A striking new genus and species of cave-dwelling frog (Amphibia: Anura: Microhylidae: Asterophryinae) from Thailand

Chatmongkon Suwannapoom 1, Montri Sumontha Corresp. 2, Jitthep Tunprasert 3, Thiti Ruangsuwan 4, Parinya Pawangkhanant 1, Dmitriy V Korost 5, Nikolay A Poyarkov Corresp. 6, 7

1 Division of Fishery, School of Agriculture and Natural Resources, University of Phayao, Phayao, Thailand
2 Department of Fishery, Ranong Marine Fisheries Station, Ranong, Thailand
3 Department of ecology, Nakhon Pathom Rajabhat University, Nakhon Pathom Mueng, Nakhon Pathom, Thailand
4 Department of Zoology, Faculty of Science, Kasetsart University, Bangkok, Thailand
5 Petroleum Geology Department, Geological Faculty, Moscow State University, Moscow, Russia
6 Biological Faculty, Department of Vertebrate Zoology, Moscow State University, Moscow, Russia
7 Laboratory of Tropical Ecology, Joint Russian-Vietnamese Tropical Research and Technological Center, Hanoi, Vietnam

Corresponding Authors: Montri Sumontha, Nikolay A Poyarkov
Email address: montri.sumontha@gmail.com, n.poyarkov@gmail.com

We report on a discovery of *Siamophryne troglodytes* Gen. et sp. nov. – a new troglophilous genus and species of microhylid frog from a limestone cave in the tropical forests of western Thailand. To assess its phylogenetic relationships we studied 12S rRNA-16S rRNA mtDNA fragment with final alignment comprising up to 2591 bp for 56 microhylid species. Morphological characterization of the new genus is based on examination of external morphology and analysis of osteological characteristics using microCT-scanning. Phylogenetic analyses place the new genus into the mainly Australasian subfamily Asterophryinae as a sister taxon to the genus *Gastrophrynoides*, the only member of the subfamily known from Sundaland. The new genus markedly differs from all other Asterophryinae members by a number of diagnostic morphological characters and demonstrates significant mtDNA sequence divergence. We provide a preliminary description of a tadpole of the new genus. Thus, it represents the only asterophryine taxon with documented free-living larval stage and troglophilous life style. Our work demonstrates that *Siamophryne troglodytes* Gen. et sp. nov. represents an old lineage of the initial radiation of Asterophryinae which took place in the mainland Southeast Asia. Our results strongly support the "out of Indo-Eurasia" biogeographic scenario for this group of frogs. To date, the new frog is only known from a single limestone cave system in Sai Yok District of Kanchanaburi Province of Thailand; its habitat is affected by illegal bat guano mining and other human activities. As such, *Siamophryne troglodytes* Gen. et sp. nov. is likely to be at high risk of habitat loss. Considering high ecological specialization and a small known range of the new taxon, we propose a IUCN Red List status of Endangered.
(EN) for it.
A striking new genus and species of cave-dwelling frog (Amphibia: Anura: Microhylidae: Asterophryinae) from Thailand

Chatmongkon Suwannapoom¹, Montri Sumontha²*, Jitthep Tunprasert³, Thiti Ruangsuwan⁴, Parinya Pawangkhanant¹, Dmitriy V. Korost⁵, Nikolay A. Poyarkov, Jr.⁶,⁷*

1 Division of Fishery, School of Agriculture and Natural Resources, University of Phayao, Phayao, Thailand
2 Department of Fishery, Ranong Marine Fisheries Station, Ranong, Thailand
3 Department of ecology, Nakhon Pathom Rajabhat University, Nakhon Pathom Mueng, Nakhon Pathom, Thailand
4 Department of Zoology, Faculty of Science, Kasetsart University, Bangkok, Thailand
5 Petroleum Geology Department, Geological Faculty, Moscow State University, Moscow, Russia
6 Biological Faculty, Department of Vertebrate Zoology, Moscow State University, Moscow, Russia
7 Laboratory of Tropical Ecology, Joint Russian-Vietnamese Tropical Research and Technological Center, Hanoi, Vietnam

* Corresponding authors: Nikolay A Poyarkov, Montri Sumontha

Email address: n.poyarkov@gmail.com, montri.sumontha@gmail.com

Abstract

We report on a discovery of Siamophryne troglodytes Gen. et sp. nov. – a new troglobilous genus and species of microhylid frog from a limestone cave in the tropical forests of western Thailand. To assess its phylogenetic relationships we studied 12S rRNA−16S rRNA mtDNA fragment with final alignment comprising up to 2591 bp for 56 microhylid species. Morphological characterization of the new genus is based on
examination of external morphology and analysis of osteological characteristics using microCT-scanning. Phylogenetic analyses place the new genus into the mainly Australasian subfamily Asterophryinae as a sister taxon to the genus *Gastrophrynoides*, the only member of the subfamily known from Sundaland. The new genus markedly differs from all other Asterophryinae members by a number of diagnostic morphological characters and demonstrates significant mtDNA sequence divergence. We provide a preliminary description of a tadpole of the new genus. Thus, it represents the only asterophryine taxon with documented free-living larval stage and troglophilous life style. Our work demonstrates that *Siamophryne troglodytes* Gen. et sp. nov. represents an old lineage of the initial radiation of Asterophryinae which took place in the mainland Southeast Asia. Our results strongly support the "out of Indo-Eurasia" biogeographic scenario for this group of frogs. To date, the new frog is only known from a single limestone cave system in Sai Yok District of Kanchanaburi Province of Thailand; its habitat is affected by illegal bat guano mining and other human activities. As such, *Siamophryne troglodytes* Gen. et sp. nov. is likely to be at high risk of habitat loss. Considering high ecological specialization and a small known range of the new taxon, we propose a IUCN Red List status of Endangered (EN) for it.

**Introduction**

Microhylidae is one of the largest frog families belonging to the Ranoidea, with a pan-tropical distribution. To date, it includes 641 species (nearly 9.4% of anuran diversity) (Frost, 2017). Microhylid frogs occur on most of the continents and several large islands, their range encompasses tropical and subtropical areas of Southern and Northern America, Africa, Madagascar, South, Southeast and East Asia, Australasian islands and northern Australia. Family-level taxonomy of Microhylidae is considered to be a “phylogeneticist’s nightmare” (Peloso et al., 2017) and is the subject of numerous studies and an on-going debate. At present, 13 subfamilies are recognized based on morphological and molecular phylogenetic data (Matsui et al., 2011; Pyron & Wiens,
However, the degree of coherence between the morphological and molecular classifications of the family is quite low due to the high morphological variation and widespread convergence in microhylids, which, in many cases, likely is connected to specializations associated with a burrowing lifestyle (see de Sá et al., 2012; Peloso et al., 2017). The basal split within Microhylidae is estimated to coincide with the Cretaceous–Paleogene boundary (65 Ma) (Feng et al., 2017); previous studies argued for Mesozoic origin of Microhylidae and considered the constituent subfamilies as full families of Anura (Bossuyt & Roelants, 2009).

Each Microhylidae subfamily is restricted to a landmass derived from the breaking up of the Gondwana: the Americas (subfamilies Adelastinae, Gastrophryninae and Otophryninae), Africa (subfamilies Hoplophryninae and Phrynomerinae), Madagascar (subfamilies Cophylinae, Dyscophinae and Scaphiophryninae), India (subfamily Melanobatrachinae), East, South and Southeast Asia (subfamilies Chaperininae, Kalophryninae and Microhylinae) and Australasia (subfamily Asterophryinae) (Kurabayashi et al., 2011; de Sá et al., 2012; Peloso et al., 2017). Due to their transcontinental pantropical distribution, Microhylidae were regarded as a promising model group for biogeography studies (Savage, 1973). Most previous works, though varying on taxon sampling and molecular data, suggested that Microhylidae are of Gondwanan origin and gave evidence supporting the “Antarctic route scenario” for the Australasian subfamily Asterophryinae, as suggested for several other vertebrate taxa that are distributed in Australia (Van Bocxlaer et al., 2006; Van der Meijden et al., 2007).

According to this scenario, the basal split of Microhylidae took place in Gondwana and the ancestor of Asterophryinae dispersed to Australia via Antarctic land bridge (Hill, 2009), where the subfamily diversified (it comprises 323 recognized species to date, Frost, 2017) and subsequently dispersed to New Guinea and adjacent Australasian islands, but was unable to cross the Wallace line with exception of the genus *Oreophryne* Boettger, which is also known from the island of Bali (west from the Wallace line, see Fig. 1).
However, for the first time, Matsui *et al.* (2011) reported on the phylogenetic position of the enigmatic genus *Gastrophrynoides* Noble inhabiting Sundaland (peninsular Malaysia and northern Borneo, see Fig. 1), which was not yet assigned to any certain subfamily. Based on the analysis of 16S rRNA – 12S rRNA mtDNA data it was established as a sister lineage of the genus *Oreophryne* (Asterophryinae). Association of *Gastrophrynoides* with asterophryines was further suggested by Kurabayashi *et al.* (2011), what allowed the authors to assign *Gastrophrynoides* to the subfamily Asterophryinae and propose an alternative biogeographic scenario for the group. According to Kurabayashi *et al.* (2011), *Gastrophrynoides* separated from other asterophryines around 48 Ma, while the presence of the most basal asterophryine taxon in the Eurasian area (Sundaland) suggests that the colonization route of Asterophryinae goes from Asia to Australia, but not via Antarctica as was suggested earlier. Further studies, applying multilocus (de Sá *et al.*, 2012) and phylogenomic (Peloso *et al.*, 2017) approaches, strongly supported the placement of the Sundanese *Gastrophrynoides* as a sister group to all other genera of the subfamily inhabiting Australasia.

As Kurabayashi *et al.* (2011:9) stated: “the biogeographic findings on *Gastrophrynoides* imply the possible occurrence of further microhylid taxa with unexpected evolutionary backgrounds and give a basis for future paleontological and biogeographic studies of Asian anurans”. In 2016, during a field survey in a limestone cave system in Kanchanaburi Province of western Thailand, we encountered an unusually-looking troglophilous frog. It was assigned to the family Microhylidae due to the presence of the following traits: lack of maxillary teeth, lack of parotoid glands, and a firmisternal pectoral girdle with non-overlapping epicoracoids, well developed coracoids reaching the midline of the girdle and scapulae, a large, cartilaginous sternum, reduced clavicles and no omosternum. However, the new frog was morphologically distinct from any genus of Microhylidae known to occur in Thailand or adjacent parts of Indochina. Further detailed morphological, osteological and phylogenetic analyses indicated that the Microhylidae *Gen. sp.* from Kanchanaburi represents a new yet undescribed genus of Asterophryinae frogs, a sister taxon to *Gastrophrynoides*. We provide the description of
this new frog herein. As we demonstrate below, our discovery carries important
biogeographic implications: highlights the initial radiation of Asterophryinae, which took
place in the mainland Southeast Asia, and supports the “out of Indo-Eurasia”
biogeographic scenario for this group of frogs.

Materials and methods

Sample collection

Field work was conducted from August to October of 2016 in Sai Yok District,
Kanchanaburi Province, northern Tenasserim Region, western Thailand (approximate
geographic coordinates: 14.476° N, 98.853° E; elevation – 440 m a.s.l.). Geographic
coordinates and elevation were obtained using a Garmin GPSMAP 60CSx and recorded
in the WGS 84 datum. In total, 11 adult specimens (6 males and 5 gravid females) and a
single tadpole of a new microhylid frog were collected and photographed in life before
being euthanized using 20% solution of benzocaine prior to fixation in 96% ethanol and
were subsequently stored in 70% ethanol. The larval specimen was fixed and
subsequently stored in 4% formalin. Tissue samples for genetic analysis were taken prior
to preservation and were stored in 95% ethanol. Specimens and tissues were subsequently
deposited in the herpetological collections of the School of Agriculture and Natural
Resources, University of Phayao (AUP, Phayao, Thailand) and of the Zoological
Museum of Moscow University (ZMMU, Moscow, Russia).

Specimens collection protocols and animal use were approved by the Institutional
Ethical Committee of Animal Experimentation of the University of Phayao, Phayao,
Thailand (certificate number UP-AE59-01-04-0022 issued to Chatmongkon
Suwannapoom) and strictly complied with the ethical conditions by the Thailand Animal
Welfare Act. Field work, including collection of animals in the field and specimen
exportation was authorized by the Institute of Animals for Scientific Purpose
Development (IAD), Bangkok, Thailand (permit number U1-01205-2558, issued to
Chatmongkon Suwannapoom).
The electronic version of this article in Portable Document Format (PDF) will represent a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new names contained in the electronic version are effectively published under that Code from the electronic edition alone (see Articles 8.5-8.6 of the Code). This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix http://zoobank.org/. The LSID for this publication is: urn:lsid:zoobank.org:pub:C8BD1C1D-0553-4662-8DE5-4337CD69E3B9. The online version of this work is archived and available from the following digital repositories: PeerJ, PubMed Central and CLOCKSS.

**Laboratory methods**

For molecular phylogenetic analysis, total genomic DNA was extracted from ethanol-preserved femoral muscle tissue using standard phenol–chloroform–proteinase K (final concentration 1 mg/ml) extraction procedures with subsequent isopropanol precipitation (protocols in accordance with Hillis *et al.*, 1996 and Sambrook & David, 2001). The isolated full-genome DNA was visualized using agarose electrophoresis in presence of ethidium bromide. The total concentration of DNA in 1 μl was measured using NanoDrop 2000 (Thermo Scientific), and consequently adjusted to ca. 100 ng DNA/μL.

We amplified mtDNA fragments, covering partial sequences of 12S rRNA and 16S rRNA mtDNA genes and a complete sequence of tRNAVal mtDNA gene in order to obtain a 2591 bp-length continuous fragment of mtDNA. 16S rRNA is a molecular marker widely applied for biodiversity surveys in amphibians (Vences *et al.*, 2005a, 2005b; Vieites *et al.*, 2009). Together with 12S rRNA partial sequences these mtDNA markers were used in the most comprehensive phylogenetic studies on Microhylinae frogs published to date (Matsui *et al.*, 2011; Pyron & Wiens, 2011; de Sá *et al.*, 2012; Peloso *et al.*, 2015 and references therein), including the molecular taxonomic research on the subfamily Asterophryinae (Hoskin, 2004; Frost *et al.*, 2006; Köhler & Günther,
Amplification was performed in 20 μl reactions using ca. 50 ng genomic DNA, 10 nmol of each primer, 15 nmol of each dNTP, 50 nmol of additional MgCl₂, Taq PCR buffer (10 mM of Tris-HCl, pH 8.3, 50 mM of KCl, 1.1 mM of MgCl₂ and 0.01% gelatine) and 1 U of Taq DNA polymerase. Primers used in PCR and sequencing are summarized in Table 1. The PCR conditions included the following steps: initial denaturation – 5 min at 94°C, 43 cycles of denaturation – 1 min at 94°C, primer annealing – 1 min with TouchDown program – reducing the temperature from 65 to 55°C by 1 degree Celcius every cycle, extension – 1 min at 72 °C, and final extension – 5 min at 72°C.

