from which it is separated herein. The new genus name is feminine in gender.

Suggested common name. Pygmy Narrow-mouthed Frogs.

Taxonomic content. Nine species, including: *Nanohyla annamensis* comb. nov. (Smith, 1923); *Nanohyla annectens* comb. nov. (Boulenger, 1900); *Nanohyla arboricola* comb. nov. (Poyarkov, Vassilieva, Orlov, Galoyan, Tran, Le, Kretova & Geissler, 2014); *Nanohyla hongiaoensis* comb. nov. (Hoang, Nguyen, Luong, Nguyen, Orlov, Chen, Wang & Jiang, 2020); *Nanohyla marmorata* comb. nov. (Bain & Nguyen, 2004); *Nanohyla nanapollexa* comb. nov. (Bain & Nguyen, 2004); *Nanohyla petrigena* comb. nov. (Inger & Frogner, 1979); *Nanohyla perparva* comb. nov. (Inger & Frogner, 1979); and *Nanohyla pulchella* comb. nov. (Poyarkov, Vassilieva, Orlov, Galoyan, Tran, Le, Kretova & Geissler, 2014). Photos of *Nanohyla* gen. nov. members are presented in Fig. 11.

Diagnosis. The new genus is assigned to the subfamily Microhylinae on the basis of phylogenetic affinities and

the following combination of morphological character states: vomers small, confined to the anterior and medial margins of choanae; clavicles and, in most cases, procoracoids absent, maxillary arcade edentate (Parker 1934). *Nanohyla* gen. nov. differs from other Microhylinae genera by the following combination of osteological character states: (1) frontoparietals fused with exoccipitals; (2) exoccipitals fused with each other (incomplete fusion in *N. pulchella*); (3) neopalatines present; (4) sphenethmoids completely fused with parasphenoid (incomplete fusion in *N. pulchella*); (5) crista parotica entirely cartilaginous; (6) otic ramus of squamosal well-developed; (7) tympanic annulus well-developed; (8) transverse processes of presacral vertebrae with the following orientation: IV and V posterolaterally, II, VII and VIII anterolaterally, III and VI at right angle to body axis; (9) clavicles absent; (10) omosternum present, cartilaginous; (11) prehallux cartilaginous; (12) terminal phalanges of longest fingers and toes T-shaped. The combination of diagnostic external morphological characters includes: (13) small to extremely small frogs (adult SVL 11.8–25.8 mm); (14) snout rounded or pointed in profile; (15) supratympanic fold present; (16) ridge on posterior sides of choanae absent; (17) first finger (FI) length less than ½ FII or reduced to a nub; (18) finger discs present, at least on FII–FIV; (19) dorsal median longitudinal grooves on finger discs generally present (with the exception of *N. perparva*); (20) toes dorsolaterally flattened, prominent discs present; (21) dorsal median longitudinal grooves on toe discs present; (22) metatarsal tubercle single (inner metatarsal tubercle present, outer absent); (23) dorsomedial line absent; (24) superciliary tubercles absent; (25) tibiotarsal articulation of adpressed hindlimb reaching well beyond snout; (26) toe webbing well-developed (at least one-half webbed); (27) skin on dorsum feebly granular to tubercular; (28) tympanum externally distinct at least in males (*N. annamensis*, *N. annectens*, *N. arboricola*, *N. marmorata*, *N. nanapollexa*, *N. pulchella*) or barely distinct (*N. hongiaoensis*, *N. perparva*, *N. petrigena*); (29) terrestrial or scansorial semi-arboreal microhabitat preference.

Phylogenetic definition. The genus *Nanohyla* gen. nov. includes all species sharing a more recent common ancestor with *Nanohyla annectens* than with *Microhyla achatina* and *Glyphoglossus molossus*.

Distribution. The distribution area of *Nanohyla* gen. nov. covers montane forests of the Annamite (Truong Son) Mountains in Vietnam, eastern Laos, and north-eastern Cambodia, the Titiwangsa Mountain Range in the southernmost Thailand and peninsular Malaysia, mountains of Borneo (including Sabah and Sarawak of Malaysia, Brunei, and Kalimantan of Indonesia) and the Sulu Archipelago of the Philippines (see Fig. 1). The occurrence of *Nanohyla* gen. nov. in Cardamom Mountains in eastern Thailand (the record of “*M. annamensis*” from Khao Seabab by Taylor [1962], see Fig. 1) is questionable (see Poyarkov et al. 2014, 2020a).

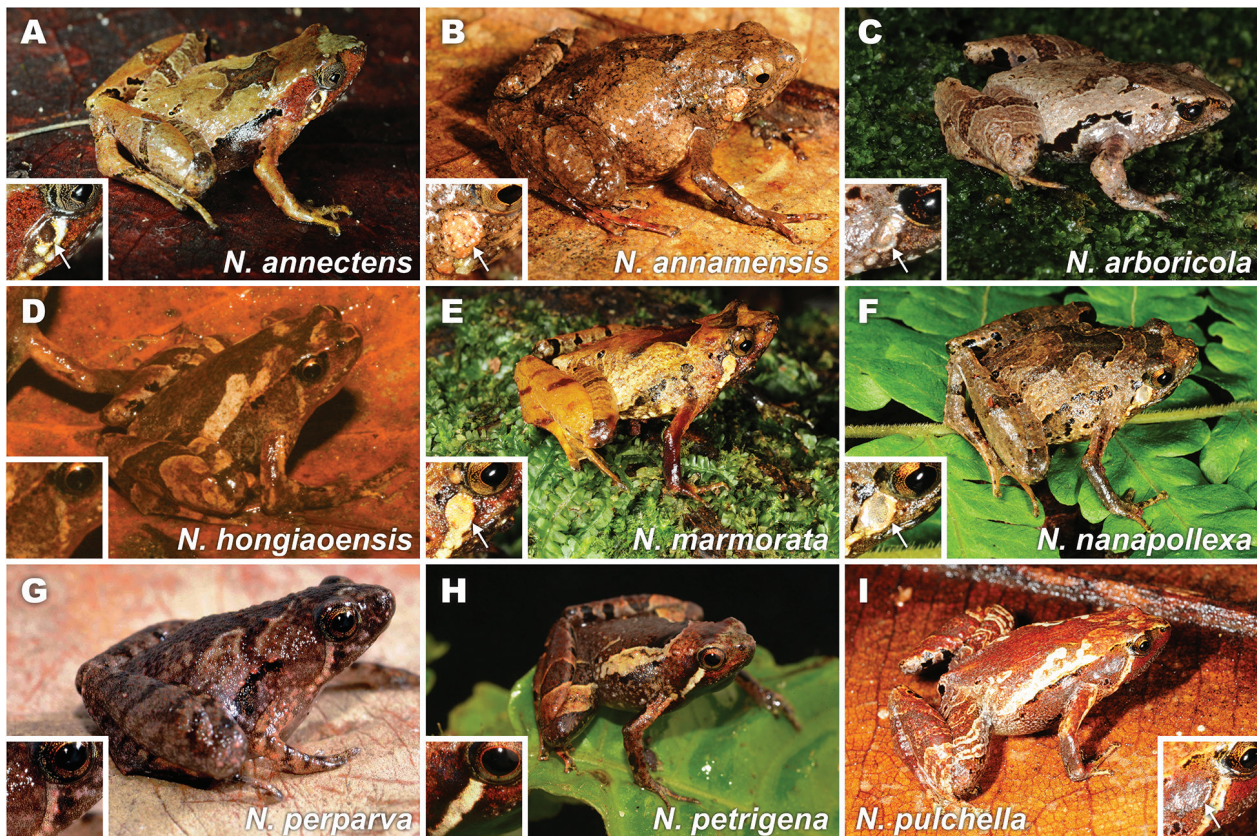


Figure 11. Members of the new genus *Nanohyla* gen. nov. in life (males): *N. annectens* from Genting Highlands, Pahang, Malaysia (A), *N. annamensis* from Bidoup – Nui Ba N.P., Lam Dong, Vietnam (B), *N. arboricola* from Chu Yang Sin N.P., Dak Lak, Vietnam (C), *N. hongiaoensis* from Bidoup – Nui Ba N.P., Lam Dong, Vietnam (D), *N. marmorata* from Kon Chu Rang N.R., Gia Lai, Vietnam (E), *N. nanapollexa* from Kon Plong, Kon Tum, Vietnam (F), *N. perparva* from Gunung Mulu, Sarawak, Malaysia (G), *N. petrigena* from Gunung Mulu, Sarawak, Malaysia (H), and *N. pulchella* from Bidoup – Nui Ba N.P., Lam Dong, Vietnam (I). Insets show tympanic area of the each species; white arrow points at the tympanic rim of the external tympanum. Photos by Nikolay A. Poyarkov (A–C, D–F, I), Vu Dang Hoang Nguyen (D), and Indraneil Das (G, H).

Morphological comparison. The new genus *Nanohyla* gen. nov. differs from its sister genus *Microhyla* Tschudi, 1838 *s. str.* by the well-developed (vs poorly-developed) otic ramus of the squamosal, frontoparietals and exoccipitals fused (vs separated or slightly fused), exoccipitals fused with each other (vs always separated), omosternum present (vs usually absent), sphenethmoid and parasphenoid fused completely or partially (vs separated), cartilaginous crista parotica (vs mineralized posteriorly), cartilaginous prehallux (vs mineralized), tympanum externally visible or barely visible (vs concealed beneath skin), inner metatarsal tubercle well-developed, outer generally absent (vs two metatarsal tubercles well-developed), and in having digits dorso ventrally flattened, FI often reduced to a nub or shortened (vs variably longer). The new genus differs from the closely related genus *Glyphoglossus* Günther, 1869 by its smaller adult size with SVL < 25mm (vs SVL > 25mm), skull longer than wide or almost equal (vs wider than long), alary process of premaxilla oriented slightly anteriorly (vs posteriorly), neopalatines present (vs obscured by vomers), vomers small, indistinct (vs large, well-developed), omosternum present (vs absent), terminal phalanges T-shaped (vs simple), tibio-tarsal articulation reaching well beyond

snout (vs to the anterior border of the eye, or less), by body habitus short, triangular-shaped (vs stout, balloon-shaped), and by inner metatarsal tubercle not enlarged (vs enlarged, shovel-shaped). *Nanohyla* gen. nov. differs from *Kaloula* Gray, 1831 by its much smaller adult body size SVL < 25 mm (vs SVL > 38 mm), procoracoids absent (vs present), postchoanal portion of vomer absent (vs present), neopalatines present (vs obscured), prehallux formed by two elements (vs one), tibio-tarsal articulation reaching well beyond snout (vs to shoulder), absence (vs presence) of ridge on posterior margin of choanae, inner metatarsal tubercle not enlarged (vs enlarged and spatulate), and by body habitus short, triangular-shaped (vs robust). The new genus can be distinguished from *Uperodon* Duméril & Bibron, 1841 by its smaller adult size, SVL < 25 mm (vs SVL > 34 mm), postchoanal portion of vomer absent (vs present), neopalatines present (vs obscured), tibio-tarsal articulation reaching well beyond snout (vs posterior border of eye, or less), absence (vs presence) of ridge on posterior margins of choanae, inner metatarsal tubercle not enlarged (vs enlarged or spatulate), and by body habitus short, triangular-shaped (vs robust and globular). *Nanohyla* gen. nov. differs from *Phrynella* Boulenger, 1887 by its smaller adult size, SVL <

25 mm (vs SVL > 30 mm), medial process of the prechoual part of vomer absent (vs present), neopalatines present (vs absent), procoracoids absent (vs present), vertebral column diplasiocoelus (vs procoelus), metatarsal tubercles separate (vs united), by tibio-tarsal articulation reaching well beyond snout (vs to tympanic region), by body habitus short, triangular-shaped (vs robust and flattened), and by generally dull brownish coloration of inguinal and dorsal surfaces (vs greenish coloration of dorsum and bright-red coloration of inguinal area, and ventral surfaces of limbs). The new genus further differs from *Metaphrynella* Parker, 1934 by its smaller adult size, SVL < 25 mm (vs SVL > 25 mm), skull longer than wide or almost equal (vs wider than long), neopalatines present (vs absent), omosternum present (vs absent), vertebral column diplasiocoelus (vs procoelus), tibio-tarsal articulation reaching well beyond snout (vs to tympanic region), absence (vs presence) of a ridge on posterior margins of choanae, metatarsal tubercles separate (vs united and enlarged), and by finger webbing absent (vs present). The new genus differs from *Mysticellus* Garg & Biju, 2019 by its short triangular-shaped body habitus (vs slender), supratympanic fold present (vs absent), finger and toe tips enlarged with prominent discs (vs slightly enlarged), toe webbing well-developed (vs rudimentary), supernumerary carpal tubercles absent (vs prominent subarticular tubercles alternating with additional smaller tubercles), and the two prominent blackish-brown ‘false-eye’ inguinal spots absent (vs present). *Nanohyla* gen. nov. differs from *Micryletta* Dubois, 1987 by its snout longer than eye diameter, and having eye less (vs more) prominent in lateral and dorsal aspects, finger and toe tips enlarged with prominent discs (vs slightly enlarged), toe webbing well-developed (vs rudimentary or absent), supernumerary carpal tubercles absent (vs present), omosternum present (vs absent), neopalatines present (vs absent), tibio-tarsal articulation reaching well beyond snout (vs to anterior border eye, or less), supratympanic fold present (vs absent), and body habitus short, triangular-shaped (vs slender). Finally, the new genus is distinguished from *Chaperina* Mocquard, 1892 by clavicles and procoracoids absent (vs present), postchoanal portion of vomer absent (vs present), omosternum present (vs absent), terminal phalanges T-shaped (vs simple), tibiotarsal articulation reaching well beyond snout (vs anterior border of eye), belly dull-colored (vs bright saffron-yellow belly with dark pattern), and by absence of spine-like projections on limbs (vs a long, narrow dermal spine projecting from calcaneus).

