Asian Herpetological Research 2018, 9(4): 224–239
DOI: 10.16373/j.cnki.ahr.180072

ORIGINAL ARTICLE

A New Species of the Asian Toad Genus *Megophrys sensu lato* (Amphibia: Anura: Megophryidae) from Guizhou Province, China

Shize LI[1], Ning XU[1], Jing LIU[1], Jianping JIANG[2], Gang WEI[3*#] and Bin WANG[1,2*#]

[1] *Department of Food Science and Engineering*, *Maotai University*, *Renhuai* 564500, *China*
[2] *CAS Key Laboratory of Mountain Ecological Restoration and Bioresource Utilization and Ecological Restoration Biodiversity Conservation Key Laboratory of Sichuan Province*, *Chengdu Institute of Biology* (*CIB*), *Chinese Academy of Sciences* (*CAS*), *Chengdu* 610041, *China*
[3] *Biodiversity Conservation Key Laboratory*, *Guiyang College*, *Guiyang* 550002, *China*

Abstract We describe a new species of the genus *Megophrys sensu lato* from Guizhou Province, China. Molecular phylogenetic analyses based on mitochondrial DNA and nuclear DNA sequences all strongly supported the new species as an independent lineage in *Megophrys* (*Panophrys*) clade. The new species is distinguished from its congeners by a combination of the following morphological characteristics: (1) small body size with SVL < 38.8 mm in male and SVL < 42.3 mm in female; (2) vomerine teeth absent; (3) tongue not notched behind; (4) a small horn-like tubercle at the edge of each upper eyelid; (5) tympanum distinctly visible, rounded; (6) two metacarpal tubercles in hand; (7) relative finger lengths: II < I < V < III; (8) toes with rudimentary webbing at bases; (9) heels overlapping when thighs are positioned at right angles to the body; (10) tibiotarsal articulation reaching the level between tympanum to eye when leg stretched forward; (11) an internal single subgular vocal sac in male; (12) in breeding male, the nuptial pads with black nuptial spines on the dorsal bases of the first and second fingers.

Keywords *Megophrys sensu lato*, new species, taxonomy, phylogenetic analysis, Guizhou Province, China

1. Introduction

Megophryidae Bonaparte, 1850 (Amphibia: Anura) is a large group of Asian toads (Frost, 2018). Delorme et al. (2006) classified this family into three subfamilies, i.e. Leptobrachiinae Delorme, Dubois, Grosjean and Ohler, 2006, Leptolalaginae Dubois, 1983 and Megophryinae Bonaparte, 1850. Frost (2018) classified all recognized species of the subfamily Megophryinae into one genus *Megophrys sensu lato* Kuhl and Van Hasselt, 1822. The Asian horned toad genus *Megophrys sensu lato* is widely distributed in the eastern and central China, throughout southeastern Asia, and extending to the islands of the Sunda Shelf and the Philippines (Frost, 2018).

Although *Megophrys sensu lato* has been widely recognized as a monophyletic group by a series of phylogenetic studies (e.g. Chen *et al.*, 2017; Mahony *et al.*, 2017; Liu *et al*., 2018), the generic and/or subgeneric classifications in this group are still under intense debate (Tian and Hu, 1983; Dubois,1987 "1986"; Rao and Yang, 1997; Lathrop, 1997; Dubois and Ohler, 1998; Delorme *et al*., 2006; Fei and Ye, 2016; Chen *et al*., 2017; Mahony *et al*., 2017; Liu *et al*., 2018; Munir *et al*., 2018). Fei and Ye (2016) classified 36 Chinese species of the subfamily Megophryinae (members of *Megophrys sensu lato*) into six genera, i.e. *Liuophrys* Fei, Ye and Jiang, 2016, *Atympanophrys* Tian and Hu, 1983 containing three subgenera (*Atympanophrys*, *Borealophrys* Fei, Ye and Jiang, 2016 and *Gigantophrys* Fei, Ye and Jiang, 2016), *Boulenophrys* Fei, Ye and Jiang, 2016, *Xenophrys* Günther, 1864 containing two subgenera (*Tianophrys* Fei and Ye, 2016 and *Xenophrys*),

# Both authors contributed equally to this work.
* Corresponding authors: Dr. Bin WANG, from Chengdu Institute of Biology, Chinese Academy of Sciences, and Prof. Gang WEI, from Guiyang College, with his research focusing on taxonomy and evolution of amphibians and reptiles.
E-mail: wangbin@cib.ac.cn (B. WANG); wg198553@126.com (G. WEI).
Received: 9 October 2018 Accepted: 1 December 2018

Ophyrophryne Boulenger, 1908 and *Brachytarsophrys* Tian and Hu, 1983, mainly based on morphology. Chen *et al*. (2017) suggested that the group contained five genera, i.e. *Atympanophrys*, *Megophrys*, *Xenophrys*, *Ophyrophryne* and *Brachytarsophrys*, based on molecular phylogenetics. Mahony *et al*. (2017) classified all members of Megophryinae into a single genus *Megophrys* including seven subgenera (*Megophrys*, *Xenophrys*, *Panophrys* Rao and Yang, 1997, *Atympanophrys*, *Ophyrophryne*, *Pelobatrachus* Beddard, 1908 "1907" and *Brachytarsophrys*) based on molecular phylogenetics and morphological comparisons. Liu *et al.* (2018) indicated *Panophrys* as a large monophyletic group through comprehensive sampling. Nevertheless, to now, it is likely that there was inadequate morphological crude to corroborate molecular phylogenetic conclusions.

Whatever, *Megophrys sensu lato* belongs to one of the most diverse groups of amphibians with currently 83 recognized species (Frost, 2018), and as note, 29 species of *Megophrys sensu lato* were described in the last decade (Deuti *et al.*, 2017; Fei *et al.*, 2009; Li *et al.*, 2014; Mahony, 2011; Mahony *et al.*, 2011; Mahony *et al*., 2013; Mahony *et al*., 2018; Mo *et al.*, 2010; Munir *et al.*, 2018; Nikolay *et al.*, 2017; Orlov *et al.*, 2015; Tapley *et al.*, 2017; Tapley *et al.*, 2018; Wang *et al.*, 2012; Wang *et al*., 2014; Wang *et al*., 2017a, b; Yang *et al.*, 2018; Zhang *et al.*, 2017; Zhao *et al.*, 2014). And even so, molecular phylogenetic studies still surprisingly indicated mass of cryptic species in the group, for example, 20 cryptic species were suggested by Chen *et al.* (2017), and 43 cryptic species were proposed just only in *Megophrys* (*Panophrys*) by Liu *et al.* (2018). Obviously, these cryptic species need to be verified and described.

During field surveys in the Leigong Mountains, Leishan County, Guizhou Province, China, some *Megophrys sensu lato* specimens were collected from the montane forests. This kind of specimens has been identified as *M. minor* (Wu *et al*., 1986), but previous molecular studies indicated the population as a cryptic species [*Megophrys* sp8 in Chen *et al*., 2017; *Megophrys* (*P.*) sp34 in Liu *et al*., 2018]. Our molecular phylogenetic analyses and morphological comparisons also supported it as a new taxon of *Megophrys sensu lato* and could be classified it into subgenus *Megophrys* (*Panophrys*). Therefore, we describe it herein as a new species.

2. Materials and Methods

2.1. Sampling Two adult females and ten adult males of the new taxon (for voucher information see Table S1) were collected from the mountain streams in the Leigong Mountain, Leishan County, Guizhou Province, China (Figure S1). Six tadpoles (voucher numbers: CIBLS20171101001–CIBLS20171101006) of the new taxon were also collected in a mountain stream where the new taxon was found. They were identified as the new taxon because they were almost identical in morphology and one representative of them was genetically close to the adult specimens of the new taxon (see the results). The stages of tadpoles were identified following Gosner (1960). All specimens were fixed in 10% buffered formalin for one day, and then later transferred to 70% ethanol. Tissue samples were taken and preserved separately in 95% ethanol prior to fixation. The specimens were deposited in Chengdu Institute of Biology, Chinese Academy of Sciences (CIB, CAS).

2.2. Molecular data and phylogenetic analyses Three adult specimens and one tadpole of the new taxon were included in molecular analyses (for voucher information see Table S2). Total DNA was extracted using a standard phenol-chloroform extraction protocol (Sambrook *et al.*, 1989). Two fragments of the mitochondrial genes encoding16S rRNA and cytochrome oxidase subunit I (COI) were amplified using the primers in Simon *et al*. (1994) and Che *et al*. (2012), respectively. They were amplified under the following conditions: 35 cycles at 95 °C for 4 min, 95 °C for 1 min, 52 °C (for 16S rRNA)/46 °C (for COI) for 40 second, and 72 °C for 1 min followed by a 10 min extension at 72 °C. The nuclear gene fragments encoding brain-derived neurotrophic factor (BDNF) and recombination activating gene 1 (RAG1) were amplified using the primers and protocols in Vieites *et al*. (2007) and Shen *et al.* (2013). All primers were presented in Table S3. PCR products were purified with spin columns and then were sequenced with primers same used in PCR. Sequencing was conducted using an ABI3730 automated DNA sequencer in Shanghai DNA BioTechnologies Co., Ltd. (Shanghai, China). All sequences were deposited in GenBank (for GenBank Accession numbers see Table S2).

For molecular analyses, the available sequence data for all related species of the genus *Megophrys sensu lato* were downloaded from GenBank, primarily from previous studies (Chen *et al*., 2017; Liu *et al*., 2018). For phylogenetic analyses, corresponding sequences of one *Leptolalax oshanensis* and one *Leptobrachium boringii* were downloaded and used as outgroups according to Chen *et al*. (2017). GenBank Accession numbers of all sequences were shown in Table S2.

Sequences were assembled and aligned using the

Clustalw module in BioEdit 7.0.9.0 (Hall, 1999) with default settings. Alignments were checked by eye and revised manually if necessary. To avoid bias in alignments, GBLOCKS 0.91.b (Castresana, 2000) with default settings was used to extract regions of defined sequence conservation from the length-variable 16S gene fragments. Non-sequenced fragments were defined as missing loci.

Gene trees were reconstructed for the mitochondrial genes concatenated data and nuclear genes concatenated data, respectively. Phylogenetic analyses were conducted using maximum likelihood (ML) and Bayesian Inference (BI) methods, implemented in PhyML 3.0 (Guindon *et al*., 2010) and MrBayes 3.12 (Ronquist and Huelsenbeck, 2003), respectively. To avoid under- or over-parameterization (Lemmon and Moriarty, 2004; McGuire *et al*., 2007), the best partition scheme and the best evolutionary model for each partition were chosen for the phylogenetic analyses using PARTITIONFINDER 1.1.1 (Robert *et al*., 2012). In the analyses, 16S, each codon position of the protein-coding genes (COI, RAG1 and BNDF) were defined, and Bayesian Inference Criteria (BIC) was used. As a result, the analyses selected the best partition scheme (i.e. 16S gene/each codon position of COI gene) and the TrN+I+G model for each partition for mitochondrial DNA dataset, and as well, selected the best partition scheme (i.e. each codon position of RAG1 and BNDF genes) and the TrN+I+G as the best model for the second codon position of nuclear genes and the GTR+G +I model as the best model for the other two codon position of RAG1 and BNDF genes. For the ML tree, branch supports were drawn from 10000 non-parametric bootstrap replicates. In BI analyses, two runs each with four Markov chains were run for 60 million generations with sampling every 1000 generations. The first 25% of generations were removed as the "burn-in" stage followed by calculation of Bayesian posterior probabilities and the 50% majority-rule consensus of the post burn-in trees sampled at stationarity. Finally, pairwise COI gene sequence divergence with uncorrected *p*-distance model was estimated using MEGA 6.06 (Tamura *et al.*, 2011) to determine the genetic distance between *Megophrys sensu lato* species.

2.3. Morphological comparisons All twelve adult specimens and six tadpole specimens of the new taxon collected in this work were measured. The terminology and methods followed Fei *et al*. (2009). Measurements were taken with a dial caliper to 0.1 mm. Nineteen morphometric characters of adult specimens were measured: SVL, snout-vent length (distance from the tip of the snout to the posterior edge of the vent); HDL, head length (distance from the tip of the snout to the articulation of jaw); HDW, maximum head width (greatest width between the left and right articulations of jaw); SL, snout length (distance from the tip of the snout to the anterior corner of the eye); ED, eye diameter (distance from the anterior corner to the posterior corner of the eye); IOD, interorbital distance (minimum distance between the inner edges of the upper eyelids); IND, internasal distance (minimum distance between the inner margins of the external nares); TYD, maximal tympanum diameter; LAL, length of lower arm and hand (distance from the elbow to the distal end of the Finger IV); LW, lower arm width (maximum width of the lower arm); FIL, first finger length (distance from base to tip of finger I); FIIL, second finger length (distance from base to tip of finger II); FIIIL, third finger length (distance from base to tip of finger III); FIVL, fourth finger length(distance from base to tip of finger IV); THL, thigh length (distance from vent to knee); TL, tibia length (distance from knee to tarsus); TW, maximal tibia width; TFL, length of foot and tarsus (distance from the tibiotarsal articulation to the distal end of the Toe IV); and FL, foot length (distance from tarsus to the tip of fourth toe). A total of 10 morphometric characters of larvae were measured: TOL, total length; SVL, snout-vent length; BH, maximum body height; BW, maximum body width; SL, snout length (distance from the anterior corner of the eye to the tip of the snout); SS, snout to spiraculum (distance from spiraculum to the tip of the snout); MW, mouth width (distance between two corners of mouth); TBW, maximum width of tail base; TAL, tail length (distance from base of vent to the tip of tail); and TH, tail height (maximum height between upper and lower edges of tail).