PCR products were visualized using 1.5% agarose electrophoresis in presence of ethidium bromide. If distinct bands were obtained, products were purified prior to cycle sequencing using 2 μl of ExoSapIt (Amersham), diluted in the ratio 1:4, per 5 μl of PCR product. A 10 μl sequencing reaction included 2 μL of template, 2.5 μl of sequencing buffer, 0.8 μl of 10 pmol primer, 0.4 μl of BigDye Terminator version 3.1 Sequencing Standard (Applied Biosystems) and 4.2 μl of water. The cycle sequencing reaction included 35 cycles consisting of the following steps: 10 sec at 96°C, 10 sec at 50°C and 4 min at 60°C. Cycle sequencing products were purified by ethanol precipitation. Sequence data collection and visualization was performed on an ABI 3730xl automated sequencer (Applied Biosystems). The obtained sequences were deposited in the GenBank under the accession numbers MG682553–MG682559 (Table 2).

Phylogenetic analyses

For phylogenetic analyses we used the 12S rRNA and 16S rRNA Microhylidae dataset of Matsui et al. (2011) with addition of available sequences from other Microhylidae genera that are distributed in Southeast Asia and Australasia together with the newly obtained sequences of Microhylidae Gen. sp. from Kanchanaburi Province of Thailand. Data on sequences and specimens used in molecular analyses is summarized in Table 2. In total, sequences of the 12S rRNA and 16S rRNA mtDNA fragments of 56 microhylid representatives and one non-microhylid outgroup taxon were subjected to the
final analyses, including seven samples of Microhylidae *Gen. sp.* from Kanchanaburi Province and 44 samples of Asian and Australasian microhylids representing all major lineages of the family inhabiting this region. The subfamily Asterophryinae was represented by approximately 26 species belonging to the following genera: *Aphantophryne* Fry, *Asterophrys* Tschudi, *Austrochaperina* Fry, *Barygenys* Parker, *Callulops* Boulenger, *Choerophryne* Van Kampen, *Cophixalus* Boettger, *Copiula* Méhely, *Gastrophrynoides*, *Genyophryne* Boulenger (now treated as a junior synonym of *Sphenophryne* Peters & Doria according to Rivera *et al.*, 2017), *Hylophorbus* Macleay, *Liophryne* Boulenger (included in the genus *Sphenophryne* by Rivera *et al.*, 2017), *Mantophryne* Boulenger, *Metamagusia* Günther (treated as a junior synonym of *Asterophrys* by Rivera *et al.*, 2017), *Oninia* Günther, Stelbrink & von Rintelen, *Oreophryne*, *Oxydactyla* Van Kampen (considered as synonym of *Sphenophryne* by Rivera *et al.*, 2017), *Paedophryne* Kraus, *Pseudocallulops* Günther (included in the genus *Asterophrys* by Rivera *et al.*, 2017), *Sphenophryne* and *Xenorhina* Peters. Other subfamilies included Microhylinae represented by genera *Glyphoglossus* Gunther, *Kaloula* Gray, *Metaphrynella* Parker, *Microhyla* Tschudi, *Micryletta* Dubois, *Phrynella* Boulenger and *Uperodon* Duméril & Bibron (14 species in total), Kalophryninae with a single genus *Kalophrynus* Tschudi (two species), Melanobatrachinae with a single monotypic genus *Melanobatrachus* Beddome, and Chaperininae with a single monotypic genus *Chaperina* Mocquard. Five outgroup sequences of non-Asian Microhylidae included: Dyscophinae (genus *Dyscophus* Grandidier; two species), Gastrophryninae (genus *Gastrophryne* Fitzinger; one species), Phrynomerinae (genus *Phrynomantis* Peters; one species), Scaphiophryninae (genus *Scaphiophryne* Boulenger; one species) subfamilies. MtDNA sequence of *Rhacophorus schlegelii* (Günther) (*Rhacophoridae*; Sano *et al.*, 2005) was used as a non-microhylid outgroup.

Nucleotide sequences were initially aligned using ClustalX 1.81 software (Thompson *et al.*, 1997) with default parameters, and then optimized manually in BioEdit 7.0.5.2 (Hall, 1999) and MEGA 6.0 (Tamura *et al.*, 2013). Mean uncorrected genetic distances (*p*-distances) between sequences were determined using MEGA 6.0.
MODELTEST v.3.06 (Posada & Crandall, 1998) was applied to estimate the optimal evolutionary models to be used for the data set analysis. The best-fitting model was determined to be the (GTR+I+G) model of DNA evolution, as suggested by the Akaike Information Criterion (AIC). Both 12S and 16S rRNA gene fragments were treated as a single partition due to the relatively short sequence length and similar features (i.e., mitochondrial rRNA).

Phylogenetic trees were inferred using two different methods: Maximum Likelihood (ML) and Bayesian Inference (BI). The ML analysis was conducted using Treefinder (Jobb et al., 2004). Confidence in tree topology was evaluated by non-parametric bootstrap analysis (BS) with 1000 replicates (Felsenstein, 1985). The BI analysis was conducted using MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003); Metropolis-coupled Markov chain Monte Carlo (MCMCMC) analyses were run with one cold chain and three heated chains for four million generations and were sampled every 1,000 generations. Five independent MCMCMC runs were performed and 1,000 trees were discarded as burn-in. Confidence in the tree topology was assessed using posterior probability (PP) (Huelsenbeck & Ronquist, 2001). We a priori regarded tree nodes with bootstrap (BS) values of 75% or greater and posterior probabilities (PP) values over 0.95 as sufficiently resolved, those with BS values in the range between 75% and 50% (PP between 0.95 and 0.90) were regarded as tendencies, those with BS below 50% (PP below 0.90) were considered to be unresolved (Huelsenbeck & Hillis, 1993).

**Adult morphology**

Sex of adult individuals was determined using gonadal dissection. All measurements were taken to the nearest 0.02 mm (and subsequently rounded to a 0.1 mm precision) from preserved specimens using digital calliper under a light dissecting microscope; morphometrics were acquired according to Poyarkov et al. (2014): (1) snout–vent length (SVL; measured from the tip of the snout to cloaca); (2) head length (HL; measured from the tip of snout to hind border of jaw angle); (3) snout length (SL; measured from the anterior corner of eye to the tip of snout); (4) eye length (EL;
measured as the distance between anterior and posterior corners of the eye); (5) nostril–
eye length (N–EL; measured as the distance between the anterior corner of the eye and
the nostril center); (6) head width (HW; measured as the maximum width of head on the
level of mouth angles in ventral view); (7) internarial distance (IND; measured as the
distance between the central points of nostrils); (8) interorbital distance (IOD; measured
as the shortest distance between the medial edges of eyeballs in dorsal view); (9) upper
eyelid width (UEW; measured as the maximum distance between the medial edge of
eyeball and the lateral edge of upper eyelid); (10) forelimb length (FLL; measured as the
length of straightened forelimb to the tip of third finger); (11) lower arm and hand length
(LAL; measured as the distance between elbow and the tip of third finger); (12) hand
length (HAL; measured as the distance between the proximal end of outer palmar
(metacarpal) tubercle and the tip of third finger); (13) inner palmar tubercle length (IPTL;
measured as the maximum distance between proximal and distal ends of inner palmar
tubercle); (14) outer palmar tubercle length (OPTL; measured as the maximum diameter
of outer palmar tubercle); (15) hindlimb length (HLL; measured as the length of
straightened hindlimb from groin to the tip of fourth toe); (16) tibia length (TL; measured
as the distance between the knee and tibiotarsal articulation); (17) foot length (FL;
measured as the distance between the distal end of tibia and the tip of fourth toe); (18)
inner metatarsal tubercle length (IMTL; measured as the maximum length of inner
metatarsal tubercle); (19) first toe length (1TOEL), measured as the distance between the
distal end of inner metatarsal tubercle and the tip of first toe; (20–23) second to fifth toe
lengths (measured as the outer lengths for toes II–IV, as the inner length for toe V; 2–
5TOEL); (24) first finger width (1FW), measured at the distal phalanx; (25–27) finger
disk diameters (2–4FDW); (28–32) toe disk diameters (1–5TDW); (33–36) finger lengths
(1–3FLO, 4FLI; for outer side (O) of the first, inner side (I) of the fourth, measured as the
distance between the tip and the junction of the neighbouring finger); (37) Tympanum
length, measured as the maximum tympanum diameter (TMP); (38) Tympanum-eye
distance (TEY). Terminology for describing eye coloration in living individuals is in
accordance with Glaw & Vences (1997); subarticular tubercle formulas follow those of Savage (1975).

The morphological characters for comparison and the data on their states in other Microhylidae representatives were taken from the following studies: Burton (1986), Chan et al. (2009), Günther & Richards (2016), Günther (2009, 2017), Günther et al. (2010, 2012a, 2012b, 2014, 2016), Köhler & Günther (2008), Kraus & Allison (2003), Kraus (2010, 2011, 2013a, 2013b, 2014, 2016, 2017), Menzies & Tyler (1977), Parker (1934), Richards & Iskandar (2000), Richards et al. (1992, 1994), Rittmeyer et al. (2012), Zweifel (1972, 2000), Zweifel et al. (2003).

**Larval morphology**

Morphological description of larval stages follows Poyarkov et al. (2015, 2017), and Vassilieva et al. (2014, 2017) and includes the following 16 measurements: total length (ToL); body length (BL); tail length (TaL); maximum body width (BW); maximum body height (BH); maximum tail height (TH); snout to vent length (SVL); snout to spiracle distance (SSp); maximum upper tail fin height (UF); maximum lower tail fin height (LF); internarial distance (IN); interpupilar distance (IP); rostro-narial distance (RN); naro-pupilar distance (NP); eye diameter (ED) and mouth width (MW). Tadpoles were staged according to the table of Gosner (1960).

**Osteology**

Micro-CT scanning protocols followed Scherz et al. (2016). Micro-CT scanning was conducted at the Petroleum Geology Department, Faculty of Geology, Lomonosov Moscow State University using a SkyScan 1172 desktop scanner (Bruker microCT, Kontich, Belgium) equipped with a Hamamatsu 10Mp digital camera. Specimen was mounted on a polystyrene baseplate and placed inside a hermetically closed polyethylene vessel. Scans were conducted with a resolution of 3.7 µm at 100 keV voltages and current of 100 mA with rotation step 0.2° with the use of oversize mode in which 4 blocks of sub scan data were connected vertically to obtain a general tomogram. Data processing was performed using Skyscan software: NRecon (reconstruction) and CTan/CTVol (3D model producing and imaging). Osteological terminology follows Scherz et al. (2016).
Micro-CT does not render cartilage, cartilage structures were therefore omitted from the osteological descriptions.

**Results**

**Sequence variation**

The studied 12S rRNA – 16S rRNA mtDNA fragment consisted of 2,591 sites: 1,070 sites were conserved and 1,394 sites were variable, 1,070 of which were found to be parsimony-informative. Hypervariable regions with poor local alignment were removed using Gblocks v0.91b (Castresana, 2000); of the original 1,556 aligned positions, 2,253 were retained in final analyses. The transition–transversion bias (R) was estimated to be equal to 2.18. Nucleotide frequencies were A=34.23%, T=22.89%, C=24.85%, and G=18.04% (all data given only for the Microhylidae ingroup).

**Phylogenetic relationships**

Results of phylogenetic analyses are shown in Figure 2. Bayesian and Maximum Likelihood analyses yielded essentially similar topologies that slightly differed only in associations at several poorly supported basal nodes. We achieved high resolution of phylogenetic relationships among major lineages of the subfamily Asterophryinae, with several sufficiently resolved major nodes (PP=1.0; BS=100%: Fig. 2). However, phylogenetic relationships between the subfamilies of Microhylidae, or within the Austro-Papuan radiation of Asterophryinae were poorly resolved with low or insignificant levels of support (BPP<0.95; BS<75%) for major basal nodes.

The general topology of the phylogenetic relationships of microhylid frogs resulting from our analyses is consistent with the results reported in recent studies by Matsui *et al.* (2011), Kurabayashi *et al.* (2012), Pyron & Wiens (2011), de Sá *et al.* (2012), the mtDNA dataset of Peloso *et al.* (2015) and Rivera *et al.* (2017). Asterophryinae generic taxonomy is currently in a state of flux; we use the taxonomy proposed by Rivera *et al.* (2017), who synonymized genera *Genyophryne*,...
Metamagusia, Pseudocallulops, Liophryne and Oxydactyla, but also provide traditional generic affiliation for these groups in brackets (see Fig. 2).

The Bayesian inference tree (Fig. 2) suggests the following set of genealogical relationships among the assessed microhylid taxa. Phylogenetic relationships among the subfamilies of Microhylidae are essentially unresolved; monophyly of the Dyscophinae, Kalophryninae and Asterophryinae subfamilies is well-supported (1.0/100; hereafter, the node support values are given for BI PP/ML BS, respectively).

Asterophryinae consists of two major well-supported (1.0/100) reciprocally monophyletic clades:

1. Asterophryinae 1, or “core” Asterophryinae, includes all presently known genera of the subfamily that inhabit Australasia east of the Wallace line and the island of Bali (see line B1 on Fig. 1; range of Asterophryinae 1 is marked in red). Phylogenetic relationships among genera within the clade Asterophryinae 1 remain essentially unresolved (Fig. 2); they are discussed in more details in a multilocus study of Rivera et al. (2017).

2. The second clade includes the genus Gastrophrynoides known to date only from Sundaland – peninsular Malaysia and Borneo (lineage Asterophryinae 2 on Fig. 2; range on Fig. 1 is marked in blue), and the newly discovered microhylid from Kanchanaburi Province in western Thailand (lineage Asterophryinae 3 on Fig. 2; locality on Fig. 1 is marked in green).

Thus, our phylogenetic analyses indicate that the newly discovered Microhylidae Gen. sp. from Kanchanaburi Province in western Thailand falls into the radiation of Asterophryinae sensu lato and is placed as a sister lineage to the genus Gastrophrynoides with high levels of node support.