Larval morphology. Description of the larval stages of the *Nanohyla* gen. nov. members are sparse and often not detailed. Poyarkov et al. (2014) provided descriptions, photos and illustrations of tadpole morphology for *N. annamensis*, *N. arboricola* and *N. pulchella*. Vassilieva et al. (2017) provided a detailed description of development, larval morphology and anatomy for *N. arboricola*. Le et al. (2016) provided a brief description of tadpole morphology of *N. marmorata*. Leong (2004) provided a short description and photographs of larval and meta-

morph morphology for *N. annectens*. Brief descriptions and figures depicting larvae of *N. petrigena* and *N. perparva* are found in the original description of these species by Inger and Frogner (1979), as well as in Inger and Steubing (2005) and Haas et al. (2020). Larval stages of *N. hongiaoensis* and *N. nanapollexa* remain unknown.

As with almost all larvae in Microhylidae, labial teeth and mandibles are absent from the oral discs of *Nanohyla* tadpoles. Most species of *Nanohyla* have larval morphology resembling that of many pond-breeding *Microhyla* species (Poyarkov et al. 2014) with rather short-tailed transparent or semi-transparent Orton’s type II tadpoles (Orton 1953), that are mid-water column (neustonic) feeders with comparatively unexpanded lower labium and anteriorly directed terminal mouths, lateral orientation of eyes, spiraculum located in a medial position on the venter, spiracular flap with crenulate margins, and tail lacking terminal filament (Altig and Johnston 1989; Donnelly et al. 1990; Leong 2004). In contrast, many species of *Microhyla* s. str. are surface suspension feeders, and demonstrate greatly expanded lower labium and dorso-terminal mouth orientation; they may have terminal filament on tail and smooth margins of spiracular flap (e.g., Leong 2004; Hendrix et al. 2008; Poyarkov et al. 2014).

A peculiar exception is the case of *N. arboricola*, which is an obligate phytotelm-breeding species that reproduces in water-filled tree hollows (Vassilieva et al. 2017). The oophagous tadpoles of this species differ from larvae of pond-dwelling *Microhyla* and *Nanohyla* species in many aspects, including external morphology (extremely long tails, dorsolateral position of the eyes, dark pigmentation), morphology of digestive tract (large, extensible stomach with comparatively short intestine), and characteristic oral morphology (Vassilieva et al. 2017). *Nanohyla nanapollexa* was suggested as phytotelm-breeder as a single specimen of this species was recorded in a water-filled tree hollow (Gorin et al. 2020), although the details of reproductive biology and tadpole morphology of this species are still unknown.

Taxonomic comment. *Microhyla pulverata* Bain & Nguyen, 2004 was considered a junior synonym of *N. marmorata* based on the phylogenetic results of Gorin et al. (2020); the same study also reported on three putative candidate species within *N. arboricola*, *N. perparva*, and *N. petrigena*, indicating that our knowledge on diversity of *Nanohyla* is still incomplete.

Certain variation in diagnostically important characters of *Nanohyla* gen. nov. requires further comments. Bain and Nguyen (2004) reported on significant variation in size and shape of the outer metatarsal tubercle in *N. marmorata* which was reported to vary from almost indistinct to “conical.” We have examined a large series of *N. marmorata* (see Poyarkov et al. 2014; Nguyen et al. 2019) and found that in this species the outer metatarsal tubercle usually is not discernable or is indistinct; we assume that this discrepancy might be explained with the differences in preservation of specimens examined by

us and by Bain and Nguyen (2004). Hoang et al. (2020) reported two metatarsal tubercles in their diagnosis of *N. hongiaoensis*, however in the holotype description they refer to the outer metatarsal tubercle as “indistinct;” it is also not discernable in their photo of holotype’s foot (Hoang et al. 2020:fig. 3F). In all the remaining species of *Nanohyla* gen. nov. it is absent, and we therefore consider this state to be diagnostic for the genus (in comparison to *Microhyla s. str.*, which has two metatarsal tubercles in all species but *M. maculifera*, see comment below). It is not clear why Bain and Nguyen (2004), or Poyarkov et al. (2014; and other preceding studies) did not recognize the presence of externally visible tympanum in most of species of the genus (Fig. 11). In species of *Nanohyla* gen. nov., smaller tubercles and other dermal structures of the skin become flattened and less distinct after fixation and preservation; this has also been reported in other anurans (Poyarkov et al. 2015, 2017, 2019; Nguyen et al. 2018, 2019, 2020). It is likely that the presence of the tympanum was artifactually concealed from Bain and Nguyen (2004), since their description was based exclusively on museum specimens. In some species of *Nanohyla* gen. nov., we were not able to detect an externally visible tympanum (*N. hongiaoensis*, *N. perparva*, *N. petrigena*). It is not clear whether this reflects an actual character state in these species, or if this apparent state relates to the small sample size of specimens and photographs available to us. Further studies are needed to clarify variation of the external tympanum in *Nanohyla* gen. nov.

Microhyla Tschudi, 1838

Synonymy (fide Frost 2020).

Microhyla Tschudi, 1838. **Type species.** “*Hylaplesia achatina* Boie, 1827” (*nomen nudum*) (= *Microhyla achatina* Tschudi, 1838), by monotypy.

Microhyla Duméril & Bibron, 1841. *Ex errore*.

Siphneus Fitzinger, 1843. **Type species:** *Engystoma ornatum* Duméril & Bibron, 1841.

Dendromanis Gistel, 1848. *Nomen substitutum* for *Microhyla* Tschudi, 1838.

Diploelma Günther, 1859. *Nomen substitutum* for *Siphneus* Fitzinger, 1843.

Scaptophryne Fitzinger, 1861 “1860.” **Type species:** *Scaptophryne labyrinthica* Fitzinger, 1861 “1860” (*nomen nudum*).

Copea Steindachner, 1864. **Type species:** *Copea fulva* Steindachner, 1864.

Ranina David, 1872 “1871.” **Type species:** *Ranina symmetrica* David, 1871, by monotypy. Junior homonym of *Ranina* Lamarck, 1801.

Etymology. The genus name is derived from the Greek μικρός (*mikros*), meaning “small,” and “*Hylas*” (for origin of this name see above).

Common name. Narrow-mouthed Frogs.

Taxonomic content. 42 species: *M. achatina* Tschudi, 1838; *M. aurantiventris* Nguyen, Poyarkov, Nguyen, Nguyen, Tran, Gorin, Murphy & Nguyen, 2019; *M. beilunensis* Zhang, Fei, Ye, Wang, Wang & Jiang, 2018; *M. berdmorei* (Blyth, 1856); *M. borneensis* Parker, 1928; *M. butleri* Boulenger, 1900; *M. chakrapanii* Pillai, 1977; *M. darevskii* Poyarkov, Vassilieva, Orlov, Galoyan, Tran, Le, Kretova & Geissler, 2014; *M. darreli* Garg, Suyesh, Das, Jiang, Wijayathilaka, Amarasinghe, Alhadi, Vineeth, Aravind, Senevirathne, Meegaskumbura & Biju, 2019; *M. eos* Biju, Garg, Kamei & Maheswaran, 2019; *M. fanjingshanensis* Li, Zhang, Xu, Lv & Jiang, 2019; *M. fissipes* Boulenger, 1884; *M. fodiens* Poyarkov, Gorin, Zaw, Kretova, Gogoleva, Pawangkhanant & Che, 2019; *M. gad-jahmadai* Atmaja, Hamidy, Arisuryanti, Matsui & Smith, 2018; *M. heymonsi* Vogt, 1911; *M. irrawaddy* Poyarkov, Gorin, Zaw, Kretova, Gogoleva, Pawangkhanant & Che, 2019; *M. karunaratnei* Fernando & Siriwardhane, 1996; *M. kodial* Vineeth, Radhakrishna, Godwin, Anwasha, Rajashekhar & Aravind, 2018; *M. laterite* Seshadri, Singal, Priti, Ravikanth, Vidisha, Saurabh, Pratik & Gururaja, 2016; *M. malang* Matsui, 2011; *M. mantheyi* Das, Yaakob & Sukumaran, 2007; *M. mihintalei* Wijayathilaka, Garg, Senevirathne, Karunaratna, Biju & Meegaskumbura, 2016; *M. minuta* Poyarkov, Vassilieva, Orlov, Galoyan, Tran, Le, Kretova & Geissler, 2014; *M. mixtura* Liu & Hu in Hu et al. 1966; *M. mukhlesuri* Hasan, Islam, Kuramoto, Kurabayashi & Sumida, 2014; *M. mymensinghensis* Hasan, Islam, Kuramoto, Kurabayashi & Sumida, 2014; *M. nepenthicola* Das & Haas, 2010; *M. nilphamariensis* Howlader, Nair, Gopalan & Merilä, 2015; *M. okinavensis* Stejneger, 1901; *M. orientalis* Matsui, Hamidy & Eto, 2013; *M. ornata* (Duméril & Bibron, 1841); *M. palmipes* Boulenger, 1897; *M. picta* Schenkel, 1901; *M. pineticola* Poyarkov, Vassilieva, Orlov, Galoyan, Tran, Le, Kretova & Geissler, 2014; *M. pulchra* (Hallowell, 1861); *M. rubra* (Jerdon, 1854); *M. sholigari* Dutta & Ray, 2000; *M. superciliaris* Parker, 1928; *M. taraiensis* Khatiwada, Shu, Wang, Thapa, Wang & Jiang, 2017; *M. tatrix* Suwannapoom, Pawangkhanant, Gorin, Juthong & Poyarkov, 2020; *M. zeylanica* Parker & Osman-Hill, 1949; and, tentatively, *M. maculifera* Inger, 1989.

Revised diagnosis. *Microhyla s. str.* differs from all other Microhylinae genera by the following combination of osteological characters: (1) frontoparietals generally separated from exoccipitals (partially fused in *M. mukhlesuri*, *M. picta* and *Microhyla* sp. 2); (2) exoccipitals separate; (3) neopalatines present (in *M. berdmorei*, *M. butleri*, *M. minuta*, *M. orientalis*, *M. pineticola*, *M. superciliaris* and *M. tatrix*) or absent (in *M. achatina*, *M. heymonsi*, *M. fissipes*, *M. malang*, *M. mukhlesuri*, *M. nepenthicola*, *M. nilphamariensis*, *M. okinavensis*, *M. picta*, *M. pulchra* and *Microhyla* sp. 2); (4) sphenethmoids not fused to parasphenoid; (5) crista parotica ossified posteriorly; (6) otic ramus of squamosal poorly developed; (7) tympanic annulus well-developed (reduced in *M. heymonsi*, *M. nepenthicola*, *M. nilphamariensis*, *M. orientalis*, *M. pinet-*

icola, *M. superciliaris* and *M. tatrix*); (8) orientation of transverse processes of presacral vertebrae VI–VIII anterolateral, other vertebrae with inconsistent orientation; (9) clavicles absent; (10) omosternum absent (cartilaginous omosternum present only in *M. pulchra*); (11) prehal-lux cartilaginous; (12) terminal phalanges of the longest fingers T-shaped (in *M. achatina*, *M. berdmorei*, *M. butleri*, *M. fissipes*, *M. heymonsi*, *M. malang*, *M. minuta*, *M. nepenthicola*, *M. nilphamariensis* and *M. pineticola*), knobbed (in *M. minuta*, *M. mukhlesuri*, *M. nilphamariensis*, *M. superciliaris* and *M. tatrix*), or simple (in *M. okinavensis*, *M. orientalis*, *M. picta* and *M. pulchra*), terminal phalanges of the longest toe T-shaped (in *M. achatina*, *M. berdmorei*, *M. butleri*, *M. heymonsi*, *M. malang*, *M. nepenthicola* and *M. pineticola*), knobbed (in *M. minuta*, *M. mukhlesuri*, *M. nepenthicola*, *M. superciliaris* and *M. tatrix*), or simple (in *M. fissipes*, *M. okinavensis*, *M. orientalis*, *M. picta*, *M. pulchra* and *Microhyla* sp. 2). The combination of diagnostic external morphological characters includes: (13) body size medium to extremely miniaturized (adult SVL 12.8–45.8 mm); (14) snout rounded or pointed in profile; (15) supratympanic fold present; (16) ridge on posterior margins of choanae absent; (17) FI length greater than $\frac{1}{2}$ FII; (18) discs present on every finger, only FII–FIV, or absent; (19) dorsomedial grooves on fingers present or absent; (20) toe discs present or absent; (21) dorsomedial grooves on toes present or absent; (22) two metatarsal tubercles (except *M. maculifera* with a single metatarsal tubercle); (23) dorsomedial line present or absent; (24) superciliary tubercles present (*M. palmipes* and *M. superciliaris*) or absent (all remaining species); (25) tibiotarsal articulation reaching well beyond snout (in *M. berdmorei*, *M. darevskii*, *M. mantheyi* and *M. tatrix*) or less; (26) toe webbing from basal to developed to discs; (27) skin on dorsum from smooth to tubercular; (28) tympanum externally indistinct; (29) terrestrial or subfossorial microhabitat preference.