We compared morphological characters of the new taxon with other *Megophrys sensu lato* species. Comparative data were obtained from the literature for *M. aceras* (Boulenger, 1903), *M. actuta* (Li *et al.*, 2014), *M. ancrae* (Mahony *et al.*, 2013), *M. auralensis* (Ohler *et al*., 2002), *M. baluensis* (Boulenger, 1899a), *M. baolongensis* (Ye *et al*., 2007), *M. binchuanensis* (Ye and Fei, 1995), *M. binlingensis* (Fei *et al.*, 2009), *M. boettgeri* (Boulenger, 1899b), *M. brachykolos* (Inger and Romer, 1961), *M. carinense* (Boulenger, 1889), *M. caudoprocta* (Shen, 1994), *M. cheni* (Wang and Liu, 2014), *M. chuananensis* (Fei *et al.*, 2001), *M. damrei* (Mahony, 2011), *M. daweimontis* (Rao and Yang, 1997), *M. dringi* (Inger *et al.*, 1995), *M. edwardinae* (Inger, 1989), *M. elfina* (Poyarkov *et al.*, 2017), *M. fansipanensis* (Tapley *et al*., 2018), *M. feae* (Boulenger, 1887), *M. feii* (Yang

et al., 2018), *M. flavipunctata* (Mahony *et al*., 2018), *M. gerti* (Ohler, 2003), *M. gigantica* (Liu *et al.*, 1960), *M. glandulosa* (Fei *et al.*,1990), *M. hansi* (Ohler, 2003), *M.himalayana* (Mahony *et al*., 2018), *M.hoanglienensis* (Tapley *et al*., 2018), *M. huangshanensis* (Fei and Ye, 2005), *M. insularis* (Wang *et al*., 2017a), *M. intermedia* (Smith, 1921), *M. jingdongensis* (Fei and Ye, 1983), *M. jinggangensis* (Wang *et al*., 2012), *M. kobayashii* (Malkmus and Matsui, 1997), *M. koui* (Mahony *et al*., 2017), *M. kuatunensis* (Pope, 1929), *M. lancip* (Munir *et al*., 2018), *M. latidactyla* (Orlov *et al.*, 2015), *M. lekaguli* (Stuart *et al.*, 2006), *M. liboensis* (Zhang *et al*., 2017), *M. lini* (Wang *et al*., 2014), *M. lishuiensis* (Wang *et al.*, 2017b), *M. longipes* (Boulenger,1886), *M. major* (Boulenger, 1908), *M. mangshanensis* (Fei *et al.*,1990), *M. maosonensis* (Bourret, 1937), *M. medogensis* (Fei *et al.*, 1983), *M. megacephala* (Mahony *et al.*, 2011), *M. microstoma* (Boulenger, 1903), *M. minor* (Stejneger, 1926), *M. montana* (Kuhl and Van Hasselt, 1822), *M.monticola* (Günther, 1864), *M. nasuta* (Schlegel, 1858), *M. nankiangensis* (Hu *et al.*,1966), *M. obesa* (Li *et al.*, 2014), *M. omeimontis* (Liu, 1950), *M.oreocrypta* (Mahony *et al*., 2018), *M. oropedion* (Mahony *et al*., 2013), *M. pachyproctus* (Huang and Fei, 1981), *M. palpebralespinosa* (Bourret, 1937), *M. parallela* (Inger and Iskandar, 2005), *M. parva* (Boulenger, 1893), *M. periosa* (Mahony *et al*., 2018), *M. popei* (Zhao *et al.*, 2014), *M. robusta* (Boulenger, 1893), *M. rubrimera* (Tapley *et al.*, 2017), *M. sangzhiensis* (Jiang *et al.*, 2008), *M. serchhipii* (Mathew and Sen, 2007), *M. shapingensis* (Liu, 1950), *M. shuichengensis* (Tian and Sun, 1995), *M. spinata* (Hu *et al.*, 1973), *M. stejnegeri* (Taylor, 1920), *M. synoria* (Stuart *et al.*, 2006), *M. takensis* (Mahony, 2011), *M. tuberogranulata* (Mo *et al.*, 2010), *M. vegrandis* (Mahony *et al.*, 2013), *M. wawuensis* (Fei *et al.*, 2001), *M. wuliangshanensis* (Ye and Fei, 1995), *M. wushanensis* (Ye and Fei, 1995), *M. zhangi* (Ye and Fei, 1992) and *M. zunhebotoensis* (Mathew and Sen, 2007).

2.4. Bioacoustics analyses The advertisement calls of the new taxon from Leigong Mountain, Guizhou Province, China, were recorded in the field. SONY PCM-D50 digital sound recorder was used to record within 20 cm of the calling individuals. The sound files in wave format were resampled at 48 kHz with sampling depth 24 bits. The sonograms and waveforms were generated by WaveSurfer software (Sjöander and Beskow, 2000) from which all parameters and characters were measured. Ambient temperature was taken by a digital hygrothermograph.

3. Results

3.1. Phylogenetic analyses Aligned sequence matrix of 16S+COI and RAG1+BNDF contained 1095 bp and 1672 bp, respectively. ML and BI trees of the mitochondrial DNA dataset presented almost consistent topology (Figure 1), and as well, ML and BI trees of the nuclear DNA dataset showed almost identical topology (Figure 2), though relationships of some lineages were unresolved (Figures 1 and 2). *Megophrys sensu lato* was strongly supported as a monophyly by all analyses. In mitochondrial DNA trees, subgenera (*Panophrys*, *Xenophrys*, *Ophyrophryne*, *Atympanophrys*, *Brachytarsophrys*, *Megophrys* and *Pelobatrachus*) of *Megophrys sensu lato* suggested by Mahony *et al*. (2017) were resolved as monophyletic groups, respectively, except subgenera *Xenophrys* and *Megophrys*. In nuclear DNA trees, all of them were also resolved as monophyletic groups. However, relationships between the subgenera were not resolved in all analyses. All samples of the new taxon were strongly clustered into one independent clade which was deeply nested into the *Megophrys* (*Panophrys*) clade. In mitochondrial DNA trees, the new taxon clade was weakly clustered into a clade including *M. baolongensis*, *M. wushanensis* and *M. tuberogranulata*, but the relationships between them were not resolved except the weakly-supported sister relationship of *M. baolongensis* and *M. wushanensis*. In nuclear DNA trees, the new taxon was weakly supported as the sister of *M. baolongensis* but with almost unresolved relationships with other species in *Megophrys* (*Panophrys*) clade.

Genetic distances on COI gene with uncorrected *p*-distance model between specimens of the new taxon were < 0.5%, much lower than interspecific genetic distances in the genus *Megophrys* (1.4%–33%; Table S4). Genetic distances between the new taxon and its closely-related species, *M. wushanensis*, *M. tuberogranulata* and *M. baolongensis*, were 5.9%–9.3%, at the same level with or even higher than that between some substantial species, for example, *M. huangshanensis* vs. *M. boettgeri* (1.4%), *M. sangzhiensis* vs. *M. spinata* (4.0%), *M. baolongensis* vs. *M. wushanensis* (5.9%), *M. sangzhiensis* vs. *M. binlingensis* (6.7%) and *M. wushanensis* vs. *M. tuberogranulata* (7.1%).

3.2. Description of the new species

***Megophrys* (*Panophrys*) *leishanensis* sp. nov.**

Holotype: CIBLS20160610002 (Figure 3 A, B and C), adult male, from Leigong Mountain (26.35888° N, 108.19055° E, 1571 m a. s. l.), Leishan County, Guizhou

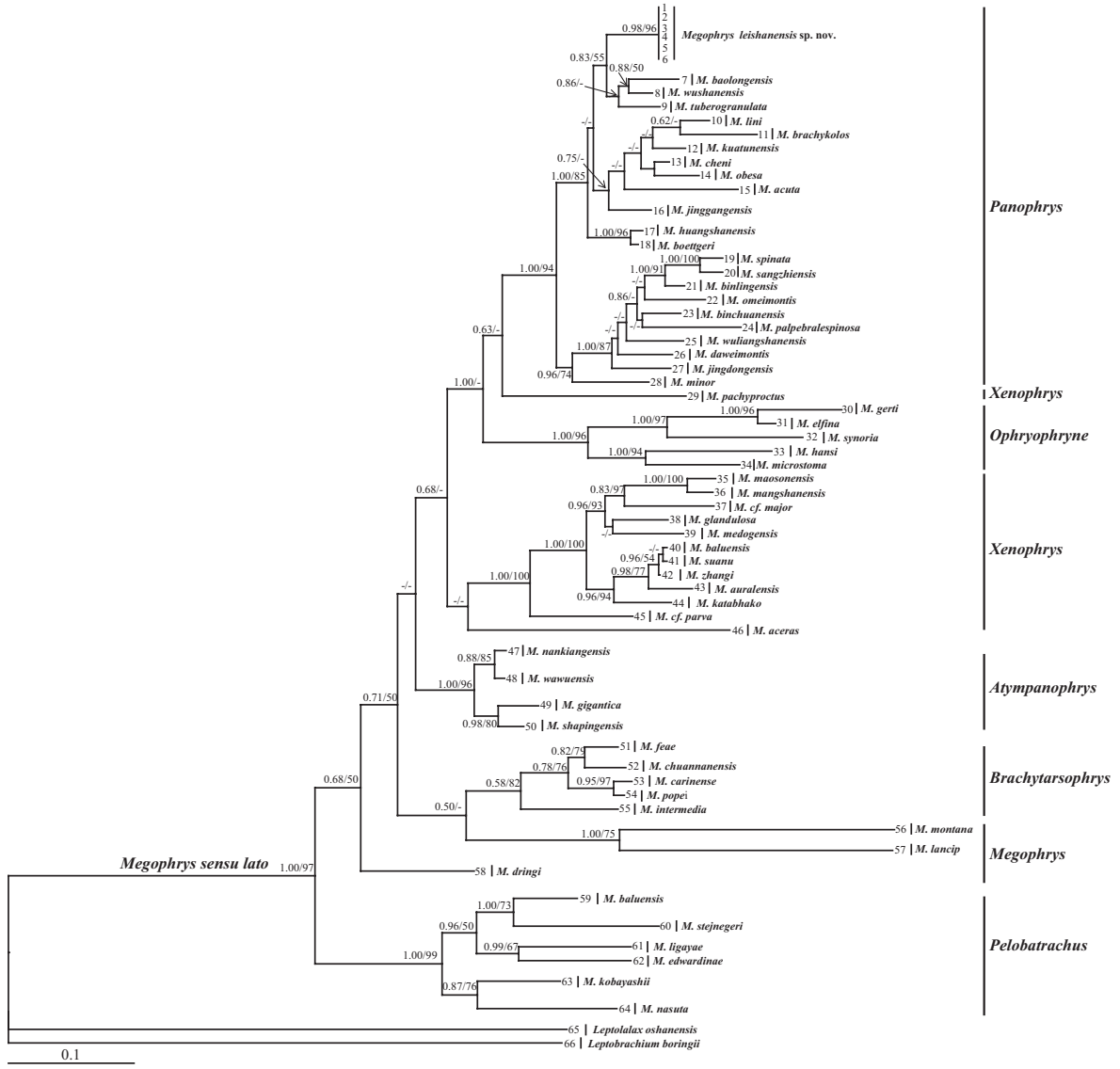

Figure 1 Maximum likelihood (ML) tree of the genus *Megophrys sensu lato* reconstructed based on the 16S rRNA and COI gene sequences. Bayesian posterior probability/ ML bootstrap support were denoted beside each node. Samples 1-66 refer to Table S2. According to Mahony *et al*. (2017), seven subgenera (*Panophrys*, *Xenophrys*, *Ophyrophryne*, *Atympanophrys*, *Brachytarsophrys*, *Megophrys* and *Pelobatrachus*) of *Megophrys sensu lato* were denoted.

Province, China, collected by Shize LI on 10 June 2016.