Genetic distances

The uncorrected genetic p-distances between the 12S rRNA – 16S rRNA gene fragments among and within the studied Microhylidae genera are shown in the Table 3. The genetic differentiation between the Microhylidae Gen. sp. from Kanchanaburi Province and other Microhylidae genera vary from 14.8% (genus Liophryne) to 20.6% of
substitutions (genus *Barygenys*). Genetic distance between the Microhylidae **Gen. sp.** and its sister lineage *Gastrophrynoides* reaches 15.6% of substitutions. These values of genetic divergence are high and correspond well to the genus level of differentiation observed in other groups of Anura (Vences et al., 2005a, 2005b; Vieites et al., 2009). No genetic variation was recorded in obtained haplotypes of 12S rRNA–16S rRNA gene of the new species (Table 3).

**Taxonomy**

Based upon the results of phylogenetic analyses of 12S rRNA – 16S rRNA mtDNA fragment sequences, the Microhylidae frog from Kanchanaburi Province represents a previously unknown highly divergent mtDNA lineage, clearly distinct from all other members of Microhylidae for which comparable genetic data were available. This lineage falls into the Australasian subfamily Asterophryinae and with high values of node support is reconstructed as a sister group to the genus *Gastrophrynoides* that inhabits Borneo and the peninsular Malaysia. Subsequent analyses of osteology and external morphology (see below) clearly indicate that the recently discovered population of Microhylidae **Gen. sp.** from Kanchanaburi Province represents a new previously undescribed genus and species which we describe herein as:

**Amphibia Linnaeus, 1758**

**Anura Fischer von Waldheim, 1813**

**Microhylidae Günther, 1858**

**Asterophryinae Günther, 1858**

**Siamophryne Gen. nov.**

**Diagnosis.** A medium-sized (19 mm < SVL < 30 mm) member of the Australasian subfamily Asterophryinae (family Microhylidae), with the following combination of morphological attributes: (1) both maxillae and dentaries eleutherognathine, no maxillary teeth; (2) vertebral column procoelous with 8 presacral vertebrae lacking neural crests; (3) no sagittal crest on cranium; (4) frontoparietals conjoined, connected by long suture;
(5) nasals wide, calcified, but not contacting each other medially; (6) vomero-palatines small, not expanded, vomerine spikes absent; (7) cultriform process of parasphenoid comparatively narrow; (8) clavicles present as slender tiny bones, lying on the procoracoid cartilage not reaching scapula or the midline; (9) omosternum absent; (10) sternum large, anterior portion consists of calcified cartilage, xiphisternum cartilaginous; (11) weak dorsal crest present on urostyle, absent on ilium; (12) terminal phalanges large T-shaped; (13) all fingers and toe discs with terminal grooves; (14) subarticular tubercles weak, discernible only at digit basis; (15) toe webbing absent; (16) tympanum distinct; (17) two transverse smooth palatal folds; (18) pupil round; (19) snout rounded, equal to eye length; (20) development with a larval stage, tadpole with peculiar dorso-ventrally compressed morphology.

**Type species.** *Siamophryne troglodytes* sp. nov.

**Other included species.** None are known at present.

**Distribution.** To date, *Siamophryne troglodytes* sp. nov. is only known from a small cave system in a karst region of Sai Yok District, Kanchanaburi Province, northern Tenasserim Region, western Thailand (see below the description of the species) (see Fig. 1).

**Discrimination from other Asterophryinae genera.** Information on character states for other Asterophryinae genera is based on Parker (1934), Zweifel (1972, 2000), Menzies & Tyler (1977), Burton (1986), Zweifel et al. (2003), Günther et al. (2010), Kraus (2010, 2017) and references therein. The new genus has eleutherognathine maxillae and dentaries and thereby is distinguished from those Asterophryinae genera which have symphignathine state of this trait in both jaws: *Asterophrys* (including the recently synonymized *Pseudocallulops* and *Metamagnusia*; see Rivera et al., 2017) (New Guinea), *Callulops* (from Sulawesi to New Guinea region), *Mantophryne* (New Guinea and Louisiade Archipelago), *Oninia* (New Guinea) and *Xenorhina* (including the recently synonymised *Xenobatrachus* Peters & Doria) (New Guinea region). The genus *Barygenys* (Papua New Guinea region) can be distinguished from the new genus by the presence of symphignathine dentaries and eleutherognathine maxillae (versus both jaws...
being eleutherognathine in *Siamophryne Gen. nov.*). The new genus lacks distinct neural
crests on presacral vertebrae, and therefore can be differentiated from the genera
*Aphantophryne* (from Philippines to New Guinea) and *Cophixalus* (from Moluccas to
New Guinea and northern Australia) (both have well-developed neural crests on presacral
vertebrae); the new genus can be further distinguished from *Aphantophryne* as it has 8
presacral vertebrae (vs. 7 presacral vertebrae in *Aphantophryne*). The genus
*Sphenophryne* sensu lato (New Guinea) has well-developed long and slender clavicles
(vs. tiny clavicles that do not reach scapula and the midline in the new genus), and broad
tomero-palatines that contact each other medially, with a post-choanal portion overlying
the palatine region (vs. vomero-palatines not expanded in the new genus); *Sphenophryne*
sensu stricto (*S. cornuta* Peters & Doria) can be further distinguished by a characteristic
spine-like projection on the upper eyelid (vs. smooth upper eyelid in the new genus), it
also has arboreal life style (vs. troglophilous life style of the new genus). The genus
*Genyophryne* (recently considered as a synonym of *Sphenophryne*; see Rivera et al.,
2017) can be distinguished from the new genus by absence of clavicles (vs. clavicles
present), stout body habitus (vs. slender body habitus) and absence of large finger discs
(vs. very large finger discs in the new genus). The genus *Liophryne* (which is also
regarded as a member of *Sphenophryne* sensu lato based on phylogenetic data of Rivera
et al., 2017) can be differentiated from the new genus by the presence of long and slender
clavicles (vs. tiny clavicles in the new genus), and by the presence of comparatively small
finger discs (vs. large broad fingers discs in the new genus). The genus *Oxydactyla* (now
regarded as a part of *Sphenophryne* sensu lato; see Rivera et al., 2017) can be
distinguished from the new genus by the absence of finger discs (vs. large finger discs
present in the new genus). The genus *Paedophryne* (New Guinea region) can be
distinguished from the new genus by a much smaller body size (SVL<20 mm, vs.
SVL≥20 mm in the new genus), by cartilaginous phalanges in the first digit (vs. ossified
phalanges in the new genus), by FI reduced to a nub (vs. well-developed FI in the new
genus), by the absence of clavicles and procoracoids (vs. presence in the new genus) and
by having 7 presacral vertebrae (vs. 8 presacral vertebrae in the new genus). By the
presence of clavicles and procoracoid cartilage, the new genus can be differentiated from the genus *Choerophryne* (New Guinea), which lacks these structures; the latter also has palatine portions of vomero-palatines fused with broad sphenethmoids (vs. not fused in the new genus). The genus *Copiula* (New Guinea) can be distinguished from the new genus by the lack of clavicles (vs. presence in the new genus), by small discs on fingers, which are smaller than those on toes (vs. large finger discs that are larger than toe discs in the new genus), by cartilaginous sternum (vs. ossified anterior portion of sternum in the new genus), and by the presence of a conspicuous rostral dermal gland (vs. rostral gland absent in the new genus). The new genus can be distinguished from *Austrochaperina* (Australia, New Guinea and New Britain) by fingers with very wide discs, much wider than penultimate phalanges, by vomero-palatines not expanded and by narrow cultriform process of parasphenoid (vs. discs on fingers absent or small, slightly different in width from penultimate phalanges, vomero-palatines expanded and broad cultriform process of parasphenoid in *Austrochaperina*). The new genus can be distinguished from *Hylophorbus* (New Guinea) by comparatively better developed nasals (vs. poorly developed nasals in *Hylophorbus*), by the presence of large finger discs (vs. discs on fingers usually absent, if present, they are much smaller than toe discs) and by completely smooth skin on dorsum (vs. shagreened to tubercular skin on dorsum in *Hylophorbus*). The genus *Oreophryne* (from Philippines and Lesser Sundas to New Guinea and New Britain, see Kraus, 2017) can be differentiated from the new genus by distinct toe webbing (vs. no toe webbing in the new genus), by arboreal or terrestrial life style (vs. troglophilous life style in the new genus), and by expanded vomero-palatines (vs. not expanded in the new genus).

From its sister genus *Gastrophrynoides* (peninsular Malaysia and Borneo) the new genus can be easily distinguished by the presence of large and wide finger discs (vs. small finger discs, slightly wider than the penultimate phalanges in *Gastrophrynoides*), by a comparatively much shorter snout and larger eye (snout length equal to eye length in the new genus vs. snout 2.5 times longer than eye in *Gastrophrynoides*) and by a distinct tympanum (vs. tympanum obscured by skin in *Gastrophrynoides*).
Finally, the sequences of the 12S – 16S rRNA mtDNA fragment for the new genus are markedly distinct from the sequences for all those Microhylidae members, for which homologous sequences are available (see Fig. 2, Table 3).

**Etymology.** The generic nomen *Siamophryne* is derived from “Siam” — the old name of present-day Thailand; referring to the range of the new genus, which to date is only known from western Thailand; and the Greek noun “phryne” (φρύνη; feminine gender), meaning “toad” in English; this root is often used in the generic names in Asterophryinae microhylid frogs. Gender of the new genus is feminine.

*Siamophryne troglo"de"tes sp. nov.*

Figs. 3–10; Table 4.

**Holotype:** AUP-00500, adult male in a good state of preservation, collected in a limestone cave in Sai Yok District, Kanchanaburi Province, western Thailand, elevation 440 m a.s.l. (approximately in the vicinity of 14°28' N, 98°51' E; exact geographic coordinates not provided for conservation purposes) (see Fig. 10); collected on October 27, 2016, by Montri Sumontha, Jitthep Tunprasert, Niruth Chomngam, and Chatmongkon Suwannapoom.

**Paratypes:** In total, 10 specimens: AUP-00501-00504, four adult males, and AUP-00505-00508, three adult females, collected on October 27, 2016, from the same locality and with the same data as the holotype; ZMMU A-5818 (field ID NAP-06651), adult male, and ZMMU A-5819 (field ID NAP-06652), adult female, collected by T. Ruangsuwan from the same locality as the holotype on August 1, 2016.

**Referred specimens:** AUP-00509, a larval specimen, Gosner stage 36, collected by T. Ruangsuwan from the same locality as the holotype on August 1, 2016.

**Diagnosis:** The only known member of the genus *Siamophryne Gen. nov.* (see Diagnosis of the genus). *Siamophryne troglo"de"tes sp. nov.* is characterized by a combination of the following traits: (1) snout-vent length of six adult males 19.1 mm to 24.9 mm, and of five adult females 25.0 mm to 27.8 mm; (2) body habitus slender, limbs very long (FLL/SVL ratio 0.69 (0.65–0.74); HLL/SVL 1.50 ratio (1.39–1.67) for both
sexes); (3) snout short, rounded, subequal to eye length (0.8–1.0 times the length of the eye); (4) eye medium-sized, eye length/snout-vent length ratio – 0.12 to 0.14; (5) tips of fingers II-IV expand to broad discs 1.5–2.5 times wider than the penultimate phalanges; toes II-IV with smaller discs slightly wider than the penultimate phalanges, tips of finger I, toe I and toe V rounded, same width as the penultimate phalanges; (6) finger discs distinctly wider than toe discs; (7) terminal phalanges distinctly T-shaped in F2–F4 and T2–T5; bobbin-shaped in finger I and toe I; (8) subarticular tubercles on fingers weak, indistinct; finger subarticular tubercle formula: 1:1:1:1; better pronounced on toes, toe subarticular tubercle formula: 1:1:1:1:1; (9) outer metatarsal tubercle absent, inner metatarsal tubercle small, rounded; (10) skin of the ventral surface completely smooth, skin of the dorsal and lateral surfaces smooth with rare flat tubercles or pustules; (11) osteological features are the same as for the genus. Other diagnostic features are given in the diagnosis of the genus.

**Description of the holotype.** Holotype in preservative is shown in Fig. 3 and Fig. 4. Medium-sized specimen, with SVL 22.1 (hereafter all measurements in mm), in a good state of preservation, however distal parts of toes II–V slightly dehydrated (Fig. 3 E); ventral surface of left thigh dissected for 5 mm and some of femoral muscles removed. Body habitus slender (Fig. 3 A); head length slightly shorter than head width (HL/HW 0.95); snout rounded both in profile (Fig. 3 C; Fig. 4 A) and in dorsal view (Fig. 3 A), shorter than the diameter of eye (SL/EL 0.91); eyes large, notably protuberant in dorsal and lateral views, pupil oval, horizontal (Fig. 3 C); dorsal surface of head flat, canthus rostralis indistinct, gently rounded; loreal region weakly concave; nostril rounded, lateral, located much closer to tip of snout than to eye; tympanum well discernable, circular, tympanic rim not elevated above the skin of temporal area, supratympanic fold absent; vomerine teeth absent, two transverse palatal folds present across the palate anteriorly to the pharynx, both of them with smooth edges, tongue spatulate and free behind, lacking papillae, vocal sac opening not discernable.
Forelimbs comparatively long, less than half length of hindlimbs (FLL/HLL 0.42); hand slightly longer than lower arm and less than half length of forelimb (HAL/FLL 0.36); fingers slender, flattened in cross-section, first finger well developed, one half length of the second finger (1FLO/2FLO 0.50); relative finger lengths: I<II<IV<III (see Fig. 3 D, Fig. 4 B). Finger webbing and dermal fringes absent. First fingertip rounded and slightly dilated, almost the same width as the basal phalanx. Tips of three outer fingers II–IV greatly dilated forming large triangular disks with distinct narrow terminal grooves; relative finger disk widths: II<IV<III; longitudinal furrow on the dorsal surface of fingers absent; flexor tendons visible through translucent skin on ventral surface of fingers; subarticular tubercles on fingers barely distinct at basis of proximal phalanges and almost indistinct under penultimate phalanges of fingers III and IV, subarticular tubercles flat, oval-shaped with unclear borders, finger subarticular tubercle formula: 1:1:1:1 (for fingers I:II:III:IV, respectively); nuptial pad absent; two palmar (metacarpal) tubercles: inner palmar tubercle small, rounded, same size as subarticular tubercles; outer palmar tubercle oval-shaped with indistinct borders, almost the same length as inner palmar tubercle (IPTL/OPTL 0.97); palmar surface smooth, supernumerary palmar tubercles absent.