Phylogenetic definition. The genus *Microhyla* s. str. includes all species that share a more recent common ancestor with *Microhyla achatina* than with *Nanohyla annectens* and *Glyphoglossus molossus*.

Distribution. Frogs of the genus *Microhyla* are widely distributed across the East (southern China, including Taiwan and Hainan islands, and Ryukyu Archipelago of Japan), Southeast (Myanmar and Indochina, Malayan Peninsula, Sumatra, Java, Bali, and Borneo), and South Asia (Bangladesh, Nepal, Indian subcontinent to north-eastern Pakistan in the west and Sri Lanka in the south) (Fig. 1).

Taxonomic comment. In the last phylogenetic revision of *Microhyla*, Gorin et al. (2020) included all species of the genus in their analysis, except *M. darevskii*, *M. fusca* Andersson, 1942, and *M. maculifera*. *Microhyla darevskii* was described from five formalin-fixed specimens and morphologically appears to be very close to the members of *M. berdmorei* species complex (Poyarkov et al. 2014).

Although the phylogenetic position of *M. darevskii* is not known, this species can be confidently assigned to the genus *Microhyla* s. str. based on morphological data. *Microhyla fusca* was described from a single specimen collected from southern Vietnam (Andersson 1942), and was recently demonstrated to be a junior synonym of *M. butleri* (Poyarkov et al. 2020a).

Microhyla maculifera remains the most enigmatic species of the group due to the lack of molecular data and uncertainties regarding morphological characters. This species was described from only two specimens (Inger 1989), and no additional specimens have been reported since that time, despite numerous field survey efforts. This small-sized species is unique among its congeners in having comparatively short hindlimbs, large and wide head, less triangular than in other *Microhyla*, comparatively stout body habitus (Fig. 12), and a single metatarsal tubercle (vs two). *Microhyla maculifera* is different from the members of the genus *Nanohyla* gen. nov. by having FI longer than $\frac{1}{2}$ of FII (vs FI shorter than $\frac{1}{2}$ of FII or reduced to a nub), lack of discs on fingers and rudimentary discs on toes (vs digital discs well-developed), absence (vs presence) of dorsal median grooves on tips of fingers and toes, having comparatively short hindlimbs with tibiotarsal articulation reaching to snout (vs to well beyond snout), and toe webbing being basal (vs well-developed; Inger 1989). Due to the lack of molecular data, the phylogenetic position and generic placement of “*Microhyla*” *maculifera* remains uncertain; we tentatively retain this species *Microhyla* s. str. pending data or future phylogenetic studies, which might suggest another arrangement.

Glyphoglossus Günther, 1869

Synonymy (*vide* Frost 2020).

Glyphoglossus Günther, 1869 “1868”. **Type species:** *Glyphoglossus molossus* Günther, 1869 “1868,” by monotypy.

Calluella Stoliczka, 1872. **Type species:** *Megalophrys guttulata* Blyth, 1856 “1855,” by original designation.

Colpoglossus Boulenger, 1904. **Type species:** *Colpoglossus brooksi* Boulenger, 1904, by monotypy.

Dyscophina Van Kampen, 1905. **Type species:** *Dyscophina volzi* Van Kampen, 1905, by monotypy.

Calliglutus Barbour & Noble, 1916. **Type species:** *Calliglutus smithi* Barbour & Noble, 1916, by monotypy.

Kalluella Gee & Boring, 1929. *Ex errore*.

Etymology. The genus name is derived from the Ancient Greek γλυφή (*gluphé*), meaning “a carving,” and Greek γλῶσσα (*glossa*), meaning “tongue.”

Common name. Balloon Frogs.

Taxonomic content. Nine species, including: *G. brooksii* (Boulenger, 1904); *G. capsus* (Das, Min, Hsu, Hertwig

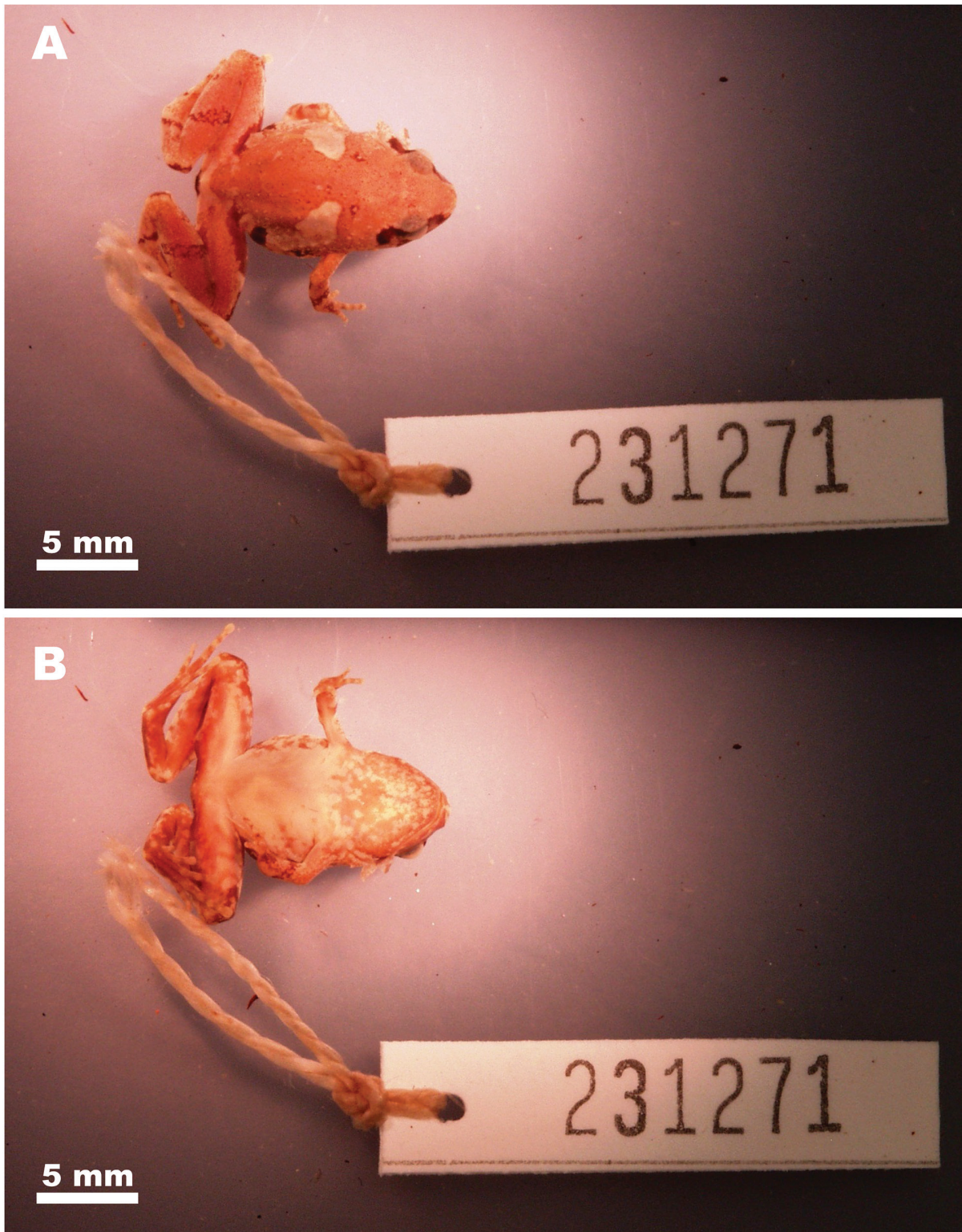


Figure 12. Holotype of *Microhyla maculifera* Inger, 1989 (FMNH 231271, adult male) in dorsal (A) and ventral (B) aspects. Scale bar denotes 5 mm. Field Museum of Natural History. FMNH 231271. Created by Field Museum of Natural History, Amphibian and Reptile Collection and licensed under CC-BY-SA 4.0.

& Haas, 2014); *G. flavus* (Kiew, 1984); *G. guttulatus* (Blyth, 1856); *G. minutus* (Das, Yaakob & Lim, 2004); *G. molossus* Günther, 1869; *G. smithi* (Barbour & Noble, 1916); *G. volzi* (Van Kampen, 1905); and *G. yunnanensis* (Boulenger, 1919).

Revised diagnosis. *Glyphoglossus* Günther, 1869 differs from other Microhylinae genera by the combination of the following osteological characters: (1) frontoparietals separated from exoccipitals (fused to them in *G. molossus*); (2) exoccipitals separated from each other;

(3) neopalatines obscured by a postchoanal portion of vomers; (4) sphenethmoids separated from parasphenoid; (5) crista parotica ossified; (6) otic ramus of squamosal well-developed; (7) tympanic annulus well-developed; (8) orientation of transverse processes of presacral vertebrae as follows: IV and V posterolateral, II, VII and VIII anterolateral, III and VI at right angle to body axis (in *G. molossus* IV posterolateral, II, VI–VIII anterolateral, III and V at right angle to body axis); (9) clavicles present (absent in *G. molossus*); (10) omosternum absent; (11) prehallux ossified; (12) terminal phalanges of the longest finger and toe simple. The combination of diagnostic external morphological characters includes: (13) large to medium-sized frogs (adult SVL 30.9–94.9 mm); (14) snout rounded or bluntly flattened; (15) supratympanic fold present; (16) ridge on posterior margins of choanae poorly developed or absent; (17) first finger (FI) length greater than $\frac{1}{2}$ FII; (18) discs on digits absent; (19) two metatarsal tubercles; (20) dorsomedial line absent; (21) superciliary tubercles absent; (22) tibiotarsal articulation of the adpressed hind-limb reaching eye or shorter; (23) toe webbing moderately developed (at least one-third webbed, in *G. molossus* three-quarters webbed); (24) skin on dorsum from feebly granular to tubercular; (25) external tympanum invisible; (26) fossorial microhabitat preference.

Phylogenetic definition. The genus *Glyphoglossus* includes all species sharing a more recent common ancestor with *Glyphoglossus molossus* than with *Microhyla achatina* and *Nanohyla annectens*.

Distribution. From south-western China across Indochina to Myanmar, Thai-Malay Peninsula, islands of Sumatra and Borneo (Fig. 1).

Taxonomic comment. Until recently *Glyphoglossus* was considered to be a monotypic genus, until it was synonymized with *Calluella* based on phylogenetic data of Peloso et al. (2016). However, available phylogenetic studies (Tu et al. 2018; Garg and Biju 2019; Gorin et al. 2020) have not all included comprehensive sampling of Sundaland species (e.g., *C. volzi*, *C. smithi*, *C. flavus*, and *C. brooksi*). In our opinion, the variable taxonomic sampling included in previous analyses (Matsui et al. 2011; Peloso et al. 2016; Tu et al. 2018; Garg and Biju 2019; Gorin et al. 2020) creates uncertainty which, along with the significant morphological disparity among *G. molossus* and the other species of *Glyphoglossus* examined (Parker et al. 1934), suggests that the generic taxonomy of the group may not be fully resolved.