Paratype: nine adult males and two adult females from Leigong Mountain, Leishan County, Guizhou Province, China, collected by Shize LI. Nine males: CIBLS20141004004 collected on 4 October 2014, CIBLS20160610001, CIB LS20160610003, CIBLS20160610004 and CIBLS20160610006 collected on 10 June 2016, CIBLS20171001001, CIBLS20171001003, CIBLS20171001004 and CIBLS20171001005 collected on 1 October 2017; two adult females CIBLS20160610005 and CIBLS20171001002 collected on 10 June 2016 and 1 October 2017, respectively.

Diagnosis: *Megophrys leishanensis* sp. nov. is assigned to the genus *Megophrys sensu lato* based on molecular phylogenetic analyses and the following generic diagnostic characters: snout shield-like; projecting beyond the lower jaw; canthus rostralis distinct; chest gland small and round, closer to the axilla than to midventral line; femoral gland on rear of thigh; vertical pupils (Fei *et al.*, 2009).

The new species could be identified from its congeners by a combination of the following morphological characters: (1) small body size (SVL in male < 38.8

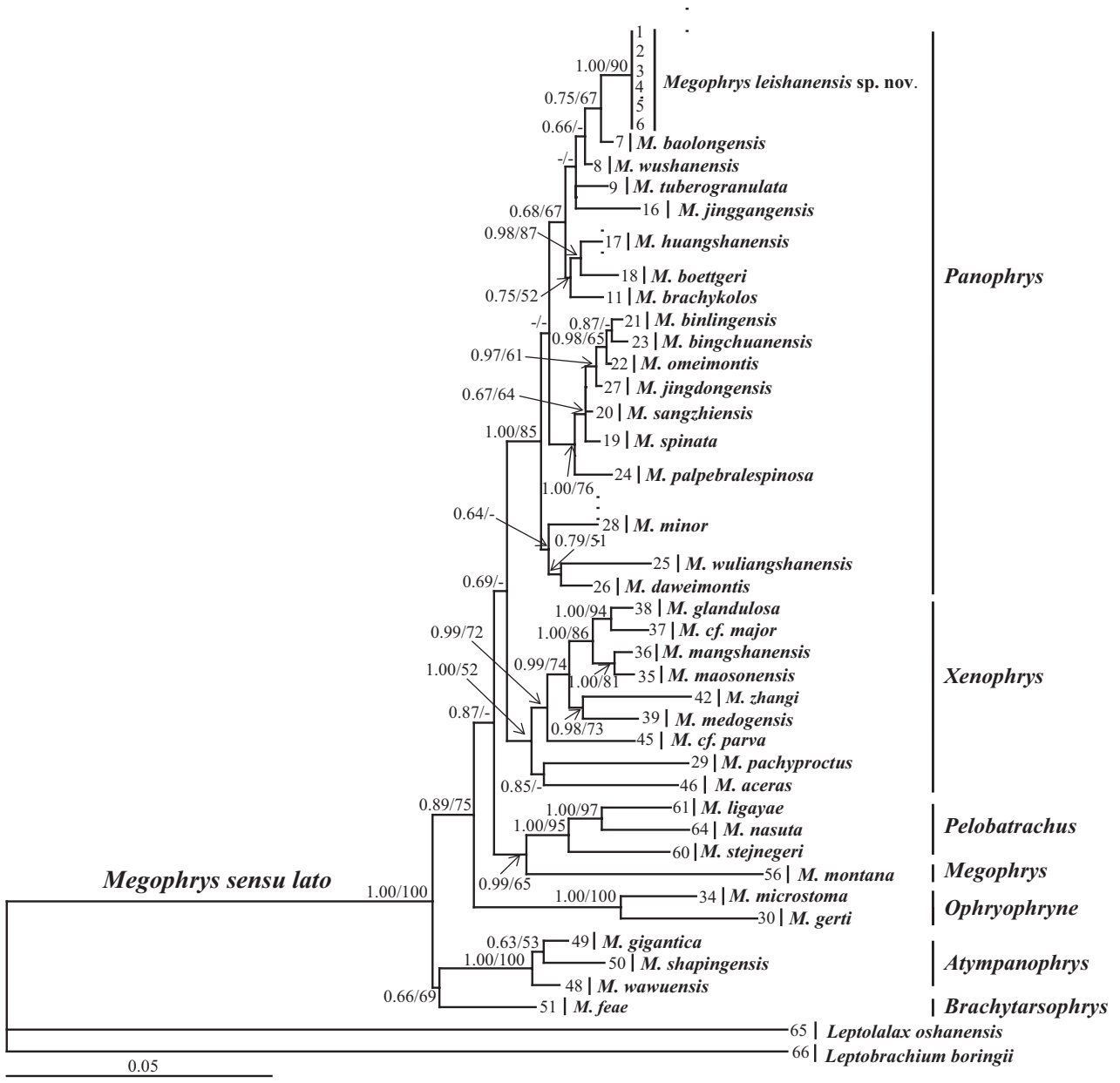

Figure 2 Maximum likelihood (ML) tree of the genus *Megophrys sensu lato* reconstructed based on the nuclear DNA sequences of RAG1 and BNDF genes. Bayesian posterior probability/ML bootstrap support were denoted beside each node. Samples 1-66 refer to Table S2. According to Mahony *et al*. (2017), seven subgenera (*Panophrys*, *Xenophrys*, *Ophyrophryne*, *Atympanophrys*, *Brachytarsophrys*, *Megophrys* and *Pelobatrachus*) of *Megophrys sensu lato* were denoted.

mm and SVL in female < 42.3 mm); (2) vomerine teeth absent; (3) tongue not notched behind; (4) a small horn-like tubercle at the edge of each upper eyelid; (5) tympanum distinctly visible, rounded; (6) two metacarpal tubercles in hand; (7) relative finger lengths: II < I < V < III; (8) toes with rudimentary webbing at bases; (9) heels overlapped when thighs are positioned at right angles to the body; (10) tibiotarsal articulation reaching the level between tympanum to eye when leg stretched forward; (11) an internal single subgular vocal sac in male; (12) in breeding male, the nuptial pads with black nuptial spines on the dorsal bases of the first and second fingers.

Description of holotype: SVL 37.5 mm; head width slightly larger than head length (HDW/HDL ratio about 1.1); snout obtusely pointed, protruding well beyond the margin of the lower jaw; loreal region vertical and concave; canthus rostralis well-developed; top of head flat; an small horn-like tubercle at the edge of the upper eyelid; eye large and convex, eye diameter 40.5% of head

length; pupils vertical; nostril orientated laterally, closer to snout than eye; tympanum distinct, TMP/EYE ratio 0.60; vomerine ridges and vomerine teeth absent; margin of tongue smooth, not notched behind.

Forelimbs slender, the length of lower arm and hand 39.6% of SVL; fingers slender, relative finger lengths: II < I < V < III; tips of digits globular, and digits without lateral fringes in fingers; subarticular tubercle distinct at the base of each fingers; two metacarpal tubercles, inner palmar tubercle moderate and elliptical and outer palmar tubercle smaller (Figure 3 D).

Hindlimbs slender (TL/SVL = 0.47); heels overlapped when thighs are positioned at right angles to the body, tibiotarsal articulation reaching the level between tympanum to eye when leg stretched forward; tibia length longer than thigh length; relative toe lengths I < II < V < III < IV; tips of toes round, slightly dilated; subarticular tubercle absent; toes with rudimentary webbing at bases, without lateral fringes; inner metatarsal tubercle elliptical; no outer metatarsal tubercle (Figure 3 E).

Dorsal skin rough, with numerous granules; several large warts scattered on flanks; an small horn-like tubercle at the edge of each upper eyelid; tubercles on the dorsum forming an X-shaped weak ridge and two discontinuous dorsolateral parallel ridges on either side of the X-shaped ridge; a dark brown butterfly-shaped marking on dorsum of head and between the eyes; several tubercles on the flanks and dorsal surface of thighs and tibias and forming four transverse tubercle rows; supratympanic fold distinct (Figure 3 A).

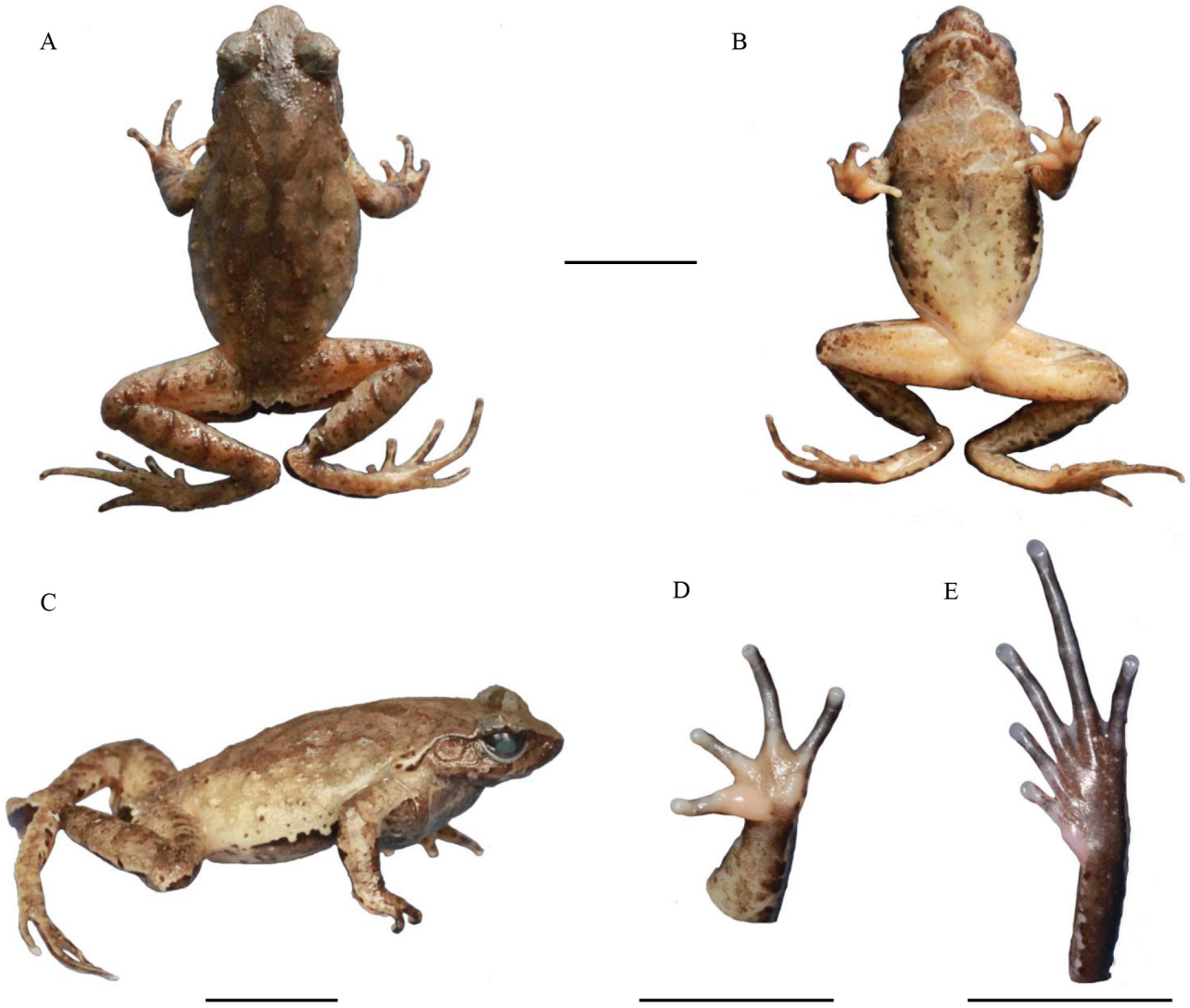

Figure 3 The holotype specimen (CIBLS20160610002) of *Megophrys leishanensis* sp. nov. A, dorsal view. B, ventral view. C, lateral view. D, ventral view of hand. E, ventral view of foot. Scale bar equals to 10 mm.

Ventral surface smooth; chest gland small and round, closer to the axilla than to midventral line; femoral gland on rear of thigh; posterior end of the body protruding distinctly and appearing as an arc-shaped swelling, above the anal region (Figure 3 B).

An internal single subgular vocal sac (Figure 4 C); in breeding season, presence of nuptial pads (Figure 4 F) with black nuptial spines (Figure 4 G) dorsally on the base of the first and second fingers.

Coloration of holotype in life: A dark brown butterfly-shaped marking on dorsum of head and between the eyes; an X-shaped marking on the dorsum, four dark transverse bands on the dorsal surface of the thigh and shank; six dark brown and five white vertical bars on the lower and upper lip; ventral surface of body olive with brown and white spots; lower lip grey; several white blotches on the belly; ventral surface of limbs dark brown with white spots; palms and soles uniform pinkish, tip of digits pale grey; inner metatarsal tubercle and two metacarpal tubercles pinkish; pectoral and femoral glands white (Figure 4 A, B and C).