Hindlimbs long and slender, tibia length is half of snout–vent length (TL/SVL 0.49); tibiotarsal articulation of adpressed limb reaching the eye level; foot shorter than tibia (FL/TL 0.89); relative toe lengths I<II<V<III<IV; tarsus smooth, tarsal fold absent; tips of all toes slightly dilated forming small spatulate disks on all toes except toe I (Fig. 3 E, Fig. 4 C), each disk with weak terminal groove similar to that on fingers, relative toe disk widths: I<II<V<III<IV; toes slightly flattened in cross-section, dermal fringes absent; toe webbing absent between all toes; subarticular tubercles on toes more distinct than on fingers, oval-shaped, elevated, toe subarticular tubercle formula: 1:1:1:1:1 (for toes I:II:III:IV:V, respectively); single metatarsal tubercle: inner metatarsal tubercle rounded, flattened.

Skin on dorsal and dorsolateral surfaces smooth with rarely scattered flat tubercles (Fig. 3 A), tubercles getting larger and more prominent on dorsal surfaces of hindlimbs;
dorsal surface of forelimbs smooth with few small tubercles on forearm; upper eyelids smooth; ventral sides of trunk, head and limbs completely smooth (Fig. 3 B); dermal ridges or skin macroglands absent.

**Measurements of holotype (in mm).** SVL 22.1; HL 6.7; SL 2.7; EL 3.0; N-EL 1.9; HW 7.1; IND 2.3; IOD 2.2; UEW 1.8; FLL 15.4; LAL 10.4; HAL 5.6; IPTL 1.0; OPTL 1.1; HLL 37.1; TL 10.8; FL 9.6; IMTL 0.9; 1TOEL 2.0; 2TOEL 4.7; 3TOEL 7.4; 4TOEL 9.6; 5TOEL 7.3; 1FW 1.0; 2FDW 1.2; 3FDW 1.3; 4FDW 1.3; 1TDW 0.2; 2TDW 0.2; 3TDW 0.3; 4TDW 0.5; 5TDW 0.3; 1FLO 1.9; 2FLO 3.7; 3FLO 5.6; 4FLI 4.5; TMP 1.2; TEY 0.8.

**Coloration of holotype in life.** Coloration rather uniform: dorsal surface of head and trunk chocolate brown; dorsal surface of limbs ochre-brown; ventral surface of limbs and throat light orange-pink, lateral sides of body grey to grey-brown; ventral surfaces of body pinkish-grey. Fingers and toes grey with dark brown mottling. Tympanum greyish with slight brown mottling. Tubercles on dorsal surfaces of body, limbs and head copper-brown. Iris uniform dark brown; pupil black; sclera bluish-grey.

**Coloration of holotype in preservative.** Coloration in preservative is shown on Fig. 3. After preservation in ethanol, dorsal coloration changed to greyish-brown, upper eyelids dark-grey (Fig. 3 A), ventral surface of chest, belly, limbs turn yellowish-grey (Fig. 3 B); iris coloration faded and turned black.

**Morphological variation of the type series.** Measurements of the type series that show variation in morphometric characteristics are given in Table 4. Coloration of paratype in life is shown in Fig. 5. Specimens show no significant variation in coloration. Specimens vary in the body size: females (SVL 25.0–27.8; mean 26.3±0.7; n=5) are larger than males (SVL 19.1–24.9; mean 22.4±2.1; n=6). Paratypes *in situ* had generally darker coloration with dark grey-brown coloration of dorsum and grey coloration of ventral surfaces and body flanks (Fig. 10 C, D).

**Osteological characteristics.** Based on microtomographic data from ZMMU A-5818, adult male. The main skeletal features are shown in Figure 6. Details of skull morphology are presented in Figure 7.
Skull clearly longer than wide (Fig. 6). Frontoparietals separate along their entire length, longer than broad, narrower anteriorly than posteriorly, connected with each other medially with long suture, lack a sagittal crest, partially fused to exoccipital posteriorly (Fig. 7 A). Exoccipitals separate, contact each other medially. Nasals large but not meeting each other at midline, lacking posterior ramus, chondrified peripherally, overlying the dorsal portion of sphenethmoid (Fig. 7 A). Sphenethmoid ossified laterally and dorsally, but chondrified anteriorly and ventrally (Fig. 7 B). Prootics partially chondrified, lacking dorsal crest (Fig. 7 C). Squamosal L-shaped, well-ossified, distally chondrified, articulating on anterolateral surface of prootic (Fig. 7 C). Columella comparatively small, centrally ossified (Fig. 7 C), distally chondrified; tympanic annulus completely chondrified. Premaxilla with slender, weakly mineralized dorsal process; labial process of premaxilla well-ossified (Fig. 7 D). Maxilla largely chondrified, ossified in central part. Anterior ends of maxillaries contact labial portions of well-developed premaxillaries (eleutherognathine condition) (Fig. 7 D). Quadratojugal ossified in posterior portion. Vomers mostly chondrified plates meeting at midline, without teeth or lateral processes; parts edging choanae weakly ossified (Fig. 7 C). Mentomeckelians ossified, connected to dentaries and to each other by strips of cartilage (Fig. 7 B); dentaries not fused (eleutherognathine condition). Parasphenoid smooth; cultriform process of parasphenoid rather narrow, terminates at the level of sphenethmoid with an anterior notch (Fig. 7 B). Hyoid plate completely cartilaginous, anterolateral (alar) processes of hyoid plate present, recurved, posterolateral processes thinner than alary processes; posteromedial processes strongly ossified, elongated, straight, wider at proximal ends, chondrified at distal ends (Fig. 6 B).

Eight nonimbricate procoelous presacral vertebrae (PSV), stout, length approximately from one-third to one half of width; presacral vertebrae longer anteriorly, narrowing progressively to the posterior; all except the first with wide diapophyses, which are also longer anteriorly (3d PSV has the longest transverse processes), decreasing in length progressively to the posterior, with chondrified tips (Fig. 6 A, B). Diapophyses of vertebrae 2, 7 and 8 oriented anteriad, those of third and sixth – straight,
and those of fourth and fifth oriented posteriad. Neural crests on PSV absent. Sacrum with slightly expanded diapophyses. Urostyle with weak dorsal crest running along about 75% of its shaft; ilia smooth, lacking dorsal crest (Fig. 6 A).

Coracoids, scapulae and suprascapulae present; first two fully ossified; suprascapula largely chondrified. Coracoids robust with narrow distal ends oriented anteriad; proximal ends greatly expanded (Fig. 6 B). Omosternum absent. Procoracoid cartilage well-developed, extends from the scapula to the mid-line of the girdle. Clavicles present as slender, tiny, slightly recurved bones, lying on the procoracoid cartilage, not reaching scapula or the mid-line of the girdle (Fig. 6 B). Sternum large, anterior portion consists of calcified cartilage (Fig. 6 B), xiphisternum completely cartilaginous.

Bones of hands (Fig. 6 C, D) with six largely calcified carpal elements: carpal distale I small, carpal distale II–IV fused into a single large element, prepollex and a large radiale lie between a small Y-element and an elongated ulnare. Metacarpals long and fully ossified; phalangeal formula: 2-2-3-3; all phalanges ossified; distal phalanx of finger I bobbin-shaped; terminal phalanges of fingers II–IV with greatly expanded, T-shaped tips, approximately 3–4 times wider than penultimate phalanges (Fig. 6 C, D).

Foot (Fig. 6 E, F) with four tarsal elements, including ossified tarsale distale II–III, central and a prehallux; prehallux ossified. Metatarsals fully ossified, long and relatively more massive than metacarpals; phalangeal formula: 2-2-3-4-3; all phalanges ossified (Fig. 6 E, F). Terminal phalanges of all toes with slightly expanded, T-shaped tips; equal in width to penultimate phalanges on toes I and V, slightly exceeding the width of penultimate phalanges on toes II–IV (Fig. 6 E, F).

**Larval morphology.** Tadpole morphology description is based on a single larval specimen AUP-00509, Gosner stage 36, collected from the type locality. External appearance and coloration of the tadpole in life is shown in Fig. 8; morphological details in preservative are presented in Fig. 9. Body measurements are given below.

The single encountered tadpole was attributed to *Siamophryne troglodytes Gen. et sp. nov.* based on the following evidence: (1) morphological features characteristic for Microhylidae larvae in general (flattened body, mouthparts lack lateral lobes and
keratinized structures); (2) collected in a crevice in the limestone cave, where adult animals were observed (see Fig. 10 E, F); (3) species identification confirmed by mtDNA sequence of short 16S rRNA gene fragment (up to 500 bp), identical to that of adult specimens (GenBank Accession number: MG682559, see Table 2).

Standard tadpole measurements (AUP-00509, Gosner stage 36) (all in mm, taken by TR): ToL: 35.1; BL: 12.1; TaL: 21.8; BW: 7.9; BH: 5.4; TH: 4.0; SVL: 13.6; SSp: 5.6; UF: 1.0; LF: 1.0; IN: 1.2; IP: 1.7; RN: 1.9; NP: 1.9; ED: 1.0; MW: 2.6.

In dorsal view (Fig. 8 A, Fig. 9 B), body elongated (BW/BL 0.65) guitar-shaped with large bluntly rounded anterior part and a distinct narrowing behind the eyes that forms notably angular body edges; snout blunt with a shallow medial notch. From lateral view (Fig. 9 A), both head and body strongly flattened. Tail strong, with well-developed muscles, less than two times longer than the body (Tal/BL 1.80), tail width at tail basis subequal to interorbital distance; tail tip gently rounded. Tail fins very low, with even edges; dorsal fin not extending on the trunk, ventral fin same height as dorsal fin (LF/UF 0.94). A wide gular fold across the anterior half of body on the level of ca. 1/3 of body length (Fig. 8 B, Fig. 9 B). Spiracle medial, narrow, slit-like, transversal; located in the anterior half of body (SSp/SVL 0.41); covering membrane with even free margin. Vent tube medial, oblique.

Eyes dorsal, very small (ED/BL 0.08); with dorsolateral orientation of pupils. Small unpigmented narial protuberances present in nostril area. Mouth terminal, oriented antero-ventrally, not visible from dorsal view (Fig. 9 B), mouth opening transverse, slit-like from ventral view (Fig. 9 C), relatively wide (MW/BW 0.33). From lateral view (Fig. 9 A), upper labium slightly projecting and overhanging over the mouth opening; upper labium with even edge; lower labium short, with U-shaped edge. Protuberances in mouth angles, lateral lobes and keratinized mouthparts absent. No papilla seen on mouth floor or mouth roof. Lingual anlage rounded.

**Coloration.** In life at stage 36 larva almost unpigmented and *in situ* appears translucent with dark-black colored eyes (Fig. 10 E, F). In laboratory conditions, live tadpole (Fig. 8) shows semitransparent body with weak brownish pigmentation on dorsal
surfaces of body and tail; a strongly-pigmented dark stripe runs along dorsal surface from interorbital region to tail base (Fig. 8 A). Tail fins and ventral sides unpigmented, in life this allows to see heart, liver and major blood vessels from ventral view (Fig. 8 B). Limb buds pigmented with brown. In preservative, brown color fades to brown-grey (Fig. 9).

**Natural history notes.** *Siamophryne troglodytes Gen. et sp. nov.* has a troglophilous life style and to date is only known from a small limestone cave system in western Thailand. All specimens were collected within a narrow area inside a limestone cave located on elevation 440 m a.s.l. in a polydominant tropical forest in Sai Yok District, Kanchanaburi Province, western Thailand (Fig. 10 A). The cave was examined twice on the 1st of August and the 27th of October, 2016. In both cases, adult specimens of *Siamophryne troglodytes Gen. et sp. nov.* were only recorded inside the cave, at a distance of more than 25 m from the entrance, sitting on walls of the cave (Fig. 10 B, D) or hiding inside small caverns in limestone (Fig. 10 C) or under flat stones. Despite the thorough search, no animals were recorded near the cave entrance or in the forest close to the cave. Animals were active from 23:00 to 24:00, when the air temperature inside the cave was 28°C in August and 26°C in October, in both cases with 100% humidity. No calling activity was recorded during both surveys. Diet and enemies of the new frog are unknown.

Three tadpoles (one of which was collected) were observed during the survey on the 1st of August, 2016, in a small water-filled cavity in the limestone on the floor of the cave, ca. 10 m from the cave entrance (Fig. 10 E, F). The cavity was filled with water, the average depth was 4–5 cm; mosquito larvae (Chironomidae) were also observed in the same water body. Four other tadpoles (not collected) were discovered in another similar water-filled cavity inside the cave (30 m from the cave entrance).

The cave system where *Siamophryne troglodytes Gen. et sp. nov.* was discovered is inhabited by several species of bats which produce significant amount of guano that accumulates on the cave floor. According to a local guide, the locals mine this guano and that affects the ecosystem of the cave.
**Comparisons.** As for the genus, *Siamophryne troglodytes Gen. et sp. nov.* is a medium-sized frog (19 mm<SVL<30 mm) and can be easily distinguished from all other microhylids inhabiting the mainland Southeast Asia by long limbs with digits bearing large disks, with those on fingers up to 2.5 times wider than the penultimate phalanges (vs. finger tips not expanded into prominent discs in *Kalophrynus, Glyphoglossus, Microhyla* and *Micryletta*); by the absence of tibiotarsal projection and uniform dull-brownish coloration of ventral surfaces (vs. the presence of bony tibiotarsal projection and belly with bright saffron-yellow spots in *Chaperina*); by single small subarticular tubercle with indistinct borders at the basis of each finger (vs. subarticular tubercles of the fingers greatly enlarged to form accessory adhesive organs in *Phrynella* and *Metaphrynella*); by the lack of a bony ridge along the posterior border of each choana (vs. well-developed bony ridge along the posterior border of each choana in *Kaloula*), by the presence of a distinct tympanum (vs. hidden tympanum in genera *Glyphoglossus, Microhyla, Micryletta, Kaloula, Phrynella* and *Metaphrynella*).

The new species can be further distinguished from *Gastrophrynoides*, another Asterophryinae genus inhabiting the mainland Southeast Asia, by slender body habitus (vs. body habitus robust in *Gastrophrynoides*, see Fig. 2 for details), by a short rounded snout, that is 0.8–1.0 times the eye diameter (vs. long, pointed snout that is 2.6–3.0 times the diameter of the eye in *Gastrophrynoides*) and by a well-developed tympanum (vs. tympanum hidden in *Gastrophrynoides*).

**Distribution.** As for the genus. At present, *Siamophryne troglodytes Gen. et sp. nov.* is known from a single limestone karst cave in Sai Yok District of Kanchanaburi Province in western Thailand. To date, numerous surveys in the nearby karst massifs have not yielded discoveries of additional populations of the new species. However, further fieldwork in Kanchanaburi Province of Thailand and the adjacent parts of Tanintharyi Division of Myanmar are required.