Body size evolution in the *Microhyla*–*Glyphoglossus* assemblage

Among vertebrates, numerous clades of fishes, frogs, and squamate reptiles compete for the title of the smallest absolute body size, with several converging around body lengths (defined vastly differently in the three clades) of

8–12 mm (Hanken and Wake 1993). This apparent size limit has invoked the idea of physiological constraints preventing the evolution of smaller body sizes (Alexander 1996; Hedges and Thomas 2001; Scherz et al. 2019). As such, species exhibiting miniaturization provide interesting opportunities to understand the lower size limits of vertebrate physiology and development, whereas clades exhibiting miniaturized body plans offer opportunities to understand the dynamics of size evolution. Moreover, miniaturization is often associated with major morphological rearrangements (Hanken 1985; Hanken and Wake 1993; Polilov 2015), and is thought to have played a significant role in generation of some key innovations, such as the mammalian inner ear (Lautenschlager et al. 2018). It is therefore of great interest to also understand the consequences of miniaturization from a broad array of cases.

Frogs, and especially microhylids, have a particular propensity to miniaturize, with several microhylids in a variety of subfamilies achieving adult body sizes of 12 mm or smaller (Clarke 1996; Lehr and Coloma 2008; Das and Haas 2010; Rittmeyer et al. 2012; Rakotoarison et al. 2017; Scherz et al. 2019; Oliver et al. 2017). Despite this diversity, there are surprisingly few studies that have looked at miniaturization in a comparative context within the Microhylidae (e.g., de Sá et al. 2012, 2019b). Here, we have demonstrated that the Microhylinae are a particularly interesting clade of microhylids in which to study miniaturization, because they have converged repeatedly on extremely small body sizes.

Body size evolution in the *Microhyla*–*Glyphoglossus* was discussed in a study based on the maximum parsimony analysis of trait evolution, categorizing SVL into a series of bins (Gorin et al. 2020). Our analysis, which instead uses ancestral state reconstruction of continuous traits (Revell 2012) on our dated phylogeny, is largely congruent with that of Gorin et al. (2020) but provides better estimation of ancestral states and the timing of transitions in body size. Our results show clearly that this assemblage has undergone repeated miniaturization events, with *Nanohyla* miniaturizing first and independently from all *Microhyla* species; their most recent common ancestor is inferred to have been only a small frog (ca 25.3 mm in males). Within *Microhyla*, two large clades converged further toward the minimum size range, but six other lineages independently also became miniaturized (crossing the threshold of SVL < 20 mm). These replicates provide an opportunity to understand the relationship of certain morphological features with extreme body size reduction. Miniaturization of *Nanohyla* appears to have been coupled with the loss of metatarsal tubercles, whereas these are retained in even the smallest *Microhyla*. Likewise, the first finger of *Nanohyla* is often reduced to a nub, whereas it is always at least half the length of the second finger in *Microhyla*. This is reminiscent of the patterns seen in *Stumpffia* Boettger, 1881 frogs from Madagascar, where digit reduction is a hallmark of each major clade, and where the first finger is always the first to reduce (Rakotoarison et al. 2017). Unlike *Stumpffia*, however, even the smallest *Microhyla*

and *Nanohyla* do not show reduction of the second and fourth fingers, although *Microhyla tatrix* presents bizarre hand morphology with a particularly thick and long third finger (Poyarkov et al. 2020b) reminiscent of the third-finger-only phenotype seen in the smallest *Stumpffia* species. Also, they have not lost any phalanges, even when fingers are reduced in length, whereas other miniaturized frogs often show finger or toe formula reduction (Alberch and Gale 1983, 1985; Scherz et al. 2019). Still, there has been a tendency for the terminal phalanx of F1 to transition from T-shaped to knobbed to simple in miniaturization series, indicative of a strong reduction despite the lack of loss of this element.

In the vertebral column, *Microhyla nepenthicola* (Fig. 6D) and *Nanohyla arboricola* (Fig. 6E) exhibit fusion of the first two presacral vertebrae, potentially linked to their extremely small body size. In the skull, both *Nanohyla* and *Microhyla* show forward displacement of the jaw articulation in miniaturized species, but other features are unique to each group, including the long otic ramus of the squamosal of *Nanohyla* (vs the reduction of the otic ramus of *Microhyla*), or the expansion of the sphenethmoid of *Nanohyla* along the parasphenoid (vs lack of expansion in *Microhyla*). The wide array of commonalities and differences both within these clades, and in comparison between these clades and other miniaturized frogs, highlights the extent to which miniaturization occurs through a combination of determinism and contingency. *Nanohyla* and *Microhyla* apparently share the reduction of the quadratojugal and loss of its connection to the maxilla, and the resulting take-over of suspensorium support by the pterygoid (Fig. 5). This arrangement is sometimes seen in other miniaturized microhylids (e.g., *Anodonthyla eximia* Scherz, Hutter, Rakotoarison, Riemann, Rödel, Ndriantsoa, Glos, Roberts, Crottini, Vences & Glaw [Scherz et al. 2019]), but, surprisingly, in the present case the loss of quadratojugal connection to the maxilla does not appear to be related to body size; even the largest species of *Microhyla* in the *M. berdmorei* group show the pterygoid suspensorium support, but are not inferred to have passed through a period of extreme body size reduction that would be expected to result in such a degree of change. Thus, caution is always recommended when interpreting features as consequences of miniaturization, when they may have arisen through other selective pressures. Interestingly, some species within *Nanohyla* and *Microhyla* increased again in body size from an ancestral body size that was <18 mm. These species would be worthy of future investigation, because cases of post-miniaturization body-size increases can leave behind hallmarks (e.g., potentially irrevocable loss of anatomical features such as fingers), which can lead to morphological innovation (Hanken and Wake 1993).

Finally, although they are not miniaturized, it is worth briefly remarking on the osteology of *Glyphoglossus*, and especially the bizarre *G. molossus*. The osteology of *G. yunnanensis* is rather typical of a large-bodied microhylid, with long, slender limb bones and a subtriangular skull. *Glyphoglossus molossus*, however, shows extreme

osteological modification associated with its more fossorial lifestyle, from its thickened hind- and forelimb bones to its small, rounded skull, to its highly modified first presacral vertebra. The peculiar flattened snout in this species is formed by a large chondrified beard-looking structure, not co-ossified to rostral and mandibular bones. Its limb and skull modifications resemble other strong burrowers, e.g., *Breviceps gibbosus* (Linnaeus, 1758) and *Barygenys maculata* Menzies & Tyler (Menzies 2020; Van Dijk 2001).

Sexual size dimorphism in the *Microhyla*–*Glyphoglossus* assemblage

As is typical for frogs (Shine 1979), most members of the *Microhyla*–*Glyphoglossus* assemblage exhibit slight female-biased size dimorphism. There is a weak, but significant, positive correlation between log(male SVL) and sexual size dimorphism, with those species with the smallest males having the strongest female-biased size dimorphism, and dimorphism decreasing with increasing male SVL. They thus conform to Rensch's rule (Rensch 1950). This may reflect a greater constraint on female body size, associated with the cost of reproduction in these frogs, which, even in the smallest species, produce clutches of many dozens of eggs.

Only a handful of species have transitioned, apparently rapidly and independently, to male-biased dimorphism. Remarkably, even species with diminutive males can be male-biased, exemplified by *Microhyla* sp. 1. In general, male-biased dimorphism is thought to be associated with territoriality and physical combat among male frogs (Shine 1979). These transitions to male-biased dimorphism may, therefore, be associated with changes in natural history of these lineages. Yet, this condition does not appear to be evolutionarily stable, because in no cases are a pair of sister species both male-biased. At present, too little is known of the ecology of these species to understand common drivers of these changes.

Conclusions

Miniaturized amphibians are characterized by a high proportion of cryptic species, along with numerous anatomical homoplasies, muddying our estimates of their evolutionary relationships and diversity (e.g., Hanken and Wake 1993; Rovito et al. 2013; Parra-Olea et al. 2016; Rakotoarison et al. 2017; Scherz et al. 2019; Gorin et al. 2020). Integrative taxonomic approaches, optimally combining the results of molecular phylogenetic analyses with morphological, acoustic and behavioral data, represent the most promising approach for better understanding of species boundaries, diversity and evolutionary relationships in microhylid frogs, including the genus *Microhyla* (Hasan et al. 2014; Garg et al. 2019; Poyarkov et al. 2018a, 2019; Gorin et al. 2020). Many recent phylogenetic studies of miniaturized frogs demonstrate that the diversity of these

groups is unexpectedly high, at both the species and supraspecific levels, due to a combination of overlooked diversity (cryptic species) and microendemism (Oliver et al. 2017; Rakotoarison et al. 2017; Clemente-Carvalho et al. 2011; Poyarkov et al. 2018a; Zimkus et al. 2012; Blackburn et al. 2008; Lourenço-de-Moraes et al. 2018; Rodriguez et al. 2013; Köhler et al. 2008; Scherz et al. 2019). The present analysis of the *Microhyla*–*Glyphoglossus* assemblage diversity represents a case in point: miniaturized taxa, that were previously assigned ad hoc to *Microhyla s. lat.*, were demonstrated to belong to two deeply divergent clades, together closely related to the genus *Glyphoglossus*, which consists of species of a much larger body size and very different ecology. Upon closer examination of their phylogenetic relationships from molecular data, as well as morphology, ecology, and biogeography, we found that these deep clades were older than most other Microhylinae genera, and sufficiently different to justify recognition as distinct genera. This yielded the new genus *Nanohyla* gen. nov. described herein. This result further underlines the importance of genetic data, useful for independently elucidating diversity and evolutionary relationships within groups with extensive homoplasies (Mott and Vieites 2009; Heideman et al. 2011; Scherz et al. 2019).

The *Microhyla*–*Glyphoglossus* assemblage (perhaps better now called the *Microhyla*–*Nanohyla*–*Glyphoglossus* assemblage) shows highly dynamic body size evolution, and a propensity to miniaturize, with at least nine separate miniaturization events inferred across *Microhyla* and the new genus *Nanohyla*. Convergence in body size in these two genera has generated some homoplasies, but both have unique, apomorphic features. It is clear, however, that, in order to gain a comprehensive understanding of the evolution of miniaturization in these frogs, much more extensive sampling of outgroups is needed. The Microhylidae, however, form an ideal group in which to study the evolution of miniaturization, which is one of several phylogenetically recurring frog ecomorphs (Moen et al. 2015).

Acknowledgements

We express our sincere gratitude to V.F. Orlova, R.A. Nazarov, and E.A. Galoyan (ZMMU, Moscow, Russia) for permission to study specimens under their care. We deeply appreciate the help of those colleagues who provided various assistance, including specimens and materials for examination: C. Suwannapoom, E.N. Solovyeva, M. Hasan, H. Okamiya, D.M.S.S. Karunarathna, P. Pawangkhanant, A. de Silva, W. Juthong, E.A. Galoyan, K.D. Milto, L.T. Nguyen, A. Haas, D.P. Bickford, and I. Das. We are grateful to N.A. Formozov, A.N. Kuznetsov, A.A. Polilov, L.P. Korzun, V.V. Shakhparonov, I.B. Soldatova, and K.B. Gerasimov for many useful comments and suggestions on the discussion of our hypotheses and results. We thank A.A. Bannikova, I.V. Artyushin, A.B. Vassilieva, A.V. Diakova, S.E. Farisenkov, M.M. Perfilov,

T.V. Duong, A.S. Dubrovskaya, P.V. Yushchenko, and S.S. Idiiatullina for valuable comments and help during our work on this project. We are deeply thankful to N.V. Kryukova who guided and helped us with making skeletal preparations and histological staining. We are grateful to D.R. Gafurova and A.N. Khomyak for assistance with micro-CT scanning. We thank V.D. Kretova who prepared line drawings for this study, and I. Das, P. Pawangkhanant, E. N. Solovyeva, V. D. H. Nguyen, L. T. Nguyen, T. V. Nguyen and vnherps.com for providing us with photos of several species of *Microhyla* and *Nanohyla*. We thank J. Mata, A. Resetar and the Field Museum of Natural History (Chicago, USA) for providing the photo of *Microhyla maculifera*. We also thank R. Brown and one anonymous reviewer for constructive feedback on this manuscript.