Coloration of holotype in preservation: Dorsal surface fades to greyish-brown; the dark brown butterfly-shaped marking and X-shaped markings on dorsum and dark brown transverse bands on limbs and digits become more distinct; ventral surface yellowish; creamy-white substitutes the pinkish in the anterior surface of the thighs and lateral surface of the trunk and hand palm (Figure 3).

Variation: Morphometric variations of adult specimens of the new species were presented in Tables 1 and S1. An inverted triangular brown speckle between two upper eyelids with X-shaped marking on back of trunk in some adult individuals (Figure 5 A) and in some adult individuals with V- shaped marking on back of trunk (Figure 5 B); in some adult individuals a brown Y-shaped marking on the dorsum of head and disconnected with a V- shaped marking on back (Figure 5 C); in some adult individuals the inverted triangular brown speckle is connected to the X-shape marking (Figure 5 D); in some adult individuals the ventral part of posterior limb

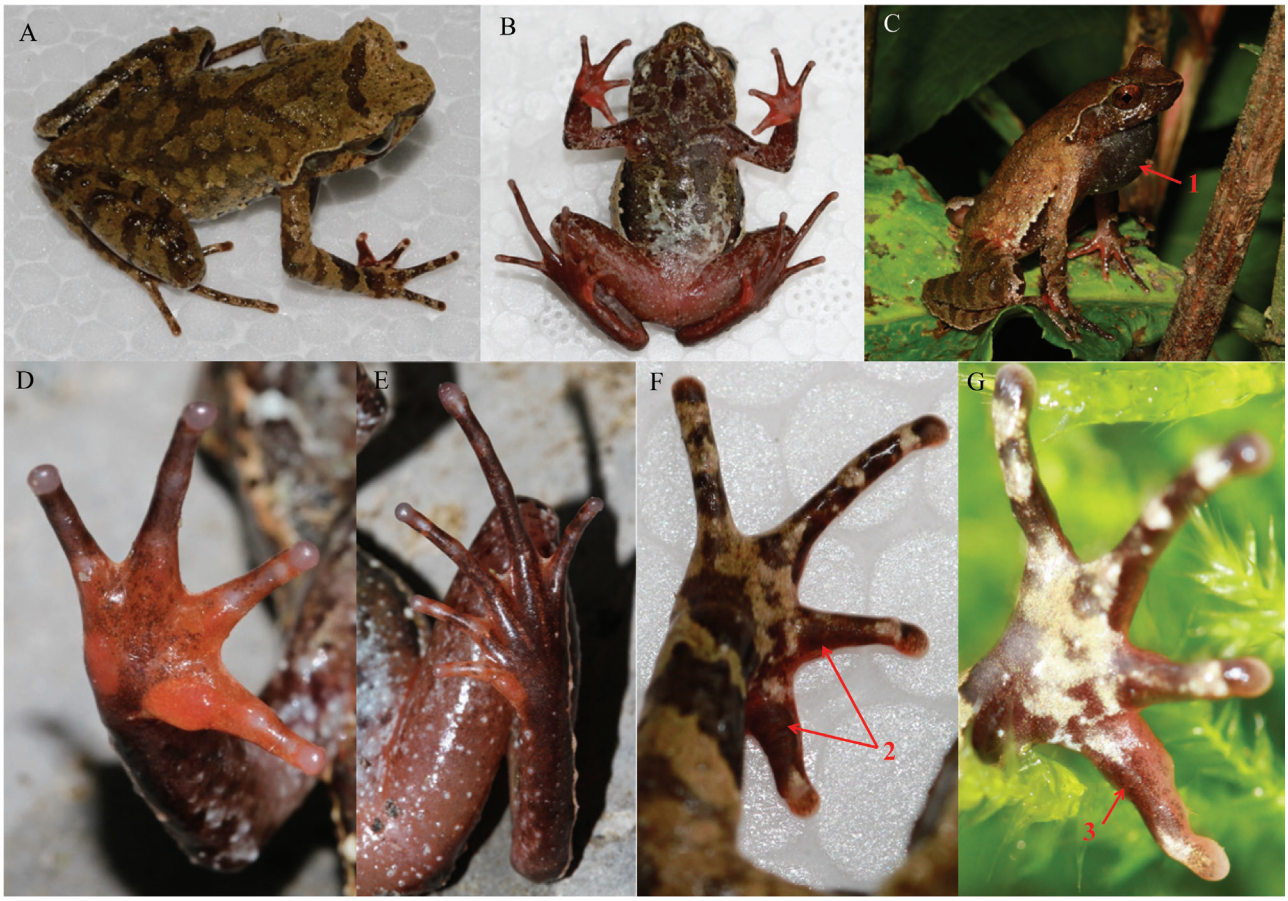

Figure 4 Photos of the holotype CIBLS20160610002 of *Megophrys leishanensis* sp. nov. in life. A, dorsal view. B, ventral view. C, lateral view showing internal single subgular vocal sac (1). D, ventral view of hand. E, ventral view of foot. F, nuptial pads on the first and second fingers (2). G, black nuptial spines (3).

Table 1 Basic statistics for measurements of the adult specimens of *Megophrys leishanensis* sp. nov.

	Male (n = 10)		Female (n = 2)
	Ranging	Mean ± SD	Ranging
SVL	30.4–38.7	34.3 ± 2.7	42.3–42.3
HDL	9.1–11.0	10.1 ± 0.7	11.3–11.7
HDW	10.5–12.0	11.4 ± 0.5	12.1–12.4
SL	3.6–4.5	4.2 ± 0.3	4.5–5.0
ED	3.3–4.3	3.9 ± 0.3	4.1–4.8
IOD	3.3–4.3	3.7 ± 0.3	3.9–4.2
IND	3.5–4.7	4.0 ± 0.4	4.1–4.3
TYD	2.0–2.6	2.3 ± 0.2	2.5–2.8
LAL	14.4–16.3	15.3 ± 0.6	18.1–18.4
LW	2.7–3.9	3.2 ± 0.5	2.8–2.9
FIL	3.2–3.9	3.5 ± 0.2	4.0–4.3
FIIL	2.8–3.5	3.2 ± 0.3	3.8–4.1
FIIIL	4.2–5.4	4.8 ± 0.4	5.4–5.8
FIVL	3.4–4.1	3.7 ± 0.2	4.2–4.3
THL	14.4–16.8	15.4 ± 0.8	17.6–17.7
TL	16.2–18.6	17.5 ± 0.9	19.2–19.2
TW	3.6–4.7	4.2 ± 0.3	4.8–5.1
TFL	21.1–25.9	23.5 ± 0.5	27.5–27.9
FL	14.9–17.3	15.9 ± 1.0	18.1–19.0

Unit: mm. See abbreviations for the morphological characters in Materials and Methods section.

with gray-purple and on the belly the white and brown patches are well demarcated (Figure 5 E); in some adult individuals the white spots on ventral part of belly are less numerous and some brick-red spots are mixed with the white spots or brown spots on ventral part of belly (Figure 5 F).

Tadpole description: The following tadpole description is based on a single specimen (CIBLS20171101001) at Stage 26, the tadpole was confirmed as *Megophrys leishanensis* sp. nov. by molecular analyses. Body slender, body and tail yellow-brown; tail height greater than body height; dorsal fin arising, behind the origin of the tail, height near mid-length, tapering gradually to narrow, tip pointed; tail 1.9 times as long as snout-vent length; tail height 21.8% of tail length; body width slightly longer than body height(BW/ BH = 1.1); tail fins lightly colored, tail muscles with small black spots; eyes large, lateral, nostril near eyes; spiracle on the left side of the body and not distinct; oral disk terminal, lips expanded and directed upwardly into a umbelliform oral disk; transverse width of expanded funnel 34.3% of snout-vent length (Figure 6).

Variation: Measurements of tadpoles were presented in Table 2. All specimens of tadpole were similar in morphology and color pattern, but different from mouth width at different Gosner's stage, the mouth width is decrease gradually from stage 25 to 27. At stage 25 the MW/SVL is 43.6%, at stage 26 is 4.3–36.9% and at stage 26 is 32.1%.

Secondary sexual characteristics: Adult females with SVL 42.3 mm, larger than adult males with SVL 30.4–38.7 mm (Table 1). Adult males have a single subgular vocal sac (Figure 3 C). In breeding male, the nuptial pads on the dorsal bases of the first finger and second fingers with black nuptial spines (Figure 3 F and G).

Advertisement calls: The advertisement call of *Megophrys leishanensis* **sp. nov.** was recorded from the holotype CIBLS20160610002 from the banks of the streamlet, and the ambient air temperature was 18.9 °C. The sonograms and waveforms are shown in Figure 7. One strophe consists of a series of syllables of a pulsed structure. The call consists of a few strophes of 5.450–7.210 s duration (mean ± SD: 6.303 ± 0.881, n = 5). Each strophe contains 12–14 syllables (mean ± SD: 13 ± 0.816, n = 5). Each syllable has a duration of 0.100–0.109 s (mean ± SD: 0.105 ± 0.003, n = 37). The interval between syllables has a duration of 0.309–0.692 s (mean ± SD: 0.409 ± 0.075, n = 36). Syllables are repeated in series at a rate of 1.15–3.24 times (mean ± SD: 2.60 ± 0.383, n = 36) per second. Syllable intervals gradually increase from the beginning to the middle of the strophe then decrease to end. Amplitude modulation within strophe is apparent, beginning with moderately high energy pulses, increasing slightly to a maximum by approximately mid strophe, and

Table 2 Measurements of the tadpoles of *Megophrys leishanensis* sp. nov.

Voucher number	Gosner's stage	TOL	SVL	BH	BW	SS	SL	MW	TAL	TH	TBW
CIBLS20171101001	26	30.9	10.8	3.4	3.8	5.0	2.5	3.7	20.6	4.5	2.0
CIBLS20171101002	26	31.0	10.0	3.0	4.0	5.1	2.2	3.6	19.3	5.4	1.8
CIBLS20171101003	27	33.0	10.9	3.5	4.0	6.0	2.6	3.5	21.4	5.7	1.9
CIBLS20171101004	26	28.4	10.3	3.3	3.7	4.8	2.6	3.7	18.8	5.2	1.9
CIBLS20171101005	25	27.7	9.4	2.7	3.1	4.7	3.0	4.1	17.6	4.3	1.4
CIBLS20171101006	26	27.0	8.4	3.1	3.3	4.2	2.7	3.1	16.6	4.5	1.1

Unit: mm. See abbreviations for the morphological characters in Materials and Methods section.

Figure 5 Color variation in *Megophrys leishanensis* sp. nov. in life. A, dorsal view of the male specimen CIBLS20141004004. B, dorsal view of the male specimen CIBLS20160610001. C, dorsolateral view of the male specimen CIBLS20171001001. D, dorsal view of the female specimen CIBLS20160610005. E, ventral view of the male specimen CIBLS20141004004. F, ventral view of the female specimen CIBLS20171001002.

subsequently decreasing towards the end of each strophe. Calls have a broad frequency range of 1180–7660 kHz.

Morphological comparisons

By having small body size, *Megophrys leishanensis* **sp. nov.** differs from *M. aceras*, *M. auralensis*, *M. binlingensis*, *M. carinense*, *M. caudoprocta*, *M. chuananensis*, *M. damrei*, *M. feae*, *M. flavipunctata*, *M. gigantica*, *M. glandulosa*, *M. himalayana*, *M. intermedia*, *M. jingdongensis*, *M. lekaguli*, *M. liboensis*, *M. longipes*, *M. major*, *M. mangshanensis*, *M. maosonensis*, *M. medogensis*, *M. megacephala*, *M. omeimontis*, *M. oreocrypta*, *M. periosa*, *M. popei*, *M. sangzhiensis*, *M. shapingensis*, *M. shuichengensis*, *M. spinata* and *M. takensis* (maximum SVL < 42.3 mm in the new species vs. minimum SVL > 45 mm in the latter).

By lacking vomerine teeth, *Megophrys leishanensis* **sp. nov.** differs from *M. aceras*, *M. ancrae*, *M. carinense*, *M.*

Figure 6 Tadpole stage of *Megophrys leishanensis* sp. nov. Dorsal view (A) and ventral view (B) of specimen CIBLS20171101001 in life.