**Conservation status.** This species was unknown to science and even to local people for a surprisingly long time despite intense human activity in this region; that suggests a possibly very limited range. Despite our numerous attempts to find additional
localities of the new species in adjacent limestone areas, we failed to detect other populations of this troglophilous frog. In this regard, we consider a disclosure of the detailed collecting locality information premature. In the case of narrow-ranged species of amphibians, the locality information should be released only after effective conservation measures have been taken and legal protection status have been established (see discussion in Hou et al., 2014). Until then, this information can be requested from the School of Agriculture and Natural Resources, University of Phayao. Currently, the only known habitat of *Siamophryne troglodytes* Gen. et sp. nov. is endangered due to illegal guano mining with the use of explosives, which is destroying the cave ecosystem. Given the available information, we suggest *Siamophryne troglodytes* Gen. et sp. nov. to be considered as an Endangered (EN) species following IUCN’s Red List categories (IUCN Standards and Petitions Subcommittee, 2016).

**Etymology.** The specific name “*troglodytes*” is a Latin adjective in the nominative singular meaning “cave-dweller”, derived from the Greek “τρωγλοδύτης”, with “*trogle*” meaning “hole, mouse-hole” and “*dyein*” meaning “go in, dive in”; referring to the troglophilous biology of the new species, which was recorded only in a limestone karst cave system.

**Suggested common names.** We recommend the following common names for the new species: “*Tenasserim Cave Frog*” (English); “*Eung Tham Tenasserim*” (Thai).

**Discussion**

**Systematics and biogeography**

In the present paper, we report a new lineage of Asterophryinae microhylid frogs from western Thailand, Tenasserim region of mainland Southeast Asia. As predicted by Kurabayashi et al. (2011), *Siamophryne troglodytes* represents an ancient lineage of Asterophryinae frogs distributed deep in the mainland Southeast Asia (Indo-Burma); it is reconstructed as a sister lineage to *Gastrophrynoides* – the only asterophryine found in the areas derived from the Eurasian landmass (Sundaland) until now. Our discovery of *Siamophryne troglodytes* has a significant biogeographic importance as, for the first time,
it documents the presence of Asterophryinae lineage north of the Isthmus of Kra (see Fig. 1) and thus strongly supports the Asian dispersal scenario for the group, suggested by Kurabayashi et al. (2011). According to this scenario, the splitting of the lineage that gave birth to the ancestors of Asterophryinae, Microhylinae and Dyscophinae occurred in the Indian landmass during the late Cretaceous (around 70 Ma). Following the collision of the India plate and the Eurasia during the Eocene (around 48 Ma, Kurabayashi et al., 2011), Asterophryinae common ancestor would have colonized mainland Southeast Asia and split into Gastrophrynoides + Siamophryne lineage and the ancestor of the “core” Asterophryinae 1 lineage. During the late Oligocene (around 25 Ma) the “core” Asterophryinae 1 ancestors further dispersed eastwards from mainland Asia to Australasian landmass via islands and/or short sea straits, where they undergone a comparatively recent and fast adaptive radiation following the orogenetic processes in the Fold Belt and the Australian Craton of New Guinea (Rivera et al., 2017) and subsequently colonized northern Australia and adjacent smaller islands.

Thus, our work demonstrates that Siamophryne troglodytes represents an old lineage of initial radiation of Asterophryinae sensu lato, which took place in the mainland Southeast Asia. The discovery of Siamophryne troglodytes goes in line with the biogeographic pattern reported for the Australasian frog family Ceratobatrachidae (Natatanura) by Yan et al. (2016): until recently, this family was only known from the South-West Pacific to the island archipelagos of South Asia, with primary centers of species diversity in Philippines and Solomon-Bismarck Archipelago. However, a recent phylogenetic study greatly extended the westernmost border of geographic distribution of the primarily Australasian family Ceratobatrachidae into western Thailand (Tenasserim) and Himalaya and assigned early branching events in this family to the lineages that are now exclusively represented by the mainland species with ranges restricted to Himalaya and Tibet (Yan et al., 2016). Hence, the biogeographic scenarios for at least two most speciose frog families of the Australasia suggest that their origin and early cladogenesis in the mainland Southeast Asia was followed by dispersal into Australasian archipelago and subsequent radiation. Our study encourages further field research and phylogenetic
studies on Southeast Asian frogs, since they may yield discoveries of new taxa and unexpected biogeographic patterns.

**Natural history and reproductive biology**

Limestone caves provide unique combinations of ecological features, such as rocky vertical substrates, climatic stability, low illumination, relaxed predation, and reduced prey base. Cave-dwelling taxa of amphibians and reptiles are often characterized by unique morphological adaptations, which are supposed to increase locomotion efficiency on vertical and inverted surfaces within cave-like microhabitats. In cave-dwelling ecomorphs of *Cyrtodactylus* geckoes these often include comparatively shorter trunks; longer and thinner limbs; flatter, narrower, and sometimes longer heads; and relatively larger eyes (Grismer & Grismer, 2017). *Siamophryne troglodytes*, as compared to its sister genus *Gastrophrynoides*, shows a number of morphological differences, which might be connected to its troglobilious life style. These include slender body habitus with notably longer limbs (see Fig. 2 for comparison), long and thin fingers and toes with tips expanded to large discs (see Fig. 5), slightly longer head (HL/SVL ratio 0.31 (0.30–0.33; N=11) in *Siamophryne troglodytes* vs. 0.28 (0.27–0.29; N=3) in *Gastrophrynoides immaculatus*), and comparatively larger eyes (EL/HL ratio 0.41 (0.37–0.46; N=11) in *Siamophryne troglodytes* vs. 0.20 (0.19–0.20; N=3) in *Gastrophrynoides immaculatus*). Higher distances between the opposing hands and opposing feet with enlarged digit disks and larger eyes might facilitate locomotion on vertical and inverted walls of limestone caves in low illumination conditions.

The life cycle of many Asterophryinae species is still unknown and females of most species are rare in museum collections. For the Australo-Papuan “core” Asterophryinae lineage there is sufficient evidence that allows to assume that all of the members of that lineage have direct development – a life cycle with metamorphosis taking place within the egg (Menzies, 2006; Günther *et al.*, 2012b); this contrasts sharply with the Southeast Asian Microhylinae and Kalophryninae, all of which have free-living tadpoles. The reproductive biology and development of *Gastrophrynoides* is completely unknown (Parker, 1934; Chan *et al.*, 2009); to our knowledge, no information on female
or egg morphology in this genus is available either. Our work shows that *Siamophryne troglodytes* possesses a life cycle with a free-living larval stage; tadpole morphology of the new genus is very peculiar and was not reported for any other microhylid so far. This is the first report of a free-living tadpole for *Asterophryinae*, as well as the first known *Asterophryinae* with a troglophilous life style. Our description is based on a single larval specimen, further studies on the reproductive biology and development of *Siamophryne troglodytes* as well as of the genus *Gastrophrynoides* are required to understand the details of life cycle in this lineage of *Asterophryinae*. It is assumed that the divergence between the “core” *Asterophryinae* lineage and the *Siamophryne + Gastrophrynoides* lineage took place no later than the Eocene (Kurabayashi *et al.*, 2011). Further research might reveal more significant differences in morphology and natural history of these lineages.

**Conservation**

Our study adds a new genus of frogs to the batrachofauna of Thailand and Indochina; according to our knowledge, *Siamophryne troglodytes* is endemic to a small limestone cave system in Sai Yok District of Kanchanaburi Province in western Thailand. This small area is known for an exceptionally high number of endemic species of squamates discovered by previous herpetofaunal surveys, including five endemic gecko species and two endemic species of snakes (see Sumontha *et al.*, 2017). The reasons behind such exceptional herpetofaunal endemism are yet unclear; it is remarkable that most reptiles that are endemic to Sai Yok District are associated with limestone habitats; this is also the case for *Siamophryne troglodytes*. Like the other representatives of Thai endemic herpetofauna that live in caves or their direct surroundings, the newly discovered genus has to cope with a high degree of human disturbance (Pauwels *et al.*, 2016), in this case – due to illegal mining of bat guano that causes destruction and modification of the cave ecosystem. This may pose a serious threat for *Siamophryne troglodytes* in the future; immediate assessment of its conservation status on national and international levels, conservation measures at the type locality that will minimize the
modification of the natural habitat by humans, as well as intensive surveys for the new localities are essential for the protection of this relict frog lineage.

The discovery of Siamophryne troglodytes further demonstrates the key role of limestone karst areas as arks of highly endangered biodiversity. Geological structure, erosion and subterranean water drainages of limestone areas permanently provide unique diversity of microrefugia with numerous shelters, like cracks and caves (Clements et al., 2006). These humid microhabitats might provide an efficient environmental buffer for small vertebrates during periods of climate change (Glaw et al., 2006). Isolated limestone massifs throughout the world are known as hotspots of vertebrate endemism and persistence (Oliver et al., 2017a). Karst areas act as microrefugia for relict amphibian species (see Sket, 1997; Min et al., 2005; Glaw et al., 2006; Milto et al., 2013). Karsts are also known as important "biodiversity arks" for both surface and cave faunas (Clements et al., 2006) with numerous new species of amphibians and reptiles being discovered from limestone areas (e.g. see Köhler et al., 2010; Nazarov et al., 2014; Rakotoarison et al., 2017; Grismer & Grismer, 2017; Grismer et al., 2017 and references therein). Ironically, though acting as major biodiversity hotspots, limestone karst areas are critically endangered due to intensive deforestation and cement manufacturing; their continued exploitation for limestone cannot be stopped (Clements et al., 2006). Our study thus calls for further focused survey and conservation efforts on karst herpetofauna in Southeast Asia.

Conclusions

Siamophryne troglodytes, a new genus and species of microhylid frogs from western Thailand, belongs to the subfamily Asterophryinae, which is most diverse in Australasia. Siamophryne and its sister genus Gastrophrynoides are the only two asterophryine lineages found in the areas derived from the Eurasian landmass. Our work demonstrates that Siamophryne troglodytes represents an old lineage of the initial radiation of Asterophryinae which took place in the mainland Southeast Asia. Our results strongly support the “out of Indo-Eurasia” biogeographic scenario for this group of frogs.
To date, the new frog is the only known asterophryine with a free-living tadpole and troglobilious life style. Further studies might reveal new members of Asterophryinae in the mainland Southeast Asia.

**Acknowledgements**

We would like to thank the Laboratory Animal Research Center, University of Phayao and The Institute of Animal for Scientific Purposes Development (IAD) Thailand for the permission to work in the field and Niruth Chomngam, Chaowalit Songsangchote and Akrachai Aksormneam for help during the field work. We are most grateful to Yu Lee (Chinese Culture University, Taipei) for providing photos of *Gastrophrynoides*. NAP thanks Valentina D. Kretova (Biological Faculty, Lomonosov Moscow State University) for help with preparation of figures, Vladislav Gorin (Biological Faculty, Lomonosov Moscow State University) for help and assistance in the lab, Duong Van Tang (Biological Faculty, Lomonosov Moscow State University) for help with phylogenetic analyses and Alexandra A. Elbakyan for help with accessing required literature. We are indebted to Evgeniya N. Solovyeva (Zoological Museum of Moscow University) for help with primer design and to Egill Scallagrimsson and Natalia Ershova for proofreading. We express our sincere gratitude to Gabriela Parra Olea and the three anonymous reviewers for their useful suggestions on the earlier version of the manuscript.

**References**

Blackburn DC, Siler CD, Diesmos AC, McGuire JA, Cannatella DC, Brown RM. 2013. An adaptive radiation of frogs in a southeast asian island archipelago. *Evolution* 67(9):2631–2646.

Bossuyt F, Roelants K. 2009. Anura. Hedges SB, Kumar S eds. *The Timetree of Life*: 357–364. New York, USA, Oxford University Press.

Burton TC. 1986. A reassessment of the Papuan subfamily Asterophryinae (Anura: Microhylidae). *Records of the South Australian Museum* 19:405–450.
Castresana J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* **17**:540–552. DOI: 10.1093/oxfordjournals.molbev.a026334

Chan KO, Grismer LL, Ahmad N, Belabut DM. 2009. A new species of *Gastrophrynoides* (Anura: Microhylidae): an addition to a previously monotypic genus and a new genus for Peninsular Malaysia. *Zootaxa* **2124**:63–68.

Clements R, Sodhi NS, Schilthuizen M, Ng PKL. 2006. Limestone Karsts of Southeast Asia: Imperiled Arks of Biodiversity. *BioScience* **56**(09):733-746.

De Sá RO, Streicher JW, Sekonyela R, Forlani MC, Loader SP, Greenbaum E, Richards SJ, Haddad CFB. 2012. Molecular phylogeny of microhylid frogs (Anura: Microhylidae) with emphasis on relationships among New World genera. *BMC Evolutionary Biology* **12**(241):1–21.

Deng X, Wang S, Liang X, Jiang J, Wang B, Deng L. 2015. The complete mitochondrial genome of *Kaloula rugifera* (Amphibia, Anura, Microhylidae). *Mitochondrial DNA*: 1–2.

Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**(4):783–791. DOI:10.2307/2408678

Feng YJ, 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 USA*; **114**(29):E5864–E5870. DOI:10.1073/pnas.1704632114. Epub 2017 Jul 3.

Glaw F, Vences M. 1997. Anuran eye colouration: definitions, variation, taxonomic implications and possible functions. In: W. Bohme, W. Bischoff & T. Ziegler (Eds), *Herpetologia Bonnensis*, Bonn: 125–138.

Glaw F, Hoegg S, Vences M. 2006. Discovery of a new basal relict lineage of Madagascan frogs and its implications for mantellid evolution. *Zootaxa* **1334**:27–43.

Gosner KL. 1960. A simplified table for staging anuran embryos and larvae. *Herpetologica* **16**:183–190.