Funding

This work was supported by the Russian Foundation of Basic Research to Nikolay A. Poyarkov (RFBR grant No. 19-34-90167). Creation of dataset accessed on MorphoSource was made possible by the University of Florida (The oVert (openVertebrate) Thematic Collection Network (TCN); NSF DBI-1701714; NSF DBI-1702263). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

- Alberch P, Gale EA (1983) Size dependence during the development of the amphibian foot: colchicine-induced digital loss and reduction. *Journal of Embryology and Experimental Morphology* 76(1): 177–197.
- Alberch P, Gale EA (1985) A developmental analysis of an evolutionary trend: digital reduction in amphibians. *Evolution* 39(1): 8–23. <https://doi.org/10.1111/j.1558-5646.1985.tb04076.x>
- Alexander RM (1996) Biophysical problems of small size in vertebrates. *Symposia of the Zoological Society of London* 69: 3–14.
- Altig R, Johnston GF (1989) Guilds of anuran larvae: relationships among developmental modes, morphologies, and habitats. *Herpetological Monographs* 3: 81–109.
- Andersson LG (1942) A small collection of frogs from Annam collected in the years 1938–39 by Bertil Björkegren. *Arkiv for Zoologi* 34A(6): 1–11. <https://doi.org/10.2307/1466987>
- Atmaja VY, Hamidy A, Arisuryanti T, Matsui M, Smith EN (2019) A new species of *Microhyla* (Anura: Microhylidae) from Sumatra, Indonesia. *Treubia* 45: 25–46. <http://dx.doi.org/10.14203/treubia.v45i0.3625>
- Bain RH, Nguyen QT (2004) Three new species of narrow-mouth frogs (Genus: *Microhyla*) from Indochina, with comments on *Microhyla annamensis* and *Microhyla palmipes*. *Copeia* 2004(3): 507–524. <https://doi.org/10.1643/CH-04-020R2>
- Barbour T, Noble GK (1916) New amphibians and a new reptiles from Sarawak. *Proceedings of the New England Zoölogical Club*. Cambridge, Massachusetts 6: 19–22.
- Biju SD, Garg S, Kamei RG, Maheswaran G (2019) A new *Microhyla* species (Anura: Microhylidae) from riparian evergreen forest in the eastern Himalayan state of Arunachal Pradesh. *India Zootaxa* 4674(1): 100–116.

- Blackburn DC (2008) Biogeography and evolution of body size and life history of African frogs: Phylogeny of squeakers (*Arthroleptis*) and long-fingered frogs (*Cardioglossa*) estimated from mitochondrial data. *Molecular Phylogenetics and Evolution* 49(3): 806–826. <https://doi.org/10.1016/j.ympev.2008.08.015>
- Blyth E (1856) Report for October meeting, 1855. *Journal of the Asiatic Society of Bengal* 24: 711–723.
- Boettger O (1881) Diagnoses reptilium et batrachiorum novorum ab ill. Antonio Stumpff in insula Nossi-Bé Madagascariensi lectorum. *Zoologischer Anzeiger* 4(87): 358–362. [in Latin]
- Boie F (1827) Bemerkungen über Merrem's Versuch eines Systems der Amphibien, I. Lieferung: Ophidier. *Isis van Oken* 20: 508–566. [in German]
- Boring AM, Liu CC (1937) Studies of the rainfrogs, *Kaloula borealis*. III. An analysis of the skeletal features. *Peking Nature History Bulletin* 12(1): 43–47.
- Boulenger GA (1884) Descriptions of new species of reptiles and batrachians in the British Museum. Part. II. *Journal of Natural History* 13(77): 396–398. <https://doi.org/10.1080/00222938409459259>
- Boulenger GA (1897) Descriptions of new Malay frogs. *Journal of Natural History* 19(109): 106–108. <https://doi.org/10.1080/00222939708680508>
- Boulenger GA (1882) Catalogue of the Batrachia Salientia s. Ecaudata in the Collection of the British Museum. Second Edition. Taylor and Francis, London, 256 pp.
- Boulenger GA (1887) On new batrachians from Malacca. *Annals and Magazine of Natural History, Series 5* 19: 345–348. <https://doi.org/10.1080/00222938709460254>
- Boulenger GA (1900) Descriptions of new batrachians and reptiles from the Larut Hills, Perak. *Annals and Magazine of Natural History, Series 7* 6: 186–193. <https://doi.org/10.1080/00222930008678356>
- Boulenger GA (1904) Description of a new genus of frogs of the family Dyscophidae, and list of the genera and species of that family. *Annals and Magazine of Natural History, Series 7* 13: 42–44. <https://doi.org/10.1080/00222930409487053>
- Boulenger GA (1919) Description of a new dyscophid frog from Yunnan. *Annals and Magazine of Natural History, Series 9* 3: 548–548. <https://doi.org/10.1080/00222931908673856>
- Bourret R (1942) Les Batraciens de l'Indochine. Institut Océanographique de l'Indochine, Hanoi (in French).
- Chandramouli SR, Dutta SK (2015) Comparative osteology of anuran genera in the Western Ghats, Peninsular India. *Alytes* 32: 67–81.
- Clarke BT (1996) Small size in amphibians – its ecological and evolutionary implications. *Symposia of the Zoological Society of London* 69: 201–224.
- Clemente-Carvalho RBG, Klaczko J, Perez SI, Alves ACR, Haddad CFB, dos Reis SF (2011) Molecular phylogenetic relationships and phenotypic diversity in miniaturized toadlets, genus *Brachycephalus* (Amphibia: Anura: Brachycephalidae). *Molecular Phylogenetics and Evolution* 61(1): 79–89. <https://doi.org/10.1016/j.ympev.2011.05.017>
- Das I, Haas A (2010) New species of *Microhyla* from Sarawak: Old World's smallest frogs crawl out of miniature pitcher plants on Borneo (Amphibia: Anura: Microhylidae). *Zootaxa* 2571(1): 37–52. <https://doi.org/10.11646/zootaxa.2571.1.2>
- Das I, Yaakob NS, Sukumaran J (2007) A new species of *Microhyla* (Anura: Microhylidae) from the Malay Peninsula. *Hamadryad* 31(2): 304–314.
- Das I, Yaakob NS, Lim BL (2004) A new species of *Calluella* Stoliczka, 1872 (Anura: Microhylidae) from Taman Negara, Pahang State, peninsular Thailand. *Raffles Bulletin of Zoology, Singapore* 52(1): 257–260.
- Das I, Min PY, Hsu WW, Hertwig ST, Haas A (2014) Red Hot Chili Pepper. A new *Calluella* Stoliczka, 1872 (Lissamphibia: Anura: Microhylidae) from Sarawak, East Malaysia (Borneo). *Zootaxa* 3785(4): 550–560. <https://doi.org/10.11646/zootaxa.3785.4.4>
- David A (1872) Rapport adressé a MM. les Professeurs-Administrateurs du Museum d'histoire naturelle. *Nouvelles Archives du Muséum d'Histoire Naturelle, Paris* 7: 75–100. [in French]
- de Sá RO, Streicher JW, Sekonyela R, Forlani MC, Loader SP, Greenbaum E, Richards S, Haddad CF (2012) Molecular phylogeny of microhylid frogs (Anura: Microhylidae) with emphasis on relationships among New World genera. *BMC Evolutionary Biology* 12(1): e241. <https://doi.org/10.1186/1471-2148-12-241>
- de Sá RO, Trueb L (1991) Osteology, skeletal development, and chondrocranial structure of *Hamptophryne boliviana* (Anura: Microhylidae). *Journal of Morphology* 209(3): 311–330. <https://doi.org/10.1002/jmor.1052090307>
- de Sá RO, Tonini JFR, van Huss H, Zaher H, Haddad CF (2019a) The unique traits of the subgenus *Unicus* within *Chiasmocleis* Méhely, 1094 (Anura: Microhylidae). *Zootaxa* 4646(3): 585–590. <https://doi.org/10.11646/zootaxa.4646.3.8>
- de Sá RO, Tonini JF, van Huss H, Long A, Cuddy T, Forlani MC, Peloso PL, Zaher H, Haddad CF (2019b) Multiple connections between Amazonia and Atlantic Forest shaped the phylogenetic and morphological diversity of *Chiasmocleis* Mehely, 1904 (Anura: Microhylidae: Gastrophryninae). *Molecular Phylogenetics and Evolution*, 130: 198–210. <https://doi.org/10.1016/j.ympev.2018.10.021>
- Donnelly MA, de Sá RO, Guyer C (1990) Description of the tadpoles of *Gastrophryne pictiventris* and *Nelsonophryne aterrima* (Anura: Microhylidae), with a review of morphological variation in free-swimming microhylid larvae. *Novitates* 2976: 1–19. <https://doi.org/10.1093/molbev/mss075>
- Drummond AJ, Suchard MA, Xie D, Rambaut A (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* 29(8): 1969–1973.
- Dubois A (1987) *Miscelanea taxinomica batrachologica*, II. *Alytes*, Paris 6: 1–9.
- Duellman WE, Trueb L (1986) *Biology of Amphibians*. McGraw Hill, New York, 670 pp. <https://doi.org/10.2307/1445022>
- Duméril AHC, Bibron G (1841) *Erpétologie Générale ou Histoire Naturelle Complète des Reptiles*. Vol. 8. Librairie Encyclopedique de Roret, Paris, III, 792 pp. [in French]
- Dutta SK, Ray P (2000) *Microhyla sholigari*, a new species of microhylid frog (Anura: Microhylidae) from Karnataka, India. *Hamadryad* 25: 38–44.
- Emerson SB (1971) The fossorial frog adaptive zone: a study of convergence and parallelism in the Anura. Los Angeles, University of South California, unpublished Ph. D. thesis.
- Fei L, Hu SQ, Ye CY, Huang YZ (2009) *Fauna Sinica. Amphibia*. Volume 2. Anura. Science Press, Chinese Academy of Science, Beijing, 957 pp. [in Chinese]
- Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39(4): 783–791. <https://doi.org/10.1111/j.1558-5646.1985.tb00420.x>
- Feng Y, Blackburn DC, Liang D, Hillis DM, Wake DB, Cannatella DC, Zhang P (2017) Phylogenomics reveals rapid, simultaneous diversification of three major clades of Gondwanan frogs at the Cretaceous–Paleogene boundary. *Proceedings of the National Academy of Sciences* 114(29): 5864–5870. <https://doi.org/10.1073/pnas.1704632114>