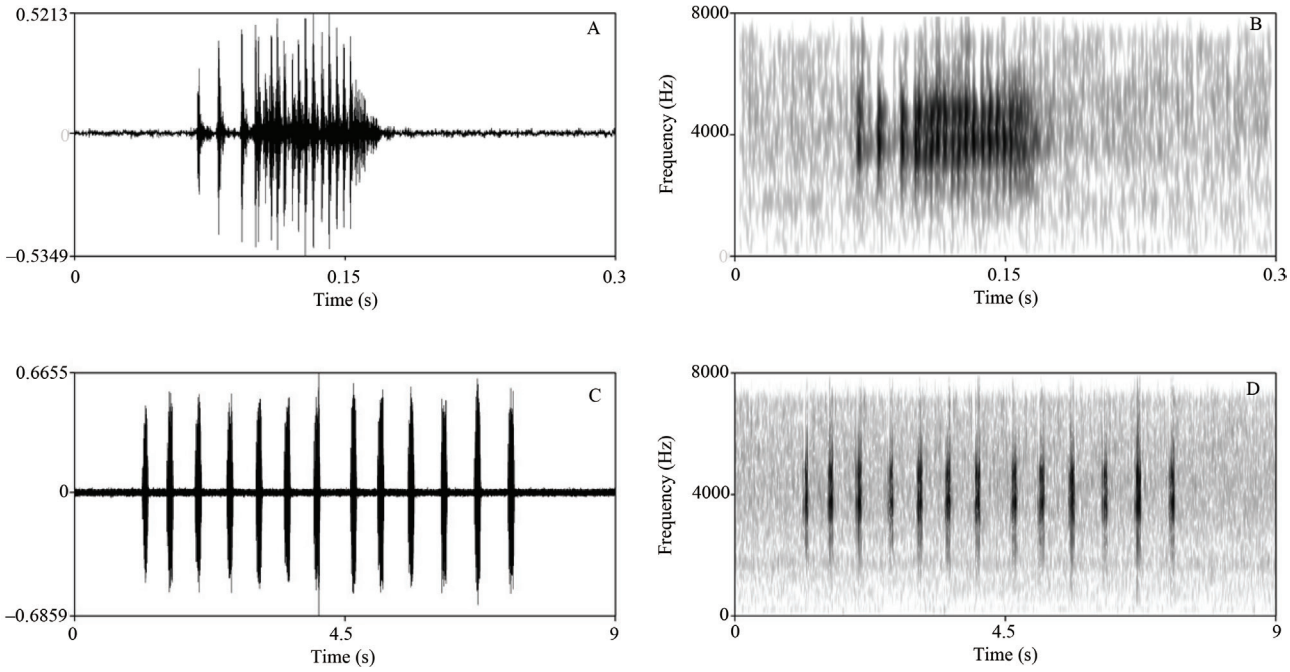

Figure 7 Advertisement call of male *Megophrys leishanensis* sp. nov., CIBLS20160610002, holotype. A, waveform showing one syllable. B, sonogram showing one syllable. C, waveform showing 13 syllables of one strophe. D, sonogram showing 13 syllables of one strophe.

baluensis, *M. caudoprocta*, *M. chuananensis*, *M. damrei*, *M. daweimontis*, *M. fansipanensis*, *M. flavipunctata*, *M. glandulosa*, *M. hoanglienensis*, *M. himalayana*, *M. insularis*, *M. intermedia*, *M. jingdongensis*, *M. jinggangensis*, *M. kobayashii*, *M. lancip*, *M. latidactyla*, *M. lekaguli*, *M. liboensis*, *M. major*, *M. mangshanensis*, *M. maosonensis*, *M. medogensis*, *M. megacephala*, *M. montana*, *M. nasuta*, *M. omeimontis*, *M. oreocrypta*, *M. oropedion*, *M. palpebralespinosa*, *M. parallela*, *M. parva*, *M. periosa*, *M. popei*, *M. robusta*, *M. rubrimera*, *M. sangzhiensis*, *M. stejnegeri*, *M. takensis*, *M. zhangi* and *M. zunhebotoensis* (vomerine teeth present in the latter).

By having a small horn-like tubercle at the edge of each upper eyelid, *Megophrys leishanensis* **sp. nov.** differs from *M. binchuanensis*, *M. binlingensis*, *M. damrei*, *M. gigantica*, *M. minor*, *M. nankiangensis*, *M. oropedion*, *M. pachyproctus*, *M. spinata*, *M. takensis*, *M. wuliangshanensis*, *M. wushanensis*, *M. zhangi* and *M. zunhebotoensis* (tubercle lacking in the latter).

By having a small horn-like tubercle at the edge of

Figure 8 Habitats of *Megophrys leishanensis* sp. nov. in the type locality Leigong Mountain, Leishan County, Guizhou Province, China. A, landscape of montane forests in the type locality. B, a mountain stream in the type locality.

each upper eyelid, *Megophrys leishanensis* **sp. nov.** differs from *M. carinense*, *M. feae*, *M. gerti*, *M. hansi*, *M.intermedia*, *M. koui*, *M. latidactyla*, *M. liboensis*, *M. microstoma*, *M. palpebralespinosa*, *M. popei*, *M. shuichengensis* and *M. synoria* (vs. having a prominent and elongated tubercle at the edge of each upper eyelid in the latter).

By a tongue not notched behind, *Megophrys leishanensis* **sp. nov.** differs from *M. ancrae*, *M. baolongensis*, *M. binlingensis*, *M. boettgeri*, *M. carinense*, *M. cheni*, *M. chuananensis*, *M. damrei*, *M. fansipanensis*, *M. feae*, *M. feii*, *M. flavipunctata*, *M. gerti*, *M. glandulosa*, *M. hoanglienensis*, *M. huangshanensis*, *M. insularis*, *M. jingdongensis*, *M. kuatunensis*, *M. liboensis*, *M. mangshanensis*, *M. maosonensis*, *M. medogensis*, *M. minor*, *M. nankiangensis*, *M. omeimontis*, *M. oropedion*, *M. pachyproctus*, *M. parallela*, *M. popei*, *M. robusta*, *M. sangzhiensis*, *M. shapingensis*, *M. shuichengensis*, *M. spinata*, *M. vegrandis*, *M. wawuensis*, *M. zhangi* and *M. zunhebotoensis* (vs. tongue notched behind in the latter).

By lacking lateral fringe in toes, *Megophrys leishanensis* **sp. nov.** differs from *M. actuta*, *M. auralensis*, *M. baolongensis*, *M. binchuanensis*, *M. boettgeri*, *M. carinense*, *M. cheni*, *M. chuananensis*, *M. elfina*, *M. feae*, *M. feii*, *M. gigantica*, *M. glandulosa*, *M. hansi*, *M. intermedia*, *M. jingdongensis*, *M. jinggangensis*, *M. kuatunensis*, *M. latidactyla*, *M. lini*, *M. major*, *M. maosonensis*, *M. nankiangensis*, *M. omeimontis*, *M. palpebralespinosa*, *M. popei*, *M. rubrimera*, *M. sangzhiensis*, *M. serchhipii*, *M. shapingensis*, *M. shuichengensis*, *M. spinata*, *M. vegrandis*, *M. zhangi* and *M. zunhebotoensis* (vs. lateral fringe present in the latter).

By toes with rudimentary webs at bases, *Megophrys leishanensis* **sp. nov.** differs from *M. brachykolos*, *M. carinense*, *M. jingdongensis*, *M. jinggangensis*, *M. lini*, *M. major*, *M. palpebralespinosa*, *M. popei*, *M. shuichengensis*, *M. spinata* (vs. at least one-fourth webbed in the latter).

By heels overlapping when thighs are positioned at right angles to the body, *Megophrys leishanensis* **sp. nov.** differs from *M. acuta*, *M. brachykolos* and *M. obesa* (vs. heels not meeting when thighs are positioned at right angles to the body in the latter).

By having an single internal subgular vocal sac in male, *Megophrys leishanensis* **sp. nov.** differs from *M. caudoprocta*, *M. shapingensis*, *M. shuichengensis* (vs. vocal sac absent in the latter).

By having nuptial pads and nuptial spines on the dorsal base of the first and second fingers in breeding male, *Megophrys leishanensis* **sp. nov.** differs from *M. acuta*, *M. feii*, *M. shapingensis* and *M. shuichengensis* (vs. nuptial pads and nuptial spines lacking in the latter).

We give more detailed compassion with the phylogenetically close species:

Megophrys leishanensis **sp. nov.** differs from *M. minor* by having a small horn-like tubercle at the edge of each upper eyelid (vs. absent in the latter), tongue not notched behind (vs. notched in the latter), tibiotarsal articulation reaching the level between tympanum to eye when leg stretched forward (vs. tibiotarsal articulation reaching the level between eye and tip of snout in the latter), having two metatarsal tubercles in each hand (vs. absent of metatarsal tubercle in hand in the latter).

Megophrys leishanensis **sp. nov.** differs from *M. baolongensis* by SVL in males < 38.8 mm (vs. SVL in males > 42 mm in the latter), tongue not notched behind

(vs. tongue feebly notched behind in the latter), lacking lateral fringes on toes (vs. having narrow lateral fringes on toes in the latter), toes with rudimentary webs at bases (vs. toes without web in the latter), heels overlapping when thighs are positioned at right angles to the body (vs. just meeting in the latter).

Megophrys leishanensis **sp. nov.** differs from *M. wushanensis* by having a small horn-like tubercle at the edge of each upper eyelid (vs. absent in the latter), lacking lateral fringes on toes (vs. lateral fringes on toes wide in the latter), heels overlapped when thighs are positioned at right angles to the body (vs. just meeting in the latter), having two metatarsal tubercles in each hand (vs. absent of metatarsal tubercle in hand in the latter).

Megophrys leishanensis **sp. nov.** differs from *M. tuberogranulata* by the SVL in females < 42.3 mm (vs. SVL in females > 50 mm in the latter), tympanum about 0.7 times of eye diameter (vs. tympanum 0.5 times of eye diameter in the latter), having a small horn-like tubercle at the edge of each upper eyelid (vs. absent in the latter), the fourth finger significantly longer than the first (vs. first and fourth fingers with almost same length in the latter); outer metatarsal tubercle absent in soles (vs. outer metatarsal tubercle flattened in soles).

Distribution and habitats: *Megophrys leishanensis* **sp. nov.** is only known from the type locality, Leigong Mountain, Leishan County, Guizhou Province, China. It inhabits high mountain forest (Figure 8 A) at elevations between 1200–1750 m a. s. l., and is more frequently found in bamboo forest and encountered in forest nearby streams (Figure 8 B). Six sympatric amphibian species, i.e. *Megophrys spinata* Liu and Hu, 1973, *Odorrana lungshengensis* Liu and Hu, 1962, *Leptolalax oshanensis* Liu, 1950, *Paramesotriton caudopunctatus* Liu and Hu, 1973, *Rana zhenhaiensis* Ye, Fei, et Matsui, 1995 and *Vibrissaphora leishanensis* Liu and Hu, 1973, were found in the type locality.

Etymology: This specific name *leishanensis* is a Latinize toponymic adjective that refers to Leigong Mountains, Leishan County, Guizhou Province, China, where the new species was collected. For the common name, we suggest Leishan horned toad (English) and Leishan Jiao Chan (Chinese).

4. Discussion

Most cryptic congeners in the genus *Megophrys sensu lato* are difficult to be distinguished from each other due to the superficial similarities in morphology: drab colorations, complicated markings and even changeable colorations and skin markings of the same individual under different environmental conditions (Fei *et al*., 2012). These result in considerable challenges in field identification, which in turn cause ambiguities in taxonomy and distributions (Wang *et al.,* 2014). *Megophrys minor* has the widest distribution in *Megophrys sensu lato*, including the provinces of Sichuan, Guizhou, Chongqin, Yunnan, Guangxi, Jiangxi and northern Vietnam (Fei *et al.*, 2012), and several new species has been separated from *M. minor* (Chen *et al*., 2017; Mahony *et al*., 2017; Liu *et al*., 2018), but most of which are not described. In this study, based on molecular phylogenetic analyses and morphological comparisons, a new species *Megophrys leishanensis* **sp. nov.** was described which was also initially identified as *M. minor*. Phylogenetic analyses based on mitochondrial DNA and nuclear DNA all suggested that the new species belonged to *Megophrys* (*Panophrys*) but was separated from its congeners. Genetic distance on COI gene between the new species and its closely-related species (*M. baolongensis, M. wushanensis and M. tuberogranulata*) was 5.9%–9.3%, matching the level about interspecific divergences in amphibians 3.1%—28.2% (Che *et al.*, 2012) and being much higher than that between many sister species (of which, most species have been completely recognized as valid species) in *Megophrys sensu lato* (Table S4). Finally, it was different from its congeners on a series of morphological characters. At all, multiple evidences support the validity of the new species.

Megophrys leishanensis **sp. nov.** was only found in Leigong Mountain, Leishan County, Gauizhou Province, China. It restrictedly inhabits the forest floor, nearby slowly flowing mountainous streams surrounded by moist evergreen at elevations between 1200 m and 1750 m. These areas are being threatened by tourism development, and the population of *Megophrys leishanensis* **sp. nov.** is declining based on the monitoring from 2014 to 2018, so future research should focus on determining the distribution and population status of the species.