Grismer LL, Grismer JL. 2017. A re-evaluation of the phylogenetic relationships of the *Cyrtodactylus condorensis* group (Squamata; Gekkonidae) and a suggested protocol for the characterization of rock-dwelling ecomorphology in *Cyrtodactylus*. *Zootaxa* **4300**(4):486–504. DOI:10.11646/zootaxa.4300.4.2

Grismer LL, Wood PL Jr, Myint Kyaw Thura, Thaw Zin, QUah ESH, Murdoch ML, Grismer MS, Aung Lin, Htet Kyaw, Ngwe Lwin. 2017. Twelve new species of *Cyrtodactylus* Gray (Squamata: Gekkonidae) from isolated limestone habitats in...
eastcentral and southern Myanmar demonstrate high localized diversity and unprecedented microendemism. *Zoological Journal of the Linnean Society* 45(306-07):251–484. DOI:10.1111/j.1096-3642.1965.tb00500.x

Günther R, Richards SJ, Bickford DP, Johnston GR. 2012b. A new egg-guarding species of *Oreophryne* (Amphibia, Anura, Microhylidae) from southern Papua New Guinea. *Zoosystematics and Evolution. Mitteilungen aus dem Museum für Naturkunde in Berlin* 88:223–230.

Günther R, Richards SJ, Dahl CS. 2014. Nine new species of microhylid frogs from the Muller Range in western Papua New Guinea (Anura, Microhylidae). *Vertebrate Zoology. Dresden* 64:59–94.

Günther R, Richards SJ, Tjaturadi B. 2016. A new species of the frog genus *Pseudocallulops* from the Foja Mountains in northwestern New Guinea (Amphibia, Microhylidae). *Russian Journal of Herpetology* 23:63–69.

Günther R, Richards SJ. 2016. Description of a striking new *Mantophryne* species (Amphibia, Anura, Microhylidae) from Woodlark Island, Papua New Guinea. *Zoosystematics and Evolution. Mitteilungen aus dem Museum für Naturkunde in Berlin* 86:245–256.

Günther R, Stelbrink B, von Rintelen T. 2010. *Oninia senglaubi*, another new genus and species of frog (Amphibia, Anura, Microhylidae) from New Guinea. *Zoosystematics and Evolution. Mitteilungen aus dem Museum für Naturkunde in Berlin* 85:171–187.

Günther R. 2009. *Metamagnusia* and *Pseudocallulops*, two new genera of microhylid frogs from New Guinea (Amphibia, Anura, Microhylidae). *Zoosystematics and Evolution. Mitteilungen aus dem Museum für Naturkunde in Berlin* 85:171–187.

Günther R. 2017. A redescription, a revalidation, and a new description within the microhylid genus *Austrochaperina* (Anura: Microhylidae). *Vertebrate Zoology. Dresden* 67(2):207–222.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In: Nucleic acids symposium series. [London]: Information Retrieval Ltd., c1979-c2000: 95–98.

Hedges SB. 1994. Molecular evidence for the origin of birds. *Proceedings of the National Academy of Sciences* 91(7):2621–2624. DOI:10.1073/pnas.91.7.2621

Hill ED. 2009. Salticidae of the Antarctic land bridge. *Peckhamia* 76.1:1–14.

Hillis DM, Moritz C, Mable BK, Graur D. 1996. Molecular systematics. Vol. 23, Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts, USA.
Hoskin CJ. 2004. Australian microhylid frogs (Cophixalus and Austrochaperina): phylogeny, taxonomy, calls, distributions and breeding biology. *Australian Journal of Zoology* **52**: 237–269.

Hou M, Wu Y-k, Yang K, Zheng S, Yuan Z-y, Li P. 2014. A missing geographic link in the distribution of the genus Echinotriton (Caudata: Salamandridae) with description of a new species from southern China. *Zootaxa* **3895**: 89–102.

Houelsenbeck JP, Hillis DM. 1993. Success of phylogenetic methods in the four-taxon case. *Systematic Biology* **42**(3):247–264. DOI:10.2307/2992463

Houelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**(8):754–755. DOI:10.1093/bioinformatics/17.8.754

Huxley TH. 1868. On the Classification and Distribution of the Alectoromorphae and Heteromorphae. *Proceedings of the Zoological Society of London*: 294–319.

Igawa T, Kurabayashi A, Usuki C, Fujii T, Sumida M. 2008. Complete mitochondrial genomes of three neobatrachian anurans: A case study of divergence time estimation using different data and calibration settings. *Gene* **407**(1–2):116–129.

IUCN Standards and Petitions Subcommittee. 2016. Guidelines for Using the IUCN Red List Categories and Criteria. Version 12. Downloaded from http://www.iucnredlist.org/documents/RedListGuidelines.pdf (accessed on 12 March 2017).

Jobb G, von Haeseler A, Strimmer K. 2004. TREEFINDER: a powerful graphical analysis environment for molecular phylogenetics. *BMC Evolutionary Biology* **4**:18.

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.

Köhler J, Vences M, D’Cruze N, Glaw F. 2010. Giant dwarfs: discovery of a radiation of large-bodied ‘stump-toed frogs’ from karstic cave environments of northern Madagascar. *Journal of Zoology* **282**:21–38.

Kraus F, Allison A. 2003. A new species of Callulops (Anura: Microhylidae) from Papua New Guinea. *Pacific Science. Honolulu* **57**:29–38.

Kraus F. 2010. New genus of diminutive microhylid frogs from Papua New Guinea. *ZooKeys* **48**:39–59.

Kraus F. 2011. New frogs (Anura: Microhylidae) from the mountains of western Papua New Guinea. *Records of the Australian Museum* **63**:53–60.
Kraus F. 2013a. Three new species of Oreophryne (Anura, Microhylidae) from Papua New Guinea. *ZooKeys* 333:93–121.

Kraus F. 2013b. A new species of Hylophorbus (Anura: Microhylidae) from Papua New Guinea. *Current Herpetology. Kyoto* 32:102–111.

Kraus F. 2014. A new species of Liophryne (Anura: Microhylidae) from Papua New Guinea. *Journal of Herpetology* 48:255–261.

Kraus F. 2016. Ten new species of Oreophryne (Anura, Microhylidae) from Papua New Guinea. *Zootaxa* 4195:1–68.

Kraus F. 2017. A new species of Oreophryne (Anura: Microhylidae) from the mountains of southeastern Papua New Guinea. *Current Herpetology. Kyoto* 36:105–115.

Kurabayashi A, Matsui M, Belabut DM, Yong H-S, Ahmad N, Sudin A, Kuramoto M, Hamidy A, Sumida M. 2011. From Antarctica or Asia? New colonization scenario for Australian-New Guinean narrow mouth toads suggested from the findings on a mysterious genus *Gastrophrynoides*. *BMC Evolutionary Biology* 11(175):1–12.

Matsui M, Hamidy A, Belabut DM, Ahmad N, Panha S, Sudin A, Khonsue W, Oh H-S, Yong H-S, Jiang J-P. 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. DOI:10.1016/j.ympev.2011.05.015

Matsui M, Shimada T, Liu W-Z, Maryati M, Khonsue W, Orlov N. 2006. Phylogenetic relationships of Oriental torrent frogs in the genus *Amolops* and its allies (Amphibia, Anura, Ranidae). *Molecular Phylogenetics and Evolution* 38(3):659–666. DOI:10.1016/j.ympev.2005.11.019

Mayr E. 1944. Wallace's Line in the Light of Recent Zoogeographic Studies. *The Quarterly Review of Biology* 19(1):1–14. DOI:10.1086/394684

Menzies JI, Tyler MJ. 1977. The systematics and adaptations of some Papuan microhylid frogs which live underground. *Journal of Zoology London* 183:431–464.

Menzies JI. 2006. *The Frogs of New Guinea and the Solomon Islands*. Pensoft Publisher Sofia. 210.

Milto KD, Poyarkov NA, Orlov NL, Nguyen TT. 2013. Two new rhacophorid frogs from Cat Ba Island, Gulf of Tonkin (Hai Phong Province, Vietnam). *Russian Journal of Herpetology* 20(4):287–300.

Min MS, Yang SY, Bonett RM, Vieites DR, Brandon RA, Wake DB. 2005. Discovery of the first Asian plethodontid salamander. *Nature* 435:87–90.

Nazarov RA, Poyarkov NA, Orlov NL, Nguyen NS, Milto KD, Martynov AA, Konstantinov EL, Chulisov AS. 2014. A review of *Cyrtodactylus* (Reptilia:
Sauria: Geckonidae) fauna of Laos with description of four new species. Proceedings of the Zoological Institute RAS 318(4):391–423.

Nguyen TL, Poyarkov NA Jr., Nguyen TT, Nguyen TA, Nguyen VH, Gorin VA, Murphy RW, Nguyen SN. 2018. A new species of the genus Microhyla Tschudi, 1838 (Amphibia: Anura: Microhylidae) from Tay Nguyen Plateau, Central Vietnam. Zootaxa, in press.

Oliver LA, Rittmeyer EN, Kraus F, Richards SJ, Austin CC. 2013. Phylogeny and phylogeography of Mantophryne (Anura: Microhylidae) reveals cryptic diversity in New Guinea. Molecular Phylogenetics and Evolution 67:600–607.

Oliver PM, Laver RJ, De Mello Martins F, Pratt RC, Hunjan S, Moritz CC. 2017a. A novel hotspot of vertebrate endemism and an evolutionary refugium in tropical Australia. Diversity and Distributions 23:53-66.

Oliver PM, Iannella A, Richards SJ, Lee MSY. 2017b. Mountain colonisation, miniaturisation and ecological evolution in a radiation of direct-developing New Guinea Frogs (Choerophryne, Microhylidae). PeerJ 5:e3077. DOI:10.7717/peerj.3077

Palumbi S, Martin A, Romano S, McMillan W, Stice L, Grabowski G. 1991. The Simple Fool’s Guide to PCR, Version 2.0, privately published document compiled by S. Palumbi. Dept. Department of Zoology, University of Hawaii, Honolulu, 96822.

Parker HW. 1934. Monograph of the frogs of the family Microhylidae. Trustees of the British Museum, London 212. DOI:10.2307/1436128

Pauwels OSG, Sumontha M, Ellis M. 2016. Les geckos cavernicoles des grottes aménagées et exploitées de Thaïlande: diversité et problématiques de conservation. In: Abstracts. 44e congrès de la Société Herpétologique de France, 2e congrès franco-belge d’Herpétologie. Société Herpétologique de France & Natagora: 36.

Peloso PL, Frost DR, Richards SJ, Rodrigues MT, Donnellan S, Matsui M, Raxworthy CJ, Biju S, Lemmon EM, Lemmon AR. 2016. The impact of anchored phylogenomics and taxon sampling on phylogenetic inference in narrow-mouthed frogs (Anura, Microhylidae). Cladistics 32(2):113–140. DOI:10.1111/cla.12118

Posada D, Crandall KA. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14(9):817–818. DOI:10.1093/bioinformatics/14.9.817

Poyarkov JNA, 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 Jr., 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. DOI:10.3897/zookeys.672.10624

Poyarkov NA Jr., Orlov NL, Moiseeva AV, Pawangkhanant P, Ruangsuwan T, Vassilieva AB, Galoyan EA, Nguyen TT, Gogoleva SS. 2015. Sorting out moss frogs: mtDNA data on taxonomic diversity and phylogenetic relationships of the Indochinese species of the genus *Theloderma* (Anura, Rhacophoridae). *Russian Journal of Herpetology* **22**(4):241–280.

Pyron RA, Wiens JJ. 2011. A large-scale phylogeny of Amphibia including over 2800 species, and a revised classification of advanced frogs, salamanders, and caecilians. *Molecular Phylogenetics and Evolution* **61**:543–583.

Rakotoarison A, Scherz MD, Glaw F, Köhler J, Andreone F, Franzen M, Glos J, Hawlitschek O, Jono T, Mori A, Ndriantsoa SH, Raminosoa NR, Riemann JC, Rödel MO, Rosa GM, Vieites DR, Crottini A, Vences M. 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.

Richards SJ, Iskandar DT. 2000. A new minute *Oreophryne* (Anura: Microhylidae) from the mountains of Irian Jaya, Indonesia. *Raffles Bulletin of Zoology. Singapore* **48**:257–262.

Richards SJ, Johnston GR, Burton TC. 1992. A new species of microhylid frogs (genus *Cophixalus*) from the Star Mountains, central New Guinea. *Science in New Guinea. Port Moresby* **18**:141–145.

Richards SJ, Johnston GR, Burton TC. 1994. A remarkable new asterophryine microhylid frog from the mountains of New Guinea. *Memoirs of the Queensland Museum* **37**:281–286.

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):1–6.

Rivera JA, Kraus F, Allison A, Butler MA. 2017. Molecular phylogenetics and dating of the problematic New Guinea microhylid frogs (Amphibia: Anura) reveals elevated speciation rates and need for taxonomic reclassification. *Molecular Phylogenetics and Evolution* **112**:1–11.
Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**(12):1572–1574. DOI:10.1093/bioinformatics/btg180

Sambrook J, David W. 2001. *Molecular cloning: a laboratory manual*, 3rd edn.. Plainview. Cold Spring Harbor Laboratory Press, New York.

Sano N, Kurabayashi A, Fujii T, Yonekawa H, Sumida M. 2005. Complete nucleotide sequence of the mitochondrial genome of Schlegel's tree frog *Rhacophorus schlegelii* (family Rhacophoridae): duplicated control regions and gene rearrangements. *Genes & Genetic Systems*. **80**(3):213–224.

Savage JM. 1973. The geographic distribution of frogs: pattern and predictions. In: Evolutionary Biology of Anurans: Contemporary Research on Major Problems. Edited by: Vial JL. Colombia, Missouri: University of Missouri Press; 351–445.

Savage JM. 1975. Systematics and distribution of the Mexican and Central American stream frogs related to *Eleutherodactylus rugulosus*. *Copeia* **1975**(2):254–306. DOI:10.2307/1442883

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**:91–106.

Sket B. 1997. Distribution of *Proteus* (Amphibia: Urodela: Proteidae) and its possible explanation. *Journal of Biogeography* **24**:263–280.

Sumontha M, Kunya K, Dangsri S, Pauwels OSG. 2017. *Oligodon saiyok*, a new limestone-dwelling kukri snake (Serpentes: Colubridae) from Kanchanaburi Province, western Thailand. *Zootaxa* **4294**(3):316–328.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* **30**(12):2725–2729. DOI:10.1093/molbev/mst197

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic acids research* **25**(24):4876–4882. DOI:10.1093/nar/25.24.4876

Trueb L. 1968. Cranial osteology of the hylid frog, *Smilisca baudini*. *University of Kansas Publications, Museum of Natural History* **18**:11–35.

Trueb L. 1973. Bones, frogs, and evolution. In: Vial, J. L. (ed.) Evolutionary biology of the anurans: Contemporary research on major problems. – University of Missouri Press, USA: 65–132.