- Fernando P, Siriwardhane M (1996) *Microhyla karunaratnei* (Anura: Microhylidae), a new species of frog endemic to Sri Lanka. *Journal of South Asian Natural History* 2(1): 135–142.
- Fitzinger LJFJ (1843) *Systema Reptilium. Fasciculus Primus*. Braumüller et Seidel, Wien, 106 pp. [in German]
- Fitzinger LJFJ (1861) Die Ausbeute der österreichischen Naturforscher an Säugethieren und Reptilien während der Weltumsegelung Sr. Majestät Fregatte Novara. *Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften, Mathematisch-Naturwissenschaftliche Classe* 42: 383–416. [in German]
- Frost DR (2020) *Amphibian Species of the World: an Online Reference*. Version 6.0. New York, USA: American Museum of Natural History, Electronic database. <http://research.amnh.org/herpetology/amphibia/index.html> [Accessed online 22/08/2020]
- Garg S, Biju SD (2019) New microhylid frog genus from Peninsular India with Southeast Asian affinity suggests multiple Cenozoic biotic exchanges between India and Eurasia. *Scientific Reports* 9(1): e1906. <https://doi.org/10.1038/s41598-018-38133-x>
- Garg S, Suyesh R, Das A, Jiang JP, Wijayathilaka N, Amarasinghe AAT, Alhadi F, Vineeth KK, Aravind NA, Senevirathne G, Meegaskumbura M, Biju SD (2019) Systematic revision of *Microhyla* (Microhylidae) frogs of South Asia: a molecular, morphological, and acoustic assessment. *Vertebrate Zoology* 69(1): 1–71.
- Garg S, Senevirathne G, Wijayathilaka N, Phuge S, Deuti K, Manamendra-Arachchi K, Meegaskumbura M, Biju SD (2018) An integrative taxonomic review of the South Asian microhylid genus *Uperodon*. *Zootaxa* 4384(1): 1–88. <https://doi.org/10.11646/zootaxa.4384.1.1>
- Gee NG, Boring AM (1929) Check list of Chinese Amphibia with notes on geographical distribution. *Peking Natural History Bulletin* 4: 15–51.
- Gistel J von NFX (1848) *Naturgeschichte des Thierreichs für höhere Schulen*. Hoffmann, Stuttgart, 216 pp. [in German]
- Gorin VA, Solovyeva EN, Hasan MK, Okamiya H, Karunarathna DMSS, Pawangkhanant P, de Silva A, Juthong W, Milto KD, Nguyen LT, Suwannapoom C, Haas A, Bickford DP, Das I, Poyarkov NA (2020) A little frog leaps a long way: compounded colonizations of the Indian Subcontinent discovered in the tiny Oriental frog genus *Microhyla* (Amphibia: Microhylidae). *PeerJ* 8: 2–47 [e9411]. <https://doi.org/10.7717/peerj.9411>
- Gray JE (1831) Description of two new genera of frogs discovered by John Reeves, Esq. in China. *Zoological Miscellany, Part 1*: 38.
- Günther ACLG (1859) *Catalogue of the Batrachia Salientia in the Collection of the British Museum*. Taylor and Francis, London, 160 pp.
- Günther ACLG (1869) First account of species of tailless batrachians added to the collection of the British Museum. *Proceedings of the Zoological Society of London* 1868: 478–490.
- Haas A, Das I, Hertwig ST (2020) Frogs of Borneo – The frogs of East Malaysia and their larval forms. [Electronic Database accessible at] <http://http://www.frogsofborneo.org> [Accessed online 15/08/2020]
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In: *Nucleic Acids Symposium Series*. Information Retrieval Ltd., London, c1979-c2000 41(1): 95–98.
- Hallowell E (1861) Report upon the Reptilia of the North Pacific Exploring Expedition, under command of Capt. John Rogers, U.S.N. *Proceedings of the Academy of Natural Sciences of Philadelphia* 1860: 480–510.
- Hanken J (1985) Morphological novelty in the limb skeleton accompanies miniaturization in salamanders. *Science* 229(4716): 871–874. <https://doi.org/10.1126/science.4023715>
- Hanken J, Wake DB (1993) Miniaturization of body size: organismal consequences and evolutionary significance. *Annual Review of Ecology and Systematics* 24(1): 501–519. <https://doi.org/10.1146/annurev.es.24.110193.002441>
- Harmon LJ, Weir JT, Brock CD, Glor RE, Challenger W (2008) GELGER: investigating evolutionary radiations. *Bioinformatics* 24(1): 129–131. <https://doi.org/10.1093/bioinformatics/btm538>
- Hasan M, Islam MM, Kuramoto M, Kurabayashi A, Sumida M (2014) Description of two new species of *Microhyla* (Anura: Microhylidae) from Bangladesh. *Zootaxa* 3755(5): 401–408. <https://doi.org/10.11646/zootaxa.3755.5.1>
- Hedges SB, Thomas R (2001) At the lower size limit in amniote vertebrates: A new diminutive lizard from the West Indies. *Caribbean Journal of Science* 37(3–4): 168–173.
- Heideman NJL, Mulcahy DG, Sites JW, Hendricks MGJ, Daniels SR (2011) Cryptic diversity and morphological convergence in threatened species of fossorial skinks in the genus *Scelotes* (Squamata: Scincidae) from the Western Cape Coast of South Africa: Implications for species boundaries, digit reduction and conservation. *Molecular Phylogenetics and Evolution* 61(3): 823–833. <https://doi.org/10.1016/j.ympev.2011.08.021>
- Hendrix R, Gawor A, Vences M, Ziegler T (2008) The tadpole of the narrow-mouthed frog *Microhyla fissipes* from Vietnam (Anura: Microhylidae). *Zootaxa* 1675(1): 67–68. <https://doi.org/10.11646/zootaxa.1675.1.5>
- Hennig W (1966) *Phylogenetic Systematics*. University of Illinois Press, Urbana, Illinois USA.
- Hoang VC, Nguyen TT, Luong MA, Nguyen QT, Orlov NL, Chen Y, Wang B, Jiang J (2020) A New species of *Microhyla* (Amphibia: Anura: Microhylidae) from Langbian Plateau, Central Vietnam. *Asian Herpetological Research* 11(3): 1–22.
- Howlader MSA, Nair A, Gopalan SV, Merilä J (2015) A new species of *Microhyla* (Anura: Microhylidae) from Nilphamari, Bangladesh. *PLoS ONE* 10(3): e0119825. <https://doi.org/10.1371/journal.pone.0119825>
- Hu SQ, Zhao EM, Liu CC (1966) A herpetological survey of the Tsinling and Ta-Pa Shan region. *Acta Zoologica Sinica / Dong wu xue bao, Beijing* 18: 57–89. [in Chinese with English abstract]
- Huelsenbeck JP, Hillis DM (1993) Success of phylogenetic methods in the four-taxon case. *Systematic Biology* 42(3): 247–264. <https://doi.org/10.1093/sysbio/42.3.247>
- Huelsenbeck JP, Ronquist F (2001) MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* 17(8): 754–755. <https://doi.org/10.1093/bioinformatics/17.8.754>
- Inger RF, Frogner KJ (1979) New species of narrow-mouth frogs (genus *Microhyla*) from Borneo. *The Sarawak Museum Journal* 27(48): 311–322.
- Inger RF (1989) Four new species of frogs from Borneo. *Malayan Nature Journal, Kuala Lumpur* 42: 229–243.
- Inger RF, Stuebing RB (2005) *A Field Guide to the Frogs of Borneo*, Natural History Publications (Borneo), Kota Kinabalu, 201 pp.
- Jerdon TC (1854) *Catalogue of reptiles inhabiting the Peninsula of India*. *Journal of the Asiatic Society of Bengal* 22: 522–534.
- Katoh K, Misawa K, Kuma K, Miyata T (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* 30(14): 3059–3066. <https://doi.org/10.1093/nar/gkf436>

- Khatiwada JR, Shu GC, Wang SH, Thapa A, Wang B, Jiang J (2017) A new species of the genus *Microhyla* (Anura: Microhylidae) from Eastern Nepal. *Zootaxa* 4254(2): 221–239. <https://doi.org/10.11646/zootaxa.4254.2.4>
- Kiew BH (1984) A new species of burrowing frog (*Calluella flava* sp. nov.) from Borneo. *Malayan Nature Journal*, Kuala Lumpur 37: 163–166.
- Kikinis R, Pieper SD, Vosburgh KG (2014) 3D Slicer: a platform for subject-specific image analysis, visualization, and clinical support. *Intraoperative imaging and image-guided therapy*, Springer, New York, 277–289. https://doi.org/10.1007/978-1-4614-7657-3_19
- Köhler F, Günther R (2008) The radiation of microhylid frogs (Amphibia: Anura) on New Guinea: A mitochondrial phylogeny reveals parallel evolution of morphological and life history traits and disproves the current morphology-based classification. *Molecular Phylogenetics and Evolution* 47: 353–365. <https://doi.org/10.1016/j.ympev.2007.11.032>
- Kraus F (2011) At the lower size limit for tetrapods, two new species of the miniaturized frog genus *Paedophryne* (Anura, Microhylidae). *ZooKeys* 154: 71–88. <https://doi.org/10.3897/zookeys.154.1963>
- Lamarck JB (1801) *Système des animaux sans vertèbres, ou tableau général des classes, des ordres et des genres de ces animaux; Présentant leurs caractères essentiels et leur distribution, d'après la considération de leurs rapports naturels et de leur organisation, et suivant l'arrangement établi dans les galeries du Muséum d'Histoire Naturelle, parmi leurs dépouilles conservées; Précédé du discours d'ouverture du Cours de Zoologie, donné dans le Muséum National d'Histoire Naturelle l'an 8 de la République*. Published by the author and Deterville, Paris, [viii +] 432 pp. <https://doi.org/10.5962/bhl.title.116650> [in French]
- Lanfear R, Calcott B, Ho SYW, Guindon S (2012) PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* 29(6): 1695–1701.
- Lautenschlager S, Gill PG, Luo Z-X, Fagan MJ, Rayfield EJ (2018) The role of miniaturization in the evolution of the mammalian jaw and middle ear. *Nature* 561(7724): 533–537. <https://doi.org/10.1093/molbev/mss020>
- Le TD, Hoa TH, Luong MA, Nguyen QT (2016) Advertisement call and description of the tadpole of *Microhyla marmorata* Bain & Nguyen, 2004 from Xuan Son National Park, Phu Tho Province. *Tap Chi Sinh Hoc [Journal of Biology]*, Hanoi 38(2): 154–161. <https://doi.org/10.15625/0866-7160/v38n2.7738> [in Vietnamese with English abstract]
- Lehr E, Coloma LA (2008) A minute new Ecuadorian Andean frog (Anura: Strabomantidae, *Pristimantis*). *Herpetologica* 64(3): 354–367. <https://doi.org/10.1655/07-089.1>
- Lehr E, Trueb L (2007) Diversity among New World microhylid frogs (Anura: Microhylidae): morphological and osteological comparisons between *Nelsonphryne* (Gunther 1901) and a new genus from Peru. *Zoological Journal of the Linnean Society* 149(4): 583–609. <https://doi.org/10.1111/j.1096-3642.2007.00270.x>
- Leong TM (2004) Larval descriptions of some poorly known tadpoles from Peninsular Malaysia (Amphibia: Anura). *Raffles Bulletin of Zoology* 52(2): 609–620.
- Li S, Zhang M, Xu N, Lv J, Jiang JP (2019) A new species of the genus *Microhyla* (Amphibia: Anura: Microhylidae) from Guizhou Province, China. *Zootaxa* 4624(4): 551–575. <https://doi.org/10.11646/zootaxa.4624.4.7>
- Lourenço-de-Moraes R, Dias IR, Mira-Mendes CV, Oliveira RM, Barth A, Ruas DS, Vences M, Solé M, Bastos RP (2018) Diversity of miniaturized frogs of the genus *Adelophryne* (Anura: Eleutherodactylidae): A new species from the Atlantic Forest of northeast Brazil. *PLoS ONE* 13(9): e0201781. <https://doi.org/10.1371/journal.pone.0201781>
- Matsui M, Hamidy A, Belabut DM, Ahmad N, Panha S, Sudin A, Khonsue W, Oh HS, Yong HS, Jiang JP, Nishikawa K (2011) Systematic relationships of oriental tiny frogs of the family Microhylidae (Amphibia, Anura) as revealed by mtDNA genealogy. *Molecular Phylogenetics and Evolution* 61(1): 167–176. <https://doi.org/10.1016/j.ympev.2011.05.015>
- Matsui M, Hamidy A, Eto K (2013) Description of a new species of *Microhyla* from Bali, Indonesia (Amphibia, Anura). *Zootaxa* 3670(4): 579–590. <http://dx.doi.org/10.11646/zootaxa.3670.4.9>
- Matsui M (2011) Taxonomic revision of one of the Old World's smallest frogs, with description of a new bornean *Microhyla* (Amphibia, Microhylidae). *Zootaxa* 2814(1): 33–49. <https://doi.org/10.11646/zootaxa.2814.1.3>
- McPartlin C (2010) *Osteology of Calluella guttulata* (Blyth 1855) and associated commentary on evolution in the family Microhylidae (Anura). University of Kansas, Unpublished Master of Arts thesis.
- Menzies JI (2020) The musculoskeletal system and natural history of *Barygenys maculata* (Anura, Microhylidae) a burrowing frog of New Guinea. *Transactions of the Royal Society of South Australia* 144(1): 27–65. <https://doi.org/10.1080/03721426.2020.1747142>
- Mocquard F (1892) Description de deux ophidiens et d'un batracien d'especes nouvelles. *Le Naturaliste, Série 2, Paris* 6: 35. [in French]
- Moen DS, Morlon H, Wiens JJ (2015) Testing convergence versus history: convergence dominates phenotypic evolution for over 150 million years in frogs. *Systematic Biology* 65(1): 146–160. <https://doi.org/10.1093/sysbio/syv073>
- Mott T, Vieites DR (2009) Molecular phylogenetics reveals extreme morphological homoplasy in Brazilian worm lizards challenging current taxonomy. *Molecular Phylogenetics and Evolution* 51(2): 190–200.
- Nguyen LT, Poyarkov NA, Le DT, Vo BD, Phan HT, Duong TV, Murphy RW, Nguyen SN (2018) A new species of *Leptolalax* (Anura: Megophryidae) from Son Tra Peninsula, central Vietnam. *Zootaxa* 4388(1): 1–21. <https://doi.org/10.1016/j.ympev.2009.01.014>
- Nguyen LT, Poyarkov NA, Nguyen TT, Nguyen TA, Nguyen VH, Gorin VA, Murphy RW, Nguyen SN (2019) A new species of the genus *Microhyla* Tschudi, 1838 (Amphibia: Anura: Microhylidae) from Tay Nguyen Plateau, Central Vietnam. *Zootaxa* 4543(4): 549–580. <https://doi.org/10.11646/zootaxa.4543.4.4>
- Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. *Molecular Biology and Evolution* 32(1): 268–274. <https://doi.org/10.1093/molbev/msu300>
- Nguyen TV, Duong TV, Luu KT, Poyarkov NA (2020) A new species of *Kurixalus* (Anura: Rhacophoridae) from northern Vietnam with comments on the biogeography of the genus. *Journal of Natural History* 54(1–4): 195–223. <https://doi.org/10.1080/00222933.2020.1728411>
- Noble GK, Parker HW (1926) A synopsis of the brevicipitid toads of Madagascar. *American Museum novitates*, 232: 1–21.
- Oliver PM, Iannella A, Richards SJ, Lee MSY (2017) Mountain colonisation, miniaturisation and ecological evolution in a radiation of direct-developing New Guinea frogs (Choerophryne, Microhylidae). *PeerJ* 5: e3077. <https://doi.org/10.7717/peerj.3077>