Acknowledgements We are grateful to editors and reviewers for their working on the manuscript. Collections in field were permitted by Administration of Leigong Mountain Nantioanl Nature Reserve (No. LGS20040348). This study was approved by the animal ethical committee of Chengdu Institute of Biology, Chinese Academy of Sciences, and animal experiments were carried out following the institutional guidelines (No. 2017CIBAEC0344). This work was supported by the Strategic Priority Research Program of the Chinese

Academy of Sciences (Grant No. XDB31000000), National Natural Sciences Foundation of China (NSFC-31201702), Biodiversity Conservation Key Laboratory of Guizhou province Education Department, Guiyang College, The laboratory on biodiversity conservation and applied ecology of Guiyang college, Ocean Park Conservation Foundation of Hong Kong (No. PR 1030001252), Biodiversity Conservation Program of Ministry of Ecology and Environment of China (#2110404), Guizhou Provincial Department of Education Youth Science and Technology Talents Growth Project (Nos. KY[2018]455, KY[2018]468 and KY[2018]453), The Specialty Food Resource Utilization Talent Base of Moutai University.

References

Beddard F. E. 1908 "1907". Contributions to the knowledge of the anatomy of the batrachian family Pelobatidae. Proc Zool Soc Lond, 1907: 871–911

Bonaparte C. L. J. L. 1850. Conspectus Systematum. Herpetologiae et Amphibiologiae. Editio altera reformata. Lugdini Batavorum: E. J. Brill

Boulenger G. A. 1886 "1885". Description of a new frog of the genus *Megalophrys*. Proc Zool Soc Lond, 1885: 850

Boulenger G. A. 1887. Description of a new frog of the genus *Megalophrys*. Annali del Museo Civico di Storia Naturale di Genova. Serie 2, 4: 512–513

Boulenger G. A. 1889. Description of a new batrachian of the genus *Leptobrachium,* obtain by M. L. Fea in the Karens Mountains Burma. Ann Mus Civ Stro Nat Genova, 7(2): 748–750

Boulenger G. A. 1893. Concluding report on the reptiles and batrachians obtained in Burma by Signor L. Fea dealing with the collection made in Pegu and the Karin Hills in 1887–88. Annali del Museo Civico di Storia Naturale di Genova, Serie 2, 13: 304–347

Boulenger G. A. 1899a. Descriptions of three new reptiles and a new batrachian from Mount Kina Balu, North Borneo. Ann Nat Hist, Series 7, 4: 453

Boulenger G. A. 1899b. On a collection of reptiles and batrachians made by Mr. J. D. La Touche in N.W. Fokien, China. Proc Zool Soc Lond, 159–172 + pls. XVI-XIM

Boulenger G. A. 1903. Report on the batrachians and reptiles. Annandale N., Robinson H. C., eds. Fasciculi Malayenses. Anthropological and Zoological Results of an Expedition to Perak and the Siamese Malay States 1901–1903 undertaken by Nelson Annandale and Herbert C. London, Longmans, Green & Co.: Robinson under the auspecies of the University of Edinburgh and the University of Liverpool. Volume 2, Zoology, Part 1: 131–176.

Boulenger G. A. 1908. A revision of the oriental pelobatid batrachians (genus *Megophrys*). Proc Zool Soc Lond, 78 (2): 407–430

Bourret R. 1937. Notes herpétologiques sur l'Indochine française. XIV. Les batraciens de la collection du Laboratoire des Sciences Naturelles de l'Université. Descriptions de quinze especes ou variétés nouvelles. Annexe au Bulletin Général de l'Instruction Publique Hanoi, 1937: 5–56

Castresana, J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol Biol Evol, 17: 540–552

Che J., Chen H. M., Yang J. X., Jin J.Q., Jiang K., Yuan Z. Y., Murphy R. W., Zhang Y. P. 2012. Universal COI primers for DNA barcoding amphibians. Mol Ecol Resour, 12:247–258

Chen J. M., Zhou W. W., Nikolay A., Poyarkov Jr., Stuart B. L., Brown R. M., Lathrop A., Wang Y. Y., Yuan Z. L., Jiang K., Hou M., Chen H. M., Suwannapoom C., Nguyen S. N., Duong T. V., Papenfuss T. J., Murphy R. W., Zhang Y. P., Che J. 2017. A novel multilocus phylogenetic estimation reveals unrecognized diversity in Asia toads, genus *Megophrys sensu lato* (Anura: Megophryidae). Mol Phylogenet Evol, 106: 28–43

Delorme M., Dubois A., Grosjean S., Ohler A. 2006. Une nouvelle ergotaxinomie des Megophryidae (Amphibia, Anura). Alytes, 24: 6–21

Deuti K., Grosjean S., Nicolas V., Vasudevan K., Ohler A. 2017. Nomenclatural puzzle in early *Megophrys* nomina (Anura, Megophryidae) solved with description of two new species from India (Darjeeling hills and Sikkim). Alytes, 34: 20–48

Dubois A. 1983. Classification et nomenclature supragenerique des amphibiens anoures. Bulletin Mensuel de la Société Linnéenne de Lyon, 52: 270–276

Dubois A. 1987 "1986". Miscellanea taxinomica batrachologica (I). Alytes, 5: 7–95

Dubois A., Ohler A. 1998. A new species of *Leptobrachium* (*Vibrissaphora*) from northern Vietnam, with a review of the taxonomy of the genus *Leptobrachium* (Pelobatidae, Megophryinae). Dumerilia, 4:1–32

Fei L., Hu S. Q., Ye C.Y., Huang. Y. Z. 2009. Fauna Sinica. Amphibia. Volume 2. Anura. Chinese Academy of Science. Beijing, China: Science Press. (In Chinese)

Fei L., Ye C.Y., Huang Y.Z. 1983. Two new subspecies of *Megophrys omeimontis* Liu from China (Amphibia, Pelobatidae). Acta Herpetologica Sinica. New Series, Chengdu, 2(2): 49–52 (In Chinese with English abstract)

Fei L., Ye C. Y., Huang Y. Z. 1990. Key to Chinese Amphibians. Chongqing, China: Publishing House for Scientific and Technological (In Chinese)

Fei L., Ye C. Y., Huang Y. Z. 2001. Colour Handbook Amph. Sichuan, Beijing: Science Press (In Chinese)

Fei L., Ye C.Y. 2005. Two new species of Megophryidae from China. In: Fei et al. (ed.), The Key and Illustration of Chinese. Chongqing, China: Sichuan Publishing House of Science and Technology (In Chinese)

Fei L, Ye C. Y. 2016. Amphibians of China. Volume (I). Beijing, China: Science Press.

Fei L, Ye C. Y., Jiang J. P. 2012. Colored atlas of Chinese Amphibians and their distributions. Chengdu, China: Sichuan Publishing House of Science and Technology (In Chinese)

Fei L, Ye C. Y., Jiang J. P. 2016. Genus *Liuophrys* Fei, Ye and Jiang, new genus; Subgenus *Atympanophrys* (*Borealophrys*) Fei, Ye and Jiang, new subgenus; Subgenus *Atympanophrys* (*Gigantophrys*) Fei, Ye and Jiang, new subgenus; Genus *Boulenophrys* Fei, Ye and Jiang, 2016, new genus; Subgenus

Xenophrys (*Tianophrys*) Fei, Ye and Jiang, new subgenus. In Fei L, Ye C. Y. 2016. Amphibians of China. Volume (I). Beijing, China: Science Press.

Felsenstein J. 2004. Inferring Phylogenies. Massachusetts, America: Sinauer Associates

Frost D. R. 2018. Amphibian Species of the World Version 6.0, an Oline Reference: Names assigned to genus *Megophrys*. American Museum of Natural History, New York, USA. Available from: http://research.amnh.org/vz/herpetology/amphibia/Amphibia/Anura/Megophryidae/Megophrys (accessed 23 November 2018).

Günther A. C. L. G. 1864. The Reptiles of British India. London: Ray Society by R. Hardwicke.

Guindon S, Dufayard J. F., Lefort V., Anisimova M., Hordijk W., Gascuel O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst Biol, 59(3): 307–321

Hall T. A. 1999. BIOEDIT: a user-friendly biological sequence alignment editor and

analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser, 41: 95–98

Hu S. X., Zhao E. M., Liu C. Z. 1966. A herpetological survey of the Tsinling and Ta-pa shan region. Acta Zool Sinica, 18(1): 57–89 (In Chinese)

Hu S. X., Zhao E. M., Liu C. Z. 1973. A survey of amphibians and reptiles in Kweichow province, including a herpetofauna analysis. Acta Zool Sinica, 19(2): 149–171 (In Chinese)

Huang Y.Z., Fei L. 1981. Two new species of amphibians from Xizang. Acta Zootaxonomica Sin, 6: 211–215

Inger R. F. 1989. Four new species of frogs from Borneo. Malayan Nat J, Kuala Lumpur, 42: 229–243

Inger R. F., Romer J. D. 1961. A new pelobatid frog of the genus *Megophrys* from Hong Kong. Fieldiana Zool, 39(46): 533–538

Inger R. F., Stuebing R. B., Lian T. F. 1995. New species and new records of Anurans from Boreno. Raff Bull Zool, 43(1): 115–131

Inger R. F., Iskandar D. T. 2005. A collection of amphibians from west Sumatra, with description of a new species of *Megophrys* (Amphibia: Anura). Raffles B Zool, 133–142

Jiang J. P, Ye C. Y., Fei L. 2008. A New Horn Toad *Megophrys sangzhiensis* from Hunan, China (Amphibia, Anura). Zool Res 29(2): 219–222 (In Chinese)

Kuhl H., Van Hasselt J. C. 1822. Uittreksels uit breieven van de Heeren Kuhl en van Hasselt, aan de Heeren C. J. Temminck, Th. van Swinderen en W. de Haan. Algemeene Konst-en Letter-Bode, 7: 99–104

Lathrop, A. 1997. Taxonomic review of the megophryid frogs (Anura: Pelobatoidea). Asian Herpetol Res, 7: 68–79

Lemmon A. R., Moriarty E.C. 2004. The importance of proper model assumption in Bayesian phylogenetics. Syst Biol, 53: 265–277

Li Y. L., Jin M. J., Zhao J., Liu Z. Y., Wang Y. Y., Pang H. 2014. Description of two new species of the genus *Megophrys* (Amphibia: Anura: Megophryidae) from Heishiding Natural Reserve, Fengkai, Guangdong, China, based on molecular and morphological data. Zootaxa, 3795(4): 449–471

Liu C. Z. 1950. Amphibians of Western China. Fieldiana, America Zool Mem, Chicago, 2: 1–400

Liu C. Z., Hu S. Q., Yang H. H. 1960. Amphibian of Yunnan collected in 1958. Acta Zool Sinca, 12(2): 149–174 (In Chinese with English abstract)

Liu Z. Y., Zhu T. Q., Zeng Z. C., Lyu Z. T., Wang J., Messenger K., Greenberg A. J., Gou Z. X., Yang Z. H., Shi S. H., Wang Y. Y. 2018. Prevalence of cryptic species in morphologically uniform taxa – Fast speciation and evolutionary radiation in Asian frogs. Mol Phylogenet Evol, 127: 723–731

Mahony S. 2011. Two new species of *Megophrys* Kuhl & van Hasselt (Amphibia: Megophryidae), from western Thailand and southern Cambodia. Zootaxa, 2734: 23–39

Mahony S., Sengupta S., Kamei R. G., Biju S. D. 2011. A new low altitude species of *Megophrys* Kuhl and van Hasselt (Amphibia: Megophryidae), from Assam, Northeast India. Zootaxa, 3059: 36–46

Mahony S., Teeling E .C., Biju S. D. 2013. Three new species of horned frogs, *Megophrys* (Amphibia: Megophryidae), from northeast India, with a resolution to the identity of *Megophrys boettgeri* populations reported from the region. Zootaxa, 3722(2): 143–169

Mahony S., Nicole M. F., Biju S.D., Teeling E. C. 2017. Evolutionary history of the Asian Horned Frogs (Megophryinae): integrative approaches to timetree dating in the absence of a fossil record. Mol Biol Evol, 34(3): 744–771

Mahony S., Kamei R. G., Teeling E. C., Biju S. D. 2018. Cryptic diversity within the *Megophrys major* species group (Amphibia: Megophryidae) of the Asian Horned Frogs: Phylogenetic perspectives and a taxonomic revision of South Asian taxa, with descriptions of four new species. Zootaxa, 4523: 1–96

Malkmus R., Matsui M. 1997. *Megophrys kobayashii*, ein neuer pelobatider Frosch vom Mount Kinabalu. Sauria. Berlin, 19: 31–37

Mathew R., Sen N. 2007. Description of two new species of *Megophrys* (Amphibia: Anura: Megophryidae) from North-east India. Cobra, 1: 18–28

McGuire J. A., Witt C. C., Altshule D. L., Remsen J. V. 2007. Phylogenetic systematics and biogeography of hummingbirds: Bayesian and maximum likelihood analyses of partitioned data and selection of an appropriate partitioning strategy. Syst Biol, 56: 837–856

Mo M. Y., Shen Y. H., Li H. H., Wu M. S. 2010. A new species of *Megophrys* (Amphibia: Anura: Megophryidae) from the northwestern Hunan Province, China. Curr Zool, 56(4): 432–436