Van Bocxlaer I, Roelants K, Biju SD, Nagaraju J, Bossuyt F. 2006. Late Cretaceous vicariance in Gondwanan amphibians. *PLoS One* **1**:e74.
Van der Meijden A, Vences M, Hoegg S, Boistel R, Channing A, Meyer A. 2007. Nuclear gene phylogeny of narrow-mouthed toads (Family: Microhylidae) and a discussion of competing hypotheses concerning their biogeographical origins. *Molecular Phylogenetics and Evolution* **44**:1017–1030.

Vassilieva AB, Galoyan EA, Gogoleva SS, Poyarkov NA Jr. 2014. Two new species of *Kalophrynus* Tschudi, 1838 (Anura: Microhylidae) from the Annamite mountains in southern Vietnam. *Zootaxa* **3796**(3):401–434. DOI:10.11646/zootaxa.3796.3.1

Vassilieva AB, Trounov VL, Poyarkov NA Jr., 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. DOI:10.11646/zootaxa.4247.4.4

Vences M, Thomas M, Bonett RM, Vieites DR. 2005a. Deciphering amphibian diversity through DNA barcoding: chances and challenges. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* **360**(1462):1859–1868. DOI:10.1098/rstb.2005.1717

Vences M, Thomas M, van der Meijden, A, Chiari Y, Vieites DR. 2005b. Comparative performance of the 16S rRNA gene in DNA barcoding of amphibians. *Frontiers in zoology* **2**(1):5.

Vieites DR, Wollenberg KC, Andreone F, Köhler J, Glaw F, Vences M. 2009. Vast underestimation of Madagascar's biodiversity evidenced by an integrative amphibian inventory. *Proceedings of the National Academy of Sciences* **106**(20):8267–8272. DOI:10.1073/pnas.0810821106

Wilkinson JA, Drewes RC, Tatum OL. 2002. A molecular phylogenetic analysis of the family Rhacophoridae with an emphasis on the Asian and African genera. *Molecular Phylogenetics and Evolution* **24**(2):265–273. DOI:10.1016/s1055-7903(02)00212-9

Wu X, Li Y, Zhang H, Yan L, Wu XB. 2014. The complete mitochondrial genome of *Microhyla pulchra* (Amphidia, Anura, Microhylidae). *Mitochondrial DNA*: 154–155. DOI:10.1080/23802359.2016.1144107.

Yan F, Jiang K, Wang K, Jin J-Q, Suwannapoom C, Li C, Vindum JV, Brown RM, Che J. 2016. The Australasian frog family Ceratobatrachidae in China, Myanmar and Thailand: discovery of a new Himalayan forest frog clade. *Zoological Research* **37**(1):7–14.

Zhang P, Zhou H, Chen YQ, Liu YF, Qu LH. 2005. Mitogenomic perspectives on the origin and phylogeny of living amphibians. *Systematic Biology* **54**(3):391–400.
Zweifel RG, Menzies JI, Price D. 2003. Systematics of microhylid frogs, genus *Oreophryne*, from the North Coast Region of New Guinea. *American Museum Novitates* 3415:1–31.

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.

Zweifel RG. 2000. Partition of the Australopapuan microhylid frog genus *Sphenophryne* with descriptions of new species. *Bulletin of the American Museum of Natural History* 253:1–130.
Figure 1

Known distribution of main Asterophryinae lineages and the type locality (green star) of *Siamophryne troglodytes* Gen. et sp. nov. in Kanchanaburi Province, Thailand.

Biogeographic borders: A – the Isthmus of Kra line, the approximate biogeographic border between Sundaland and Indochina; B-1 – the Wallace line (after Huxley, 1868); B-2 – the Wallace line (after Mayr, 1944); C – the Weber line; D – the Lydekker line. The majority of the Asterophryinae genera inhabit Australasia east of the Wallace line (red) and the island of Bali; *Gastrophrynoides* is confined to Sundaland (Borneo and peninsular Malaysia); *Siamophryne troglodytes* Gen. et sp. nov. is known from Indochina.
Figure 2

Bayesian inference dendrogram of Asterophryinae derived from the analysis of 2591 bp of the 12S rRNA - 16S rRNA mtDNA gene fragment.

Voucher specimen IDs and Genbank access numbers are given in Table 2. Sequence of *Rhacophorus schlegelii* is used as an outgroup. Numbers near the branches represent posterior probability (PP) or bootstrap support values (BS, 1000 replicates) for the BI/ML inferences respectively. “A” denotes the subfamily Asterophryinae sensu lato node.

Thumbnails show adult specimens of *Siamophryne troglodytes* Gen. et sp. nov. and *Gastrophrynoides* sp. (Malaysia); photos by N. A. Poyarkov and Yu Lee.
Figure 3

The male holotype of *Siamophryne troglodytes* Gen. et sp. nov. (AUP-00500) in preservative.

(A) Dorsal view; (B) ventral view; (C) lateral view of the head; (D) volar view of the right hand; (E) plantar view of the right foot. Photos by N. A. Poyarkov.

*Note: Auto Gamma Correction was used for the image. This only affects the reviewing manuscript. See original source image if needed for review.*
Figure 4

Morphological details of the male holotype of *Siamophryne troglodytes* Gen. et sp. nov. (AUP-00500) in preservative.

(A) Head in the lateral view; (B) volar view of the right hand; (C) plantar view of the right foot. Scale bar equals 2 mm. Drawings by Valentina D. Kretova.
Figure 5

Male paratype of *Siamophryne troglodytes* Gen. et sp. nov. (ZMMU A-5818) in life in dorsolateral aspect.

Photo by N. A. Poyarkov.
Figure 6

Osteology of *Siamophryne troglodytes* Gen. et sp. nov. (paratype, ZMMU A-5818).

The full skeleton is shown in (A) dorsal, and (B) ventral views; the right forelimb in (C) dorsal, and (D) volar aspects; and the right foot in (E) plantar, and (F) dorsal aspects. Digits numbered I–V. Abbreviations as follows: antbr. – os antebrachii (radius + ulna); carp.d.II-IV – carpale distale F2-F4; centr. – centrale; clav. – clavicle; cor. – coracoid bone; crur. – os cruris (tibia + fibula); fem. – femoral bone; fib. – fibulare; hm. – humeral bone; il. – ilium; mtc.I-IV – metacarpalia F1-F4; mtt.I-V – metatarsalia T1-T5; ph.d.I-IV – finger phalanges F1-F4; ph.d.I-V – toe phalanges T1-T5; pr.p.-m. – processus postero-medialis; prhl. – prehallux; prsac.v. – presacral vertebrae; rad. – radiale; sac.v. – sacral vertebra; sc. – scapula; strn. – sternum; tar.d.II-III – tarsale distale T2-T3; tib. – tibiale; uln. – ulnare; ur. – urostyle.
Figure 7

Head skeleton of *Siamophryne troglodytes* Gen. et sp. nov. (male paratype, ZMMU A-5818).

The skull is shown in (A) dorsal, (B) ventral, (C) lateral, and (D) frontal views. Scale bar equals to 1 mm. Abbreviations as follows: angspl. – angulosplenial; col. – columella; cond.oc. – occipital condylus; dent. – dentary bone; exoc. – exoccipital; fpar. – frontoparietal bone; max. – maxilla; mmk. – mentomeckelian bone; nas. – nasal bone; pmax. – premaxilla; proot. – prootic; psph. – parasphenoid; pter. – pterygoid; qj. – quadratojugal; smax. – septomaxilla; spheth. – sphenethmoid; sq. – squamosal; vom. – vomer.
Figure 8

Tadpole of *Siamophryne troglodytes* Gen. et sp. nov. in life (AUP-00509; Gosner stage 36).

(A) In dorsal and (B) in ventral aspects. Scale bar equals to 5 mm. Photos by N. A. Poyarkov.
Figure 9

Tadpole of *Siamophryne troglodytes* Gen. et sp. nov. in preservative (AUP-00509; Gosner stage 36).

(A) In lateral, (B) in dorsal, and (C) in ventral views. Scale bar equals to 5 mm. Photos by T. Ruangsuwan.

*Note: Auto Gamma Correction was used for the image. This only affects the reviewing manuscript. See original source image if needed for review.*
Figure 10

Breeding habitat of *Siamophryne troglodytes* Gen. et sp. nov. at the type locality – Sai Yok District, Kanchanaburi Province, northern Tenasserim Region, western Thailand.

(A) Entrance to the limestone cave where the frogs were recorded; (B) female *in situ* sitting on the limestone wall of the cave; (C) male *in situ* sitting in a water-filled crevice; (D) female *in situ* on the wall of the cave (photos by M. Sumontha); (E, F) tadpole *in situ* in a water-filled crevice (photos by T. Ruangsuwan).
**Table 1** (on next page)

Primers used in this study.
| Primer name | Primer sequence | Reference          |
|-------------|-----------------|--------------------|
| **12S rRNA** |                 |                    |
| Micro-1F-12stail | ACGCTAAAATGWACCCTAAAAAGT | Nguyen *et al.*, in press |
| Micro-600R-12stail | TAGAGGAGCCTGTTCTATAATCGATTC | Nguyen *et al.*, in press |
| Micro-500F-12stail | CCACTTGAACCCACGACAGCTAGRAMACAA | Nguyen *et al.*, in press |
| Micro-1200R-12stail | AGTAAAGGCGATYAAAAAATTTTCAAAAG | Nguyen *et al.*, in press |
| 12sA-L | AACTGGGATTTAGATACCCCACTAT | Palumbi *et al.*, 1991 |
| R-1169 | GTGGCTGCTTTTAGGCCCACT | Wilkinson *et al.*, 2002 |
| **16S rRNA** |                 |                    |
| L-2188 | AAAGTGGGCTTAAAAGCAGCCA | Matsui *et al.*, 2006 |
| 16H-1 | CTCCGGTCTGACTCAGATCAGTGG | Hedges, 1994 |
### Table 2 (on next page)

Specimens and sequences of *Siamophryne troglodytes* Gen. et sp. nov. and outgroup representatives of Microhylidae and Rhacophoridae used in molecular analyses.

AN – GenBank accession numbers.
| Group              | GenBank AN               | Species                                      | Specimen ID | Reference         |
|--------------------|--------------------------|----------------------------------------------|-------------|-------------------|
| Asterophryinae     | DQ283195                 | *Aphantophryne pansa*                       | ABTC 49605  | Frost et al., 2006|
| Asterophryinae     | FR832625; FR832642       | *Asterophrys turpica*                       | ZMB 70537   | Günther et al., 2010|
| Asterophryinae     | JN048979; JN049004       | *Austrochaperina guttata*                   | LSUMZ 95008 | Rittmeyer et al., 2012|
| Asterophryinae     | KC822485                 | *Austrochaperina sp.*                       | BSFS 11377  | Blackburn et al., 2013|
| Asterophryinae     | EU100119; EU100235       | *Barygenys exsul*                          | BPBM 20128  | Köhler & Günther, 2008|
| Asterophryinae     | KM509105                 | *Callulops robustus*                        | PT-506      | Peloso et al., 2015|
| Asterophryinae     | DQ283207                 | *Cheirophryne sp.*                          | ABTC 47720  | Frost et al., 2006|
| Asterophryinae     | DQ283206                 | *Copihula sp.*                              | ABTC 47881  | Frost et al., 2006|
| Asterophryinae     | AB634647; AB634705       | *Gastrophrynoides immaculatus*              | UKMHC 279   | Matsui et al., 2011|
| Asterophryinae     | DQ283209                 | *Genophryne thomsoni*                       | ABTC 49624  | Frost et al., 2006|
| Asterophryinae     | JX119248; JX119392       | *Hylophorus rufescens*                     | LSUMZ 94943  | Oliver et al., 2013|
| Asterophryinae     | DQ283199                 | *Liophryne rhododactyla*                    | ABTC 49566  | Frost et al., 2006|
| Asterophryinae     | JN048989; JN049014       | *Mantophryne lateralis*                     | LSUMZ 92102 | Rittmeyer et al., 2012|
| Asterophryinae     | KM509160                 | *Metamagnusia slateri*                      | PT-507      | Peloso et al., 2015|
| Asterophryinae     | MG682555                 | *Siamophryne troglodytes Gen. et sp. nov.* | AUP-00500  | this work         |
| Asterophryinae     | MG682556                 | *Siamophryne troglodytes Gen. et sp. nov.* | AUP-00501  | this work         |
| Asterophryinae     | MG682557                 | *Siamophryne troglodytes Gen. et sp. nov.* | AUP-00502  | this work         |
| Asterophryinae     | MG682558                 | *Siamophryne troglodytes Gen. et sp. nov.* | AUP-00503  | this work         |
| Asterophryinae     | MG682559                 | *Siamophryne troglodytes Gen. et sp. nov.* | AUP-00509  | this work         |
| Asterophryinae     | MG682553                 | *Siamophryne troglodytes Gen. et sp. nov.* | ZMMU A-5818 | this work         |
| Asterophryinae     | MG682554                 | *Siamophryne troglodytes Gen. et sp. nov.* | ZMMU A-5819 | this work         |
| Asterophryinae     | FR832634; FR832635       | *Oninia senglaubi*                          | ZMB 74608   | Günther et al., 2010|
| Asterophryinae     | KC822488                 | *Oreophryne anulata*                        | PNCMNH 1366 | Blackburn et al., 2013|
| Asterophryinae     | DQ283194                 | *Oreophryne brachypus*                      | ABTC 50081  | Frost et al., 2006|
| Asterophryinae     | AB634651; AB634709       | *Oreophryne monticola*                      | MZBAmp 16265 | Matsui et al., 2011|
| Asterophryinae     | KC822489                 | *Oreophryne variabilis*                     | TNHC 58922  | Blackburn et al., 2013|
| Asterophryinae     | EU100323; EU100207       | *Oxydactyla crassa*                         | BPBM 17061  | Köhler & Günther, 2008|
| Asterophryinae     | JN048996; JN049021       | *Paedophryne amauensis*                     | BPBM 31882  | Rittmeyer et al., 2012|
| Asterophryinae     | FR832653; FR832636       | *Pseudocallulops eurydactylus*             | ZMB 70534   | Günther et al., 2010|
| Asterophryinae     | JX119386; JX119242       | *Sphenophryne cornuta*                      | LSUMZ 94793  | Oliver et al., 2013|
| Asterophryinae     | FR832655; FR832638       | *Xenorhina cf. oxycephala*                  | ZMB 74628   | Günther et al., 2010|
| Asterophryinae     | KM509212                 | *Xenorhina obesa*                           | PT-529      | Peloso et al., 2015|
| Chaperininae       | AB598318; AB598342       | *Chaperina fusca*                           | BORN 8478   | Matsui et al., 2011|
| Dyscophinae        | AB634648; AB634706       | *Dyscophus guineti*                         | KUHE 33150  | Matsui et al., 2011|
| Dyscophinae        | AB634649; AB634707       | *Dyscophus insularis*                       | KUHE 35001  | Matsui et al., 2011|
| Family               | GenBank Accession Numbers | Species Name                          | Accession号 | Reference                      |
|---------------------|---------------------------|---------------------------------------|-------------|-------------------------------|
| Gastrophryninae     | AB634650; AB634708        | *Gastrophryne olivacea*               | KUHE 33224  | Matsui et al., 2011           |
| Kalophryninae       | AB634642; AB634700        | *Kalophrynus pleurostigma*            | MZBAmp 15295| Matsui et al., 2011           |
| Kalophryninae       | AB634645; AB634703        | *Kalophrynus subterrestris*           | KUHE 53145  | Matsui et al., 2011           |
| Melanobatrachinae   | KM509159                 | *Melanobatrachus indicus*             | IND-18      | Peloso et al., 2015           |
| Microhylinae        | AB201182; AB201193        | *Glyphoglossus molossus*              | KUHE 35182  | Matsui et al., 2011           |
| Microhylinae        | AB634626; AB634684        | *Glyphoglossus yunnanensis*           | KUHE 44148  | Matsui et al., 2011           |
| Microhylinae        | KP682314                 | *Kaloula rugifera*                    | -           | Deng et al., 2015             |
| Microhylinae        | AB634634; AB634692        | *Metaphrynella pollicaris*            | KUZ-21655   | Matsui et al., 2011           |
| Microhylinae        | AB634600; AB634658        | *Microhyla annectens*                 | -           | Matsui et al., 2011           |
| Microhylinae        | DQ512876                 | *Microhyla fissipes*                  | -           | unpublished                   |
| Microhylinae        | NC006406                 | *Microhyla heymonsi*                  | -           | Zhang et al., 2005            |
| Microhylinae        | AB303950                 | *Microhyla okinavensis*               | -           | Igawa et al., 2008            |
| Microhylinae        | AB634616; AB634674        | *Microhyla petrigena*                 | -           | Matsui et al., 2011           |
| Microhylinae        | NC024547                 | *Microhyla pulchra*                   | -           | Wu et al., 2014               |
| Microhylinae        | AB598317; AB598341        | *Micryletta inornata*                 | KUHE 20497  | Matsui et al., 2011           |
| Microhylinae        | AB634638; AB634696        | *Micryletta steinegeri*              | KUHE 35937  | Matsui et al., 2011           |
| Microhylinae        | AB634636; AB634694        | *Phrynella pulchra*                   | UKMHC 820   | Matsui et al., 2011           |
| Microhylinae        | AB634633; AB634691        | *Uperodon taprobanicus*              | KUHE 37252  | Matsui et al., 2011           |
| Phrynomerinae       | AB634652; AB634710        | *Phrynomantis bifasciatus*           | KUHE 33277  | Matsui et al., 2011           |
| Scaphiophryninae    | AB634653; AB634711        | *Scaphiophryne gottlebei*             | KUHE 34977  | Matsui et al., 2011           |
| Rhacophoridae       | AB202078                 | *Rhacophorus schlegelii*              | -           | Sano et al., 2005             |
Genetic divergence of *Siamophryne troglodytes* Gen. et sp. nov.