- Orme D, Freckleton R, Thomas G, Petzoldt T, Fritz S, Isaac N, Pearse W (2018) Caper: comparative analyses of phylogenetics and evolution in R. R package version 1.0.1. <https://CRAN.R-project.org/package=caper>.
- Orton GL (1953) The systematics of vertebrate larvae. *Systematic Zoology* 2(2): 63–75. <https://doi.org/10.2307/2411661>
- Parker HW, Osman HWC (1948) LI.—Frogs of the genus *Microhyla* from Ceylon. *Journal of Natural History* 1(10): 759–764. <https://doi.org/10.1080/00222934808653944>
- Parker HW (1928) LXIII.—The brevicipitid frogs of the genus *Microhyla*. *Journal of Natural History* 2(11): 473–499. <https://doi.org/10.1080/00222932808672911>
- Parker HW (1934) Monograph of the Frogs of the Family Microhylidae. Trustees of the British Museum, London, 208 pp.
- Parra-Olea G, Rovito SM, García-París M, Maisano JA, Wake DB, Hanken J (2016) Biology of tiny animals: three new species of minute salamanders (Plethodontidae: *Thorius*) from Oaxaca, Mexico. *PeerJ* 4: e2694. <https://doi.org/10.7717/peerj.2694>
- Peloso PLV, Frost DR, Richards SJ, Rodrigues MT, Donnellan SC, Matsui M, Raxworthy CJ, Biju SD, Lemmon EM, Lemmon AR, Wheeler WC (2016) The impact of anchored phylogenomics and taxon sampling on phylogenetic inference in narrow-mouthed frogs (Anura, Microhylidae). *Cladistics* 32(2): 113–140. <https://doi.org/10.1111/cla.12118>
- Pillai R (1977) On two frogs of the family Microhylidae from Andamans including a new species. *Proceedings of the Indian Academy of Sciences, Section-B* 86(2): 135–138.
- Polilov AA (2015) Small is beautiful: features of the smallest insects and limits to miniaturization. *Annual Review of Entomology* 60: 103–121. <https://doi.org/10.1146/annurev-ento-010814-020924>
- Poyarkov NA, Duong TV, Orlov NL, Gogoleva SS, Vassilieva AB, Nguyen LT, Nguyen VHD, Nguyen SN, Che J, Mahony S (2017) Molecular, morphological and acoustic assessment of the genus *Ophryophryne* (Anura, Megophryidae) from Langbian Plateau, southern Vietnam, with description of a new species. *ZooKeys* 672: 49–120. <https://doi.org/10.3897/zookeys.672.10624>
- Poyarkov NA, Rowley JJJ, Gogoleva SS, Vassilieva AB, Galoyan EA, Orlov NL (2015) A new species of *Leptotalax* (Anura: Megophryidae) from the western Langbian Plateau, southern Vietnam. *Zootaxa* 3931(2): 221–252. <https://doi.org/10.1080/00222933.2020.1804005>
- Poyarkov NA, Pawangkhanant P, Gorin VA, Juthong W, Suwannapoom C (2020b) A new species of miniaturized narrow-mouth frog of the genus *Microhyla* Tschudi, 1838 (Amphibia: Anura: Microhylidae) from northern Tenasserim, Thailand. *Journal of Natural History* 54(23–24): 1525–1558.
- Poyarkov NA, Solovyeva EN, Nguyen TV, Geissler P (2020a) On the taxonomic status of three enigmatic Indochinese frog species (Amphibia: Anura) described by L. G. Andersson. *Zootaxa* 4834(4): 502–522. <https://doi.org/10.11646/zootaxa.4834.4.2>
- Poyarkov NA, Vassilieva AB, Orlov NL, Galoyan EA, Tran D, Le DTT, Kretova VD, Geissler P (2014) Taxonomy and distribution of narrow-mouth frogs of the genus *Microhyla* Tschudi, 1838 (Anura: Microhylidae) from Vietnam with descriptions of five new species. *Russian Journal of Herpetology* 21(2): 89–148.
- Poyarkov NA, Gorin VA, Zaw T, Kretova VD, Gogoleva SI, Pawangkhanant P, Che J (2019) On the road to Mandalay: contribution to the *Microhyla* Tschudi, 1838 (Amphibia: Anura: Microhylidae) fauna of Myanmar with description of two new species. *Zoological Research* 40(4): 244–276. <https://doi.org/10.24272/j.issn.2095-8137.2019.044>
- Poyarkov NA, Nguyen TV, Duong TV, Gorin VA, Yang JH (2018b) A new limestone-dwelling species of *Micryletta* (Amphibia: Anura: Microhylidae) from northern Vietnam. *PeerJ* 6: e5771. <https://doi.org/10.7717/peerj.5771>
- Poyarkov NA, Suwannapoom C, Pawangkhanant P, Aksornneam A, Duong TV, Korost DV, Che J (2018a) A new genus and three new species of miniaturized microhylid frogs from Indochina (Amphibia: Anura: Microhylidae: Asterophryinae). *Zoological Research* 39(3): 130–155.
- R Core Team (2014) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. <http://www.R-project.org/>
- Rakotoarison A, Scherz MD, Glaw F, Koehler J, Andreone F, Franzen M, Glos J, Hawlitschek O, Jono T, Mori A, Ndriantsoa SH (2017) Describing the smaller majority: Integrative taxonomy reveals twenty-six new species of tiny microhylid frogs (genus *Stumpffia*) from Madagascar. *Vertebrate Zoology* 67(3): 271–398.
- Rambaut A, Suchard M, Xie W, Drummond A (2014) Tracer v. 1.6. Institute of Evolutionary Biology, University of Edinburgh. Available at: <http://tree.bio.ed.ac.uk/software/tracer/>.
- Rensch B (1950) Die Abhängigkeit der relativen Sexualdifferenz von der Körpergröße. *Bonner Zoologische Beiträge* 1: 58–69. [in German]
- Revell LJ (2012) phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution* 3(2): 217–223. <https://doi.org/10.1111/j.2041-210X.2011.00169.x>
- Rittmeyer EN, Allison A, Gründler MC, Thompson DK, Austin CC (2012) Ecological guild evolution and the discovery of the world's smallest vertebrate. *PLoS ONE* 7(1): e29797. <https://doi.org/10.1371/journal.pone.0029797>
- Rodríguez A, Poth D, Schulz S, Gehara M, Vences M. (2013) Genetic diversity, phylogeny and evolution of alkaloid sequestering in Cuban miniaturized frogs of the *Eleutherodactylus limbatus* group. *Molecular Phylogenetics and Evolution* 68(3): 541–554. <https://doi.org/10.1016/j.ympev.2013.04.031>
- Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19(12): 1572–1574.
- Rovito SM, Parra-Olea G, Hanken J, Bonett RM, Wake DB (2013) Adaptive radiation in miniature: the minute salamanders of the Mexican highlands (Amphibia: Plethodontidae: *Thorius*). *Biological Journal of the Linnean Society* 109(3): 622–643. <https://doi.org/10.1111/bj.12083>
- Savage JM (1975) Systematics and distribution of the Mexican and Central American stream frogs related to *Eleutherodactylus rugulosus*. *Copeia* 1975(2): 254–306. <https://doi.org/10.2307/1442883>
- Schenkel E (1901) Achter Nachtrag zum Katalog der herpetologischen Sammlung des Basler Museums. *Verhandlungen der Naturforschenden Gesellschaft in Basel* 13(1): 142–199. [in German]
- Scherz MD, Glaw F, Vences M, Andreone F, Crottini A (2016) Two new species of terrestrial microhylid frogs (Microhylidae: Cophylinae: *Rhombophryne*) from northeastern Madagascar. *Salamandra* 52(2): 91–106.
- Scherz MD, Hawlitschek O, Andreone F, Rakotoarison A, Vences M, Glaw F (2017) A review of the taxonomy and osteology of the *Rhombophryne serratopalpebrosa* species group (Anura: Microhylidae) from Madagascar, with comments on the value of volume rendering of micro-CT data to taxonomists. *Zootaxa* 4273(3): 301–340. <https://doi.org/10.11646/zootaxa.4273.3.1>

- Scherz MD, Hutter CR, Rakotoarison A, Riemann JC, Rödel MO, Ndrintantsoa SH, Glos J, Roberts SH, Crottini A, Vences M, Glaw F (2019) Morphological and ecological convergence at the lower size limit for vertebrates highlighted by five new miniaturised microhylid frog species from three different Madagascan genera. *PLoS ONE* 14(3): e0213314. <https://doi.org/10.1371/journal.pone.0213314>
- Scherz MD, Rakotoarison A, Hawlitschek O, Vences M, Glaw F (2015) Leaping towards a saltatorial lifestyle? An unusually long-legged new species of *Rhombophryne* (Anura, Microhylidae) from the Sotata massif in northern Madagascar. *Zoosystematics and Evolution* 91(2): 105–114. <https://doi.org/10.3897/zse.91.4979>
- Seshadri KS, Singal R, Priti H, Ravikanth G, Vidisha MK, Saurabh S, Pratik M, Gururaja KV (2016) *Microhyla laterite* sp. nov., a new species of *Microhyla* Tschudi, 1838 (Amphibia: Anura: Microhylidae) from a laterite rock formation in South West India. *PLoS ONE* 11(3): e0149727. <https://doi.org/10.1371/journal.pone.0149727>
- Shine R (1979) Sexual Selection and Sexual Dimorphism in the Amphibia. *Copeia* 1979(2): 297–306. <https://doi.org/10.2307/1443418>
- Smith M (1923) Notes on reptiles and batrachians from Siam and Indo-China (No. 2). *Journal of the Natural History Society of Siam* 6(1): 47–53.
- Steindachner F (1864) Batrachologische Mittheilungen. *Verhandlungen des Zoologisch-Botanischen Vereins in Wien* 14: 239–288. [in German]
- Stejneger L (1901) Diagnoses of eight new batrachians and reptiles from the Riu Kiu Archipelago, Japan. *Proceedings of the Biological Society of Washington* 14: 189–191.
- Stoliczka F (1872) Note on a few Barmese species of Sauria, Ophidia and Batrachia. *Proceedings of the Asiatic Society of Bengal* 1872: 143–147.
- Streicher JW, Loader SP, Varela-Jaramillo A, Montoya P, de Sá RO (2020) Analysis of ultraconserved elements supports African origins of narrow-mouthed frogs. *Molecular Phylogenetics and Evolution* 146: 106771. <https://doi.org/10.1016/j.ympev.2020.106771>
- Suwannapoom C, Sumontha M, Tunprasert J, Ruangsawan T, Pawangkhanant P, Korost DV, Poyarkov NA (2018) A striking new genus and species of cave-dwelling frog (Amphibia: Anura: Microhylidae: Asterophryinae) from Thailand. *PeerJ* 6: e4422. <https://doi.org/10.7717/peerj.4422>
- Suwannapoom C, Nguyen TV, Pawangkhanant P, Gorin VA, Chomdej S, Che J, Poyarkov NA (2020) A new species of *Micryletta* (Amphibia: Microhylidae) from southern Thailand. *Zoological Research* 41(5): 1–8.
- Tamura K, Stecher G, Peterson D, Filipiński A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 30(12): 2725–2729. <https://doi.org/10.1093/molbev/mst197>
- Taylor EH (1962) The amphibian fauna of Thailand. *University of Kansas Science Bulletin* 43: 265–599. <https://doi.org/10.5962/bhl.part.13347>
- Trifinopoulos J, Nguyen TL, von Haeseler A, Bui MQ (2016) W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Research* 44(W1): W232–W235. <https://doi.org/10.1093/nar/gkw256>
- Trueb L, Diaz R, Blackburn DC (2011) Osteology and chondrocranial morphology of *Gastrophryne carolinensis* (Anura: Microhylidae), with a review of the osteological diversity of New World microhylids. *Phyllomedusa* 10(2): 99–135. <https://doi.org/10.11606/issn.2316-9079.v10i2p99-135>
- Trueb L (1968) Cranial osteology of the hyloid frog, *Smilisca baudini*. *University of Kansas Publications, Museum of Natural History*, 18: 11–35.
- Trueb L (1973) Bones, frogs, and evolution. In: Vial JL (Ed.) *Evolutionary biology of the anurans: Contemporary research on major problems*. University of Missouri Press, USA, pp. 65–132.
- Tschudi JJV (1838) *Classification der Batrachier mit Berücksichtigung der fossilen Thiere dieser Abtheilung der Reptilien*. Petitpierre, Neuchâtel, 124 pp. [in German]
- Tu N, Yang M-H, Liang D, Zhang P (2018) A large-scale phylogeny of Microhylidae inferred from a combined dataset of 121 genes and 427 taxa. *Molecular Phylogenetics and Evolution* 126: 85–91. <https://doi.org/10.1016/j.ympev.2018.03.036>
- Van Dijk DE (2001) Osteology of the ranoid burrowing African anurans *Breviceps* and *Hemisus*. *African Zoology* 36(2): 137–141. <https://doi.org/10.1080/15627020.2001.11657131>
- Van Kampen PN (1905) *Amphibien von Palembang (Sumatra)*. *Zoologische Jahrbücher. Abteilung für Systematik, Geographie und Biologie der Tiere, Jena* 22: 701–716. [in German]
- Vassilieva AB, Galoyan EA, Gogoleva SS, Poyarkov NA (2014) Two new species of *Kalophrynus* Tschudi, 1838 (Anura: Microhylidae) from the Annamite mountains in southern Vietnam. *Zootaxa*, 3796(3): 401–434. <https://doi.org/10.11646/zootaxa.3796.3.1>
- Vassilieva AB, Trounov VL, Poyarkov NA, Galoyan EA (2017) The phytotelm tadpoles of *Microhyla arboricola* (Anura: Microhylidae) from Vietnam, with comments on reproductive biology and development. *Zootaxa* 4247(4): 413–428. <https://doi.org/10.11646/zootaxa.4247.4.4>
- Vences M, Guayasamin JM, Miralles A, de la Riva I (2013) To name or not to name: Criteria to promote economy of change in Linnaean classification schemes. *Zootaxa* 3636(2): 201–244. <https://doi.org/10.11646/zootaxa.3636.2.1>
- Vineeth KK, Radhakrishna U, Godwin R., Anwesha S, Rajashekhar KP, Aravind N (2018) A new species of *Microhyla* Tschudi, 1838 (Anura: Microhylidae) from West Coast of India: an integrative taxonomic approach. *Zootaxa* 4420(2): 151–179. <https://doi.org/10.11646/zootaxa.4420.2.1>
- Virgil (1916) *Eclogues*. *Georgics*. *Aeneid*: Books 1–6. Translated by H. Rushton Fairclough. Revised by G. P. Goold. *Loeb Classical Library* 63. Cambridge, MA: Harvard University Press. <https://doi.org/10.4159/DLCL.virgil-eclogues.1916>
- Vogt T (1911) Beitrag zur Amphibien-fauna der Insel Formosa. *Sitzungsberichte der Gesellschaft Naturforschender Freunde zu Berlin*, 1911: 179–184. [in German]
- Wake DB (2013) The enigmatic history of the European, Asian and American plethodontid salamanders. *Amphibia-Reptilia* 34(3): 323–336. <https://doi.org/10.1163/15685381-00002893>
- Walker MB, Kimmel CB (2007) A two-color acid-free cartilage and bone stain for zebrafish larvae. *Biotechnic & Histochemistry* 82(1): 23–28. <https://doi.org/10.1080/10520290701333558>
- Wells KD (2010) *The Ecology and Behavior of Amphibians*. University of Chicago Press, Chicago, 1148 pp.
- Wickham H (2016) *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag, New York NY. https://doi.org/10.1007/978-3-319-24277-4_9
- Wijayathilaka N, Garg S, Senevirathne G, Karunarathna N, Biju S, Meegaskumbura M (2016) A new species of *Microhyla* (Anura: Microhylidae) from Sri Lanka: an integrative taxonomic approach. *Zootaxa* 4066(3): 331–342. <https://doi.org/10.11646/zootaxa.4066.3.9>