Munir M., Hamidy A., Farajallah A., Smith E. N. 2018. A new *Megophrys* Kuhl and Van Hasselt (Amphibia: Megophryidae) from southwestern Sumatra, Indonesia. Zootaxa, 4442: 389–412

Poyarkov N. A., Duong Jr., T. V., Orlov N. L., Gogoleva S. I., Vassilieva A. B., Nguyen L. T., Nguyen V. D. H., Nguyen S. N., 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

Ohler H. 2003. Revision of the genus *Ophryophryne* Boulenger, 1903 (Megophryidae) with description of two new species. Alytes, 23–44

Ohler A., Swan S. R., Daltry J. C. 2002. A recent survey of the amphibian fauna of the Cardamom Mountains, Southwest Cambodia with descriptions of three new species. Raffles B Zool, 50(2): 465–481

Orlov N. L., Pyarkov Jr N. A., Nguyen T. T. 2015. Taxonomic notes on *Megophrys* frogs (Megophryidae: Anura) of Vietnam, with description of a new species. Russian Herpetol, 22: 206–218

Pope C. H. 1929. Four new frogs from Fukien Province, China. Amer Mus Nov, 352: 1–5

Rao D. Q., Yang D. T. 1997. The karyotypes of Megophryinae (Pelobatidae) with a discussion on their classification and phylogenetic relationships. Asian Herpetol Res, 7: 93–102

Robert L., Brett C., Simon Y. W. H., Stephane G. 2012. PartitionFinder: Combined Selection of Partitioning Schemes and Substitution Models for Phylogenetic Analyses. Mol Phylogenet Evol, 29(6): 1695–1701

Ronquist F. R., Huelsenbeck J. P. 2003. MrBayes3: Bayesian phylogenetic inference under mixed models. Bioinformatics,19(12): 1572–1574

Sambrook J., Fritsch E. F., Maniatis T. 1989. Molecular Cloning: A Laboratory Manual. New York, America: Cold Spring Harbor Laboratory Press

Schlegel H. 1858. Handleiding tot de Beoefening der Dierkunde. Volume 2. Breda: Koninklijke Militaire Akademie

Shen M. M., Liang D., Feng Y. J., Chen M. Y., Zhang P. 2013. A versatile and highly efficient toolkit including 102 nuclear markers for vertebrate phylogenomics, tested by resolving the higher level relationships of the Caudata. Mol Biol Evol, 30(10): 2235–2248

Shen Y. H. 1994. A new pelobatid toad of the genus *Megophrys* from China (Anura: Pelobatidae). Collection of Articles for the 60th Anniversary of the Foundation of the Zoological Society of China, Nanking: Zool Soc, 603–606 (In Chinese)

Simon C., Frati F., Beckenbach A., Crespi B., Liu H. Flook P. 1994. Evolution, weighting and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann Entomol SOC Amer, 87: 651–701

Sjöander K., Beskow J. 2000. Wavesurfer (Anura: Pelobatidae). Collection of Articles for the International Conference on Spoken Language Processing, Beijing, China, 464–467

Smith, M.A. 1921. New or little-known reptiles and batrachians from southern Annam (Indo-China). Proc Zool Soc London, 423–440

Stejneger L. 1926. Two new tailless amphibians from western China. Proc Biol Soc Washing, 39: 53–54

Stuart B. L., Chuaynkern Y., Chan-ard T., Inger R. F. 2006. Three new species of frogs and a new tadpole from eastern Thailand. Fieldiana Zool, 111: 1– 19

Tamura K, Stecher G, Peterson D, Fiipski A, Kumar S. 2011. MEGA6: molecular evolutionary genetics analysis using evolutionary distance. Mol Biol Evol, 28: 2725–2729

Tapley B., **Cutajar T., Mahony S., Nguyen C. T., Dau V. Q., Nguyen T. T., Luong H. V., Rowley J. J. L.** 2017. The Vietnamese population of *Megophrys kuatunensis* (Amphibia: Megophryidae) represents a new species of Asian horned frog from Vietnam and southern China. Zootaxa, 4344(3): 465–492

Tapley B., Cutajar T. P., Mahony S., Nguyen C. T., Dau V. Q., Luong A. M., Le D. T., Nguyen T. T., Nguyen T. Q., Portway C., Luong H. V., Rowley J. J. L. 2018. Two new and potentially highly threatened *Megophrys* Horned frogs (Amphibia: Megophryidae) from Indochina's highest mountains. Zootaxa, 4508: 301–333

Taylor E. H. 1920. Philippine Amphibia. Philipp J Sci, 16: 213–359

Tian Y. Z., Gu M. M., Sun A. Q. 2000. A new species of *Megophrys* in China (Amphibia: Pelobatidae). Acta Zootaxonomica Sin, 25: 462–466 (In Chinese)

Tian Y. Z., Sun A. Q. 1995. A new species of *Megophrys* from China (Amphibia: Pelobatidae). J Liupanshui Teach Col, 52(3): 11–15 (In Chinese)

Tian W.S., Hu Q.X. 1983. Taxonomic study on genus *Megophrys*, with descriptions of two genera. Acta Herpetol Sinica, 2:41–48 (In Chinese).

Vieites D.R., Min M. S., Wake, D. B. 2007. Rapid diversification and dispersal during periods of global warming by plethodontid salamanders. Proc Natl Acad Sci USA, 104: 19903–19907

Wang J., Liu Z. Y., Lyu Z. T., Wang Y. Y. 2017a. A new species of the genus *Megophrys* (Amphibia: Anura: Megophryidae) from an offshore island in Guangdong Province, southeastern China. Zootaxa, 4324(3): 541–556

Wang, Y. E., Liu B.Q., Jiang K., Jin W., Xu J. N., Wu C. H. 2017b. A new species of the Horn Toad of the genus *Xenophrys* from Zhejiang, China (Amphibia: Megophryidae). Chin J Zool, 52: 19–29 (In Chinese)

Wang Y. Y., Zhang T. D., Zhao J., Sung Y. H., Yang J. H., Pang H., Zhang Z. 2012. Description of a new species of the genus *Xenophrys* Guenther, 1864 (Amphibia: Anura: Megophryidae) from Mount Jinggang, China, based on molecular and morphological data. Zootaxa, 3546: 53–67

Wang Y. Y., Zhao J., Yang J. H., Zhou Z. M., Chen G. L., Liu Y. 2014. Morphology, molecular genetics, and bioacoustics support two new sympatric *Megophrys* (Amphibia: Anura Megophryidae) species in Southeast China. Plos ONE, 9: e93075

Wu L., Dong Q., Xu R. H. 1986. Amphibians of Guizhou province. Guiyang, China: Guizhou People Press (In Chinese)

Yang J. H., Wang J., Wang Y. Y. 2018. A new species of the genus *Megophrys* (Anura: Megophryidae) from Yunnan Province, China. Zootaxa, 4413: 325–338.

Ye C. Y., Fei L. 1992. A new Pelobatid toad of the genus *Megophrys*from Xizang, China. Acta Herpetol Sinica, 1–2: 50–52 (In Chinese)

Ye C. Y., Fei L. 1995. Taxonomic studies on the small type *Megophrys* in China including descriptions of the new species (subspecies) (Pelobatidae: genus *Megophrys*). Acta Herpetol Sinica, 4–5: 72–81 (In Chinese)

Ye C. Y., Fei L., Xie F. 2007. A new species of Megophryidae *Megophrys baolongensis* from China (Amphibia, Anura). Acta Herpetol Sinica, 11: 38–41

Zhang Y., Li G., Xiao N., Li J., Pan T., Wang H., Zhang B., Zhou J. 2017. A new species of the genus *Megophrys* (Amphibia: Anura: Megophryicae) from Libo County, Guizhou, China. Asian Herpetol Res, 8: 75–85

Zhao J., Yang J. H., Chen G. L., Chen C. Q., Wang Y. Y. 2014. Description of a new species of the genus *Brachytarsophrys* Tian and Hu, 1983 (Amphibia: Anura: Megophryidae) from Southern China based on molecular and morphological data. Asian Herpetol Res, 5(3): 150–160

Appendix

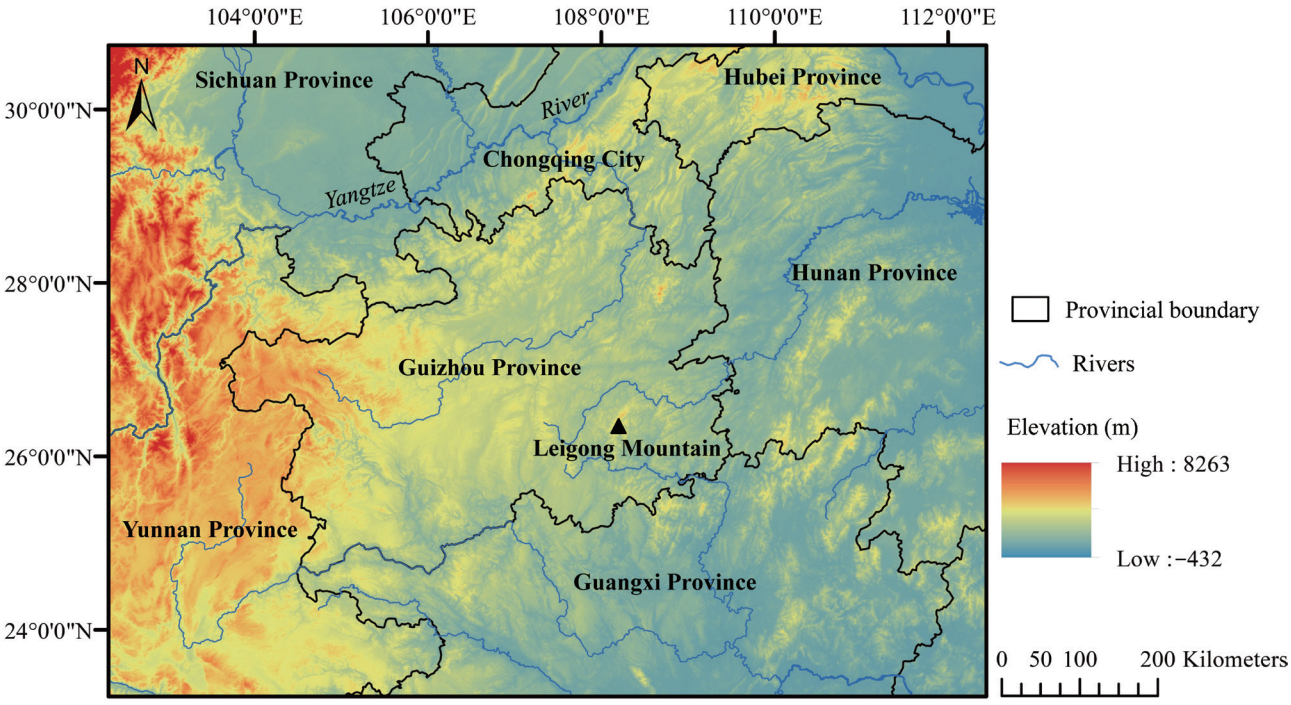

Figure S1 Geographical location of the type locality of *Megophrys leishanensis* sp. nov., Leigong Mountain, Leishan County, Guizhou Province, China.

Table S1 Measurements of the adult specimens of *Megophrys leishanensis* sp. nov.

Voucher number	Sex	SVL	HDL	HDW	SNT	IND	IOD	ED	TYD	LAL	LW	FIL	FIIL	FIIIL	FIVL	THL	TL	TW	TFL	FL
CIBLS20141004004	male	32.0	9.1	11.2	4.1	3.3	4.0	3.6	2.2	14.4	2.9	3.4	2.8	4.6	3.5	14.9	16.2	4.2	21.1	14.9
CIBLS20160610001	male	42.3	11.7	12.4	5.0	4.8	4.2	4.1	2.8	18.1	2.8	4.0	3.8	5.8	4.2	17.6	19.2	5.1	27.9	19.0
CIBLS20160610002	male	37.5	10.7	11.7	4.3	3.6	3.4	4.3	2.6	14.8	3.7	3.2	2.8	4.6	3.6	14.8	17.7	4.2	23.0	15.3
CIBLS20160610003	male	36.5	10.6	11.6	4.3	4.0	3.8	4.0	2.3	16.3	3.7	3.7	3.5	5.4	3.9	16.8	18.6	4.7	23.8	17.1
CIBLS20160610004	male	34.4	10.6	11.7	4.5	4.3	3.9	4.0	2.0	15.9	2.7	3.5	3.0	5.0	3.8	15.4	18.2	4.3	25.1	16.8
CIBLS20160610006	male	38.7	11.0	12.0	4.3	4.3	4.3	4.7	2.5	15.5	3.9	3.9	3.4	5.2	4.1	16.8	18.6	4.3	25.9	17.6
CIBLS20171001001	male	42.3	11.3	12.1	4.5	4.1	3.9	4.3	2.5	18.4	2.9	4.3	4.1	5.4	4.3	17.7	19.2	4.8	27.5	18.1
CIBLS20171001003	male	31.9	9.3	11.0	4.2	4.1	3.9	4.1	2.5	14.7	2.8	3.3	2.9	4.3	3.4	15.7	16.3	3.9	22.7	14.9
CIBLS20171001004	male	34.8	10.4	11.5	4.5	4.2	3.5	4.3	2.5	15.5	2.9	3.4	3.4	5.0	3.6	14.4	17.3	4.1	23.3	15.7
CIBLS20171001005	male	33.2	9.4	10.7	4.0	3.6	3.3	3.6	2.2	14.7	2.7	3.3	2.8	4.9	3.5	15.5	16.8	3.6	23.4	15.3
CIBLS20160610005	female	33.2	10.0	11.6	4.3	3.9	3.4	3.5	2.0	15.2	3.3	3.3	2.9	5.0	3.4	14.7	16.8	4.2	21.8	14.9
CIBLS20171001002	female	30.4	9.6	10.5	3.6	3.7	3.5	3.9	2.4	16.1	3.2	3.7	3.0	4.2	3.8	15.5	18.4	3.8	24.7	16.4

Unit: mm. See abbreviations for the morphological characters in Materials and Methods section.