Uncorrected $p$-distances (percentage) between 12S rRNA - 16S rRNA sequences of *Siamophryne troglodytes* Gen. et sp. nov. and other Microhylidae genera included in phylogenetic analyses (below the diagonal line), and standard error estimates (above the diagonal line). The mean uncorrected $p$-distances within those genera for which more than one specimen was examined are shown in the diagonal and shaded with grey.
| Genus               | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
|---------------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| *Siamophryne*       | 0.0| 1.4| 1.6| 1.6| 1.5| 1.7| 1.5| 1.6| 1.5| 1.5| 1.4| 1.4| 1.5| 1.5| 1.5| 1.3| 1.5| 1.7| 1.6| 1.5| 2.0|
| *Gastrophrynoidea*  | 15.6| — | 1.5| 1.4| 1.4| 1.5| 1.5| 1.6| 1.3| 1.4| 1.3| 1.5| 1.4| 1.5| 1.2| 1.4| 1.4| 1.5| 1.7| 1.7|
| *Aphantophrine*     | 15.9| 16.0| — | 1.3| 1.1| 1.5| 1.3| 1.4| 1.3| 1.4| 1.2| 1.4| 1.3| 1.4| 0.9| 1.4| 1.5| 1.4| 1.4| 1.5|
| *Asterophrys*       | 17.5| 18.0| 15.0| — | 1.2| 1.5| 1.4| 1.4| 1.4| 1.5| 1.5| 1.5| 1.4| 1.5| 1.1| 1.3| 1.1| 1.4| 1.6| 1.2| 1.4| 1.4|
| *Austrochaperina*   | 16.3| 17.3| 12.7| 14.4| 12.7| 1.2| 1.2| 1.3| 1.3| 1.2| 1.1| 1.1| 1.2| 1.3| 0.9| 1.2| 1.3| 1.3| 1.1| 1.3|
| *Barygenys*         | 20.6| 18.1| 15.6| 15.7| 14.7| — | 1.7| 1.6| 1.5| 1.4| 1.5| 1.5| 1.4| 1.5| 1.2| 1.5| 1.6| 1.7| 1.4| 1.6|
| *Callulops*         | 17.4| 16.1| 13.5| 13.4| 14.0| 16.8| — | 1.4| 1.3| 1.5| 1.3| 1.3| 1.2| 1.2| 1.4| 1.2| 1.3| 1.5| 1.4| 1.4| 1.5|
| *Choerophryn*       | 19.4| 19.2| 15.7| 17.9| 16.9| 18.9| 16.1| — | 1.3| 1.6| 1.3| 1.5| 1.4| 1.6| 1.3| 1.4| 1.2| 1.5| 1.5| 1.4| 1.7|
| *Cophixalis*        | 17.0| 17.6| 13.4| 15.7| 15.2| 15.1| 14.7| 15.9| — | 1.5| 1.3| 1.4| 1.4| 1.3| 1.2| 1.4| 1.1| 1.1| 1.5| 1.4| 1.2| 1.5|
| *Copiula*           | 16.8| 19.6| 15.5| 16.0| 14.0| 18.9| 17.5| 19.1| 17.5| — | 1.5| 1.5| 1.5| 1.4| 1.5| 1.4| 1.6| 1.6| 1.4| 1.5| 1.8|
| *Genyophryn*        | 16.0| 17.2| 12.5| 16.7| 14.3| 15.2| 15.9| 15.2| 14.1| 17.2| — | 1.4| 1.4| 1.3| 1.5| 1.5| 1.2| 1.6| 1.5| 1.5| 1.3| 1.8|
| *Hyphorbus*         | 16.5| 18.2| 15.7| 16.4| 16.0| 17.9| 12.9| 16.2| 16.4| 16.3| 16.4| — | 1.3| 1.3| 1.3| 1.4| 1.1| 1.3| 1.5| 1.4| 1.6| 1.9|
| *Liophryn*          | 14.8| 15.9| 11.7| 13.0| 12.4| 14.5| 11.4| 16.4| 13.2| 15.5| 14.2| 14.0| — | 1.2| 1.2| 1.4| 1.0| 1.3| 1.4| 1.4| 1.2| 1.5|
| *Mantophryn*        | 16.5| 17.4| 15.7| 14.4| 13.9| 17.1| 12.7| 16.8| 14.6| 16.2| 14.9| 12.9| — | 1.2| 1.2| 1.1| 1.3| 1.3| 1.3| 1.4| 1.7|
| *Metamagnusia*      | 16.6| 16.5| 14.5| 7.3| 13.8| 16.6| 12.9| 16.6| 14.0| 15.0| 15.2| 15.4| 12.7| 12.2| — | 1.2| 1.0| 1.3| 1.4| 1.2| 1.5|
| *Oninia*            | 16.7| 17.7| 14.7| 16.7| 16.6| 17.9| 17.3| 16.9| 17.4| 18.1| 16.4| 16.7| 15.1| 16.2| 14.7| — | 1.1| 1.5| 1.7| 1.3| 1.6|
| *Oreophryn*         | 17.8| 17.7| 14.2| 15.7| 14.5| 16.2| 15.5| 17.7| 16.1| 16.9| 16.4| 16.2| 13.5| 16.4| 14.7| 17.2| 15.1| 1.0| 1.1| 1.1| 1.2|
| *Ozydactyla*        | 16.1| 15.7| 14.4| 13.7| 12.2| 15.4| 12.2| 15.9| 11.2| 16.5| 14.7| 14.7| 9.2| 13.7| 12.7| 15.7| 14.4| — | 1.4| 1.5| 1.1| 1.7|
| *Paedophryn*        | 17.9| 17.7| 16.2| 16.7| 15.4| 16.9| 17.9| 20.1| 17.1| 18.4| 17.3| 19.4| 16.6| 17.4| 14.7| 18.8| 16.5| 15.9| — | 1.4| 1.4| 1.6|
| *Pseudocallulops*   | 17.5| 17.7| 15.7| 12.9| 15.6| 18.2| 14.5| 16.6| 16.6| 14.8| 16.2| 15.5| 15.1| 12.7| 16.6| 16.8| 16.0| 16.2| — | 1.4| 1.5|
| *Sphenophryn*       | 17.2| 19.4| 14.2| 15.2| 14.2| 15.4| 13.9| 17.6| 15.1| 16.5| 14.7| 16.7| 10.2| 13.5| 13.2| 16.4| 15.1| 11.2| 16.2| 17.2| — | 1.4|
| *Xenorhina*         | 19.5| 20.4| 16.4| 14.9| 15.6| 18.9| 15.6| 19.5| 19.0| 17.4| 19.4| 18.1| 15.9| 16.8| 14.1| 19.0| 18.3| 16.9| 18.7| 15.5| 16.7| 14.0|
**Table 4** (on next page)

Measurement data for *Siamophryne troglodytes* Gen. et sp. nov. type series.

Abbreviations: m = male, f = female, SD = standard deviation, n = number of measured specimens. For other abbreviations see Materials and methods. All measurements are in mm.
| Museum ID | Paratype males | Paratype females |
|-----------|----------------|-----------------|
| Sex       | Characters     | Characters     |                  |
| m         | AUP-00501      | f               |                  |
| m         | AUP-00502      | m               |                  |
| m         | AUP-00503      | f               |                  |
| m         | AUP-00504      | m               |                  |
| m         | NAP-00651      | f               |                  |
| Mean±SD   | Min–Max        | Mean±SD        | Min–Max         |
| AUP-00505 |                |                |                  |
| AUP-00506 |                |                |                  |
| AUP-00507 |                |                |                  |
| AUP-00508 |                |                |                  |
| NAP-00652 |                |                |                  |

1. SVL: 22.1 ± 0.2 (19.4–24.9)
2. HL: 6.7 ± 0.2 (6.3–7.7)
3. SL: 2.7 ± 0.2 (2.4–2.9)
4. EL: 3.0 ± 0.2 (2.4–2.9)
5. N-EL: 1.9 ± 0.2 (1.5–2.0)
6. HW: 7.1 ± 0.2 (6.6–6.8)
7. IND: 2.3 ± 0.2 (1.9–2.4)
8. IOD: 2.2 ± 0.2 (1.4–2.4)
9. UEW: 1.8 ± 0.2 (1.5–1.8)
10. FLL: 15.4 ± 0.2 (13.9–16.2)
11. LAL: 10.4 ± 0.2 (9.6–11.9)
12. HAL: 5.6 ± 0.2 (4.9–6.4)
13. IPTL: 1.0 ± 0.2 (0.9–1.0)
14. OPTL: 1.1 ± 0.2 (1.0–1.1)
15. HLL: 37.1 ± 0.2 (35.4–38.0)
16. TL: 10.8 ± 0.2 (10.1–12.4)
17. FL: 9.6 ± 0.2 (9.1–10.1)
18. IMTL: 0.9 ± 0.2 (0.8–1.0)
19. 1TOEL: 2.0 ± 0.2 (1.9–2.0)
20. 2TOEL: 4.7 ± 0.2 (4.6–4.9)
21. 3TOEL: 7.4 ± 0.2 (7.5–8.1)
22. 4TOEL: 9.6 ± 0.2 (9.7–10.9)
23. 7TOEL: 7.3 ± 0.2 (6.9–7.6)
24. 1FW: 1.0 ± 0.2 (0.7–1.0)
25. 2FDW: 1.2 ± 0.2 (1.3–1.4)
26. 3FDW: 1.3 ± 0.2 (1.2–1.2)
27. 4FDW: 1.3 ± 0.2 (1.4–1.4)
28. 1TDW: 0.2 ± 0.2 (0.2–0.2)
29. 2TDW: 0.2 ± 0.2 (0.2–0.2)
30. 3TDW: 0.3 ± 0.2 (0.3–0.3)
31. 4TDW: 0.5 ± 0.2 (0.5–0.6)
32. 5TDW: 0.3 ± 0.2 (0.3–0.3)
33. 1FLO: 1.9 ± 0.2 (2.2–2.1)
34. 2FLO: 3.7 ± 0.2 (3.5–4.8)

Mean±SD: Mean ± Standard Deviation
Min–Max: Minimum–Maximum
|     | 35) 3FLO | 36) 4FLI | 37) TMP | 38) TEY |
|-----|-----------|-----------|---------|---------|
|     | 5.6       | 6.4       | 5.0     | 6.9     | 6.7 | 3.2 | 5.6±1.0 | (3.2–6.9) |
|     |           |           |         |         |     |     | 6.6 | 6.7 | 5.2 | 6.2 | 4.3 | 5.8±0.8 | (4.3–6.7) |
|     | 4.5       | 4.9       | 3.9     | 6.0     | 6.2 | 2.6 | 4.7±1.0 | (2.6–6.2) |
|     |           |           |         |         |     |     | 5.5 | 4.8 | 3.2 | 5.4 | 3.1 | 4.4±1.0 | (3.1–5.5) |
|     | 1.2       | 1.5       | 1.1     | 1.4     | 1.3 | 1.1 | 1.3±0.1 | (1.1–1.5) |
|     |           |           |         |         |     |     | 1.4 | 1.5 | 1.6 | 1.1 | 1.6 | 1.4±0.2 | (1.1–1.6) |
|     | 0.8       | 0.7       | 0.6     | 0.6     | 0.7 | 0.6 | 0.7±0.1 | (0.6–0.8) |
|     |           |           |         |         |     |     | 0.6 | 0.7 | 1.0 | 0.6 | 0.6 | 0.7±0.1 | (0.6–1.0) |