- Wilcox TP, Zwickl DJ, Heath TA, Hillis DM (2002) Phylogenetic relationships of the Dwarf Boas and a comparison of Bayesian and bootstrap measures of phylogenetic support. *Molecular Phylogenetics and Evolution* 25(2): 361–371. [https://doi.org/10.1016/S1055-7903\(02\)00244-0](https://doi.org/10.1016/S1055-7903(02)00244-0)
- Wu SH (1994) Phylogenetic relationships, higher classification, and historical biogeography of the microhylid frogs (Lissamphibia: Anura: Brevicipitidae and Microhylidae). University of Michigan, unpublished Ph. D. thesis.
- Yang Z (2007) PAML 4: phylogenetic analysis by maximum likelihood. *Molecular Biology and Evolution* 24(8): 1586–1591. <https://doi.org/10.1093/molbev/msm088>
- Yuan ZY, Suwannapoom C, Yan F, Poyarkov NA, Nguyen SN, Chen HM, Chomdej S, Murphy RW, Che J (2016) Red River barrier and Pleistocene climatic fluctuations shaped the genetic structure of *Microhyla fissipes* complex (Anura: Microhylidae) in southern China and Indochina. *Current Zoology* 62(6): 531–543. <https://doi.org/10.1093/cz/zow042>
- Zhang M, Chen X, Ye C, Fei L, Li P, Jiang J, Wang B (2020) Osteology of the Asian narrow-mouth toad *Kaloula borealis* (Amphibia, Anura, Microhylidae) with comments on its osteological adaptation to fossorial life. *Acta Zoologica* 101: 366–383. <https://doi.org/10.1111/azo.12305>
- Zhang M, Fei L, Ye C, Wang Y, Wang B, Jiang J (2018) A new species of genus *Microhyla* (Amphibia: Anura: Microhylidae) from Zhejiang Province, China. *Asian Herpetological Research* 9(3): 135–148.
- Zimkus BM, Lawson L, Loader SP, Hanken J (2012) Terrestrialization, miniaturization and rates of diversification in African puddle frogs (Anura: Phrynobatrachidae). *PLoS ONE* 7(4): e35118. <https://doi.org/10.1371/journal.pone.0035118>
- Zweifel RG (1986) A new genus and species of microhylid frog from the Cerro de la Neblina Region of Venezuela and a discussion of relationships among New World microhylid genera. *American Museum Novitates* 2863: 1–24.
- Zweifel RG (1972) Results of the Archbold Expeditions. No. 97. A revision of the frogs of the subfamily Asterophryinae, Family Microhylidae. *Bulletin of the American Museum of Natural History* 148: 411–546.

Supplementary material 1

Table S1

Authors: Vladislav A. Gorin, Mark D. Scherz, Dmitriy V. Korost, Nikolay A. Poyarkov
 Data type: Microsoft Word Document (.docx)
 Explanation note: Museum voucher information, geographic localities, and GenBank accession numbers of specimens and sequences used in this study. Asterisk (*) denotes sequences that were included in the alignment for timetree calibration. Exact locality information unknown for specimens obtained via pet trade or those published in earlier works.
 Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.
 Link: <https://doi.org/10.3897/zse.97.57968.suppl1>

Supplementary material 2

Table S2

Authors: Vladislav A. Gorin, Mark D. Scherz, Dmitriy V. Korost, Nikolay A. Poyarkov
 Data type: Microsoft Word Document (.docx)
 Explanation note: Museum voucher information and geographic localities of osteological specimens examined. For abbreviations see Materials and methods; SVL given in mm.
 Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.
 Link: <https://doi.org/10.3897/zse.97.57968.suppl2>

Supplementary material 3

Table S3

Authors: Vladislav A. Gorin, Mark D. Scherz, Dmitriy V. Korost, Nikolay A. Poyarkov
 Data type: Microsoft Word Document (.docx)
 Explanation note: Body size data for the *Microhyla-Glyphoglossus* assemblage members (from Gorin et al. 2020, with modifications). For both sexes of each species maximal body size data is given. Question mark denotes “no data.” For voucher IDs of specimens see Gorin et al. (2020).
 Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.
 Link: <https://doi.org/10.3897/zse.97.57968.suppl3>

Supplementary material 4

Table S4

Authors: Vladislav A. Gorin, Mark D. Scherz, Dmitriy V. Korost, Nikolay A. Poyarkov
 Data type: Microsoft Word Document (.docx)
 Explanation note: Results of divergence time estimates. Node No. – estimated tree node, for node names see Suppl. material 8: Figure S3; divergence time given in million years before present (Ma).
 Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended

to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/zse.97.57968.suppl4>

Supplementary material 5

Table S5

Authors: Vladislav A. Gorin, Mark D. Scherz, Dmitriy V. Korost, Nikolay A. Poyarkov

Data type: Microsoft Word Document (.docx)

Explanation note: Osteological comparison of the *Microhyla–Glyphoglossus* assemblage members. For character definitions and state descriptions see Materials and methods; Latin numerals (I–VIII) refer to numbers of presacral vertebrae.

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/zse.97.57968.suppl5>

Supplementary material 6

Figure S1

Authors: Vladislav A. Gorin, Mark D. Scherz, Dmitriy V. Korost, Nikolay A. Poyarkov

Data type: Adobe PDF file

Explanation note: Comparison of bayesian inference trees of the *Microhyla–Glyphoglossus* assemblage derived from the analysis of: (A) 2478 bp of mtDNA fragment including 12S rRNA, tRNA^{Val} and 16S rRNA genes; (B) 720 bp of *BDNF* nuDNA gene; (C) the combined mtDNA + nuDNA dataset of 3207 bp including 12S rRNA, tRNA^{Val}, 16S rRNA and *BDNF* gene fragments. For voucher specimen information and GenBank accession numbers see Suppl. material 1: Table S1. Yellow, red, and blue color denotes *Microhyla* I, *Microhyla* II, and *Glyphoglossus*, respectively. Numbers at tree nodes correspond to PP/BS support values, respectively.

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/zse.97.57968.suppl6>

Supplementary material 7

Figure S2

Authors: Vladislav A. Gorin, Mark D. Scherz, Dmitriy V. Korost, Nikolay A. Poyarkov

Data type: Adobe PDF file

Explanation note: Updated mtDNA-genealogy of the *Microhyla–Glyphoglossus* assemblage. For voucher specimen information and GenBank accession numbers see Suppl. material 1: Table S1. Yellow, red, and blue color denotes *Microhyla* I, *Microhyla* II, and *Glyphoglossus*, respectively. Numbers at tree nodes correspond to PP/BS support values, respectively.

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/zse.97.57968.suppl7>

Supplementary material 8

Figure S3

Authors: Vladislav A. Gorin, Mark D. Scherz, Dmitriy V. Korost, Nikolay A. Poyarkov

Data type: Adobe PDF file

Explanation note: Bayesian chronogram resulted from *BEAST analysis of the 3207 bp-long concatenated mtDNA + nuclear DNA dataset. Node values correspond to node numbers, for estimated divergence times (in Ma) see Suppl. material 4: Table S4. Red circles correspond to calibration points used in molecular dating analysis, for details see Gorin et al. (2020). Blue bars correspond to 95%-confidence intervals.

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/zse.97.57968.suppl8>

Supplementary material 9

Figure S4

Authors: Vladislav A. Gorin, Mark D. Scherz, Dmitriy V. Korost, Nikolay A. Poyarkov

Data type: Adobe PDF file

Explanation note: Hand preparations of the three representatives of the *Microhyla–Glyphoglossus* assemblage. Pictures provided for *G. guttulatus* (A – dorsal view of

the skull, **B** – lateral view of the skull, palmar view of the hand), *M. fissipes* (**D** – dorsal view of the skull, **E** – lateral view of the skull, **F** – palmar view of the hand) and *N. marmorata* (**G** – dorsal view of the skull, **H** – lateral view of the skull, **I** – palmar view of the hand).

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/zse.97.57968.suppl9>

Supplementary material 10

Figure S5

Authors: Vladislav A. Gorin, Mark D. Scherz, Dmitriy V. Korost, Nikolay A. Poyarkov

Data type: Adobe PDF file

Explanation note: Variable states of osteological characters in the *Microhyla*–*Glyphoglossus* assemblage. (**A**) mineralized prehallux of *M. butleri*; (**B**) cartilaginous prehallux of *N. annamensis*; (**C**) ossified prehallux of *G. guttulatus*; (**D**) fan-shaped sternum of *M. picta*; (**E**) bifurcate sternum of *M. nilphamariensis*, (**F**) pectoral girdle of *M. annectens* (omosternum shown by an arrow); (**G, H, I**) – mineralized stapes of *N. pulverata*, *M. berdmorei* and miniaturized *M. minuta* (shown with an arrow) respectively; (**J, K, L**) – vertebral column of *G. guttulatus*, *M. fissipes* and *N. marmorata* respectively; (**M**) – hyoid of *M. okinavensis*; (**N, O**) – palatine region of *N. marmorata* and *M. fissipes* respectively (neopalatine shown with an arrow).

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/zse.97.57968.suppl10>