Table S2 Information of samples used in the molecular analyses in this study. *sequence was downloaded from GenBank.

Sample Number	Species	Voucher number	Locality	GenBank Accession number			
				16s	COI	RAG1	BNDF
1	*Megophrys leishanensis* sp. nov.	KIZ049172	Leigong Shan, Guizhou, China	KX811825*	KX812102*	KX812204*	KX811946*
2	*Megophrys leishanensis* sp. nov.	SYSa002213	Mt. Leigong, Guizhou, China	MH406673*	MH406113*	MH404881*	–
3	*Megophrys leishanensis* sp. nov.	CIBLS20171101001	Leigong Shan, Guizhou, China	MK005310	MK005306	MK005318	MK005314
4	*Megophrys leishanensis* sp. nov.	CIBLS20141004004	Leigong Shan, Guizhou, China	MK005307	MK005303	MK005315	MK005311
5	*Megophrys leishanensis* sp. nov.	CIBLS20171001003	Leigong Shan, Guizhou, China	MK005308	MK005304	MK005316	MK005312
6	*Megophrys leishanensis* sp. nov.	CIBLS20160610005	Leigong Shan, Guizhou, China	MK005309	MK005305	MK005317	MK005313
7	*Megophrys baolongensis*	KIZ019216	Baolong, Chongqing, China	KX811813*	KX812093*	KX812202*	KX811945*
8	*Megophrys wushanensis*	KIZ045469	Guangwu Shan, Sichuan, China	KX811838*	KX812094*	KX812203*	KX811984*
9	*Megophrys tuberogranulata*	Tissue ID: YPX10987	Badagongshan Nature Reserve, Hunan, China	KX811823*	KX812095*	KX812209*	KX811979*
10	*Megophrys lini*	SYS a002370	Suichuan, Jiangxi, China	KJ560412*	–	–	–
11	*Megophrys brachykolos*	ROM 16634	Hong Kong, China	KX811897*	KX812150*	KX812217*	KX811985*
12	*Megophrys kuatunensis*	SYS a001579	Wuyi Shan, Fujian, China	KJ560376*	–	–	–
13	*Megophrys cheni*	SYS a001427	Jinggang Shan, Jiangxi, China	KJ560391*	–	–	–
14	*Megophrys obesa*	SYS a002272	Heishiding Nature Reserve, Guangdong, China	KJ579122*	–	–	–
15	*Megophrys acuta*	SYS a001957	Heishiding Nature Reserve, Guangdong, China	KJ579118*	–	–	–
16	*Megophrys jinggangensis*	KIZ07132	Chashan Forest Farm, Jiangxi, China	KX811840*	KX812108*	KX812242*	KX811986*
17	*Megophrys huangshanensis*	KIZ022004	Huang Shan, Anhui, China	KX811821*	KX812107*	KX812216*	KX811983*
18	*Megophrys boettgeri*	Tissue ID: YPXJK033	Wuyi Shan, Fujian, China	KX811814*	KX812104*	KX812213*	KX811980*
19	*Megophrys spinata*	KIZ016100	Leigong Shan, Guizhou, China	KX811864*	KX812119*	KX812228*	KX811963*
20	*Megophrys sangzhiensis*	Tissue ID: YPX11006	Badagongshan Nature Reserve, Hunan, China	KX811856*	KX812117*	KX812226*	KX811961*
21	*Megophrys binlingensis*	KIZ025807	Wawu Shan, Sichuan, China	KX811852*	KX812115*	KX812221*	KX811970*
22	*Megophrys omeimontis*	KIZ025765	Emei Shan, Sichuan, China	KX811884*	KX812136*	KX812223*	KX811967*
23	*Megophrys binchuanensis*	KIZ019441	Jizu Shan, Yunnan, China	KX811849*	KX812112*	KX812219*	KX811952*
24	*Megophrys palpebralespinosa*	KIZ011603	Pu Hu Nature Reserve, Thanh Hoa, Vietnam	KX811888*	KX812137*	KX812236*	KX811994*
25	*Megophrys wuliangshanensis*	KIZ046812	Huangcaoling, Yunnan, China	KX811881*	KX812129*	KX812258*	KX811958*
26	*Megophrys daweimontis*	KIZ048997	Dawei Shan, Yunnan, China	KX811867*	KX812125*	KX812248*	KX811969*
27	*Megophrys jingdongensis*	KIZ-LC0805067	Huanglianshan National Nature Reserve, Yunnan, China	KX811872*	KX812131*	KX812232*	KX811973*
28	*Megophrys minor*	KIZ01939	Qingcheng Shan, Sichuan, China	KX811896*	KX812145*	KX812253*	KX812045*
29	*Megophrys pachyproctus*	KIZ010978	Beibeng, Xizang, China	KX811908*	KX812153*	KX812265*	KX811998*
30	*Megophrys gerti*	ITBCZ 1108	Nui Chua National Park, Ninh Thuan, Vietnam	KX811917*	KX812161*	KX812279*	KX812037*
31	*Megophrys elfina*	ZMMU ABV-00454	Vietnam, Lam Dong Prov., Bidoup–Nui Ba N.P., Bidoup Mt.	KY425379*	–	–	–
32	*Megophrys synoria*	FMNH 262778	Cambodia, Mondolkiri Prov., O'Reang	KY022198*	–	–	–
33	*Megophrys hansi*	KIZ010360	Phong Dien Nature Reserve, Thua Thien Hue, Vietnam	KX811913*	KX812155*	–	–
34	*Megophrys microstoma*	KIZ048799	Xiaoqiaogou Nature Reserve, Yunnan, China	KX811914*	KX812156*	KX812277*	KX812039*
35	*Megophrys maosonensis*	KIZ016045	Xiaoqiaogou Nature Reserve, Yunnan, China	KX811780*	KX812080*	KX812195*	KX812024*
36	*Megophrys mangshanensis*	KIZ021786	Nanling National Forest Park, Guangdong, China	KX811790*	KX812079*	KX812194*	KX812025*
37	*Megophrys cf. major*	KIZ024336	Ban Pang Kamphaeng Hin, Chiang Mai, Thailand	KX811770*	KX812091*	KX812191*	KX812016*
38	*Megophrys glandulosa*	KIZ048439	Husa, Yunnan, China	KX811762*	KX812075*	KX812184*	KX812012*

Sample Number	Species	Voucher number	Locality	GenBank Accession number			
				16s	COI	RAG1	BNDF
39	*Megophrys medogensis*	KIZ06621	Beibeng, Xizang, China	KX811767*	KX812082*	KX812197*	KX812017*
40	*Megophrys baluensis*	ZMH A13125	Malaysia: Sabah: Gunung Kinabalu National Park: Kogopan Trail	KJ831310*	–	–	–
41	*Megophrys sanu*	K5198/ZSI11393	–	KX894679*	–	–	–
42	*Megophrys zhangi*	KIZ014278	Zhangmu, Xizang, China	KX811765*	KX812084*	KX812200*	KX812026*
43	*Megophrys auralensis*	NCSM 79599	Aural, Kampong Speu, Cambodia	KX811807*	–	–	–
44	*Megophrys katabhako*	ZSIA11799	–	KX894669*	–	–	–
45	*Megophrys cf. parva*	KIZ048508	Mengyue, Yunnan, China	KX811797*	KX812072*	KX812181*	KX812006*
46	*Megophrys aceras*	KIZ025467	Khao Nan National Park, Nakhon Si Thammarat, Thailand	KX811925*	KX812159*	KX812262*	KX812030*
47	*Megophrys nankiangensis*	CIB ZYC517	Nanjiang, Sichuan, China	KX811900*	–	–	–
48	*Megophrys wawuensis*	KIZ025799	Wawu Shan, Sichuan, China	KX811902*	KX812062*	KX812271*	KX812041*
49	*Megophrys gigantica*	SYSa003933	Mt. Wuliang, Yunnan, China	MH406775*	MH406235*	MH405011*	–
50	*Megophrys shapingensis*	KIZ014512	Liziping Nature Reserve, Sichuan, China	KX811904*	KX812060*	KX812274*	KX811943*
51	*Megophrys feae*	KIZ046706	Huangcaoling, Yunnan, China	KX811810*	KX812056*	KX812269*	KX811938*
52	*Megophrys chuananensis*	CIB20050081	China: Zihuai, Hejiang Co., Sichuan	KM504261*	–	–	–
53	*Megophrys carinense*	Tissue ID: YPX20455	Dayao Shan, Guangxi, China	KX811811*	KX812057*	KX812268*	KX811939*
54	*Megophrys popei*	SYS a000589	China: Naling NR, Guangdong	KM504251*	–	–	–
55	*Megophrys intermedia*	ZFMK 87596	Vietnam: U Bo, Phong Nha-Ke Bang NP	HQ588950*	–	–	–
56	*Megophrys montana*	LSUMZ 81916	Sukabumi, Java, Indonesia	KX811927*	KX812163*	–	–
57	*Megophrys lancip*	MZB:Amp:22233	–	KY679891*	–	–	–
58	*Megophrys dringi*	UNIMAS 8943	Malaysia: Sarawak: Gunung Mulu National Park	KJ831317*	–	–	–
59	*Megophrys baluensis*	ZMH A13125	Malaysia: Sabah: Gunung Kinabalu National Park: Kogopan Trail	KJ831310*	–	–	–
60	*Megophrys stejnegeri*	KU 314303	Pasonanca Natural Park, Zamboanga City, Philippines	KX811922*	KX812052*	–	–
61	*Megophrys ligayae*	ZMMU NAP-05015	Palawan, Philippines	KX811919*	KX812051*	KX812169*	KX811933*
62	*Megophrys edwardinae*	FMNH 273694	Bintulu, Sarawak, Malaysia	KX811918*	KX812050*	–	–
63	*Megophrys kobayashii*	UNIMAS 8148	Malaysia: Sabah: Gunung Kinabalu National Park	KJ831313 *	–	–	–
64	*Megophrys nasuta*	KIZ019419	Malaysia, no exact locality	KX811921*	KX812054*	KX812171*	KX811935*
65	*Leptolalax oshanensis*	KIZ025778	Emei Shan, Sichuan, China	KX811928*	KX812166*	KX812284*	KX812048*
66	*Leptobrachium boringii*	Tissue ID: YPX37539	Emei Shan, Sichuan, China	KX811930*	KX812164*	KX812282*	KX812046*

Table S3 Primers used in this study.

Locus	Primer name	Sequence (5′–3′)	Source
16S rRNA	P7	CGCCTGTTTACCAAAAACAT	Simon *et al.*, 1994
	P8	CCGGTCTGAACTCAGATCACGT	
COI	Chmf4	TYTCWACWAAYCAYAAAGAYATCGG	Che *et al.,* 2012
	Chmr4	ACYTCRGGRTGRCCRAARAATCA	
RAG1	RAG1-F1	TAYCAYAARATGTAYMGNACNGT	Shen *et al.*, 2013
	RAG1-R1	GANGTRTANARCCARTGRTGYTT	
	RAG1-F2	AGGGTTTTCCCAGTCACGACACKGGGMGVCARATHTTYCARCC	
	RAG1-R2	AGATAACAATTTCACACAGGTTNGACTGNCKSGCRTTCATYTT	
BDNF	BDNF-F	ACCATCCTTTTCCTKACTATGG	Vieites *et al*., 2007
	BDNF-R	CTATCTTCCCCTTTTAATGGTC	

Table S4 Uncorrected *p*-distance between *Megophrys sensu lato* species based on COI gene sequences.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44
1	*Megophrys leishanensis* sp.nov.																																												
2	*Megophrys leishanensis* sp.nov.	0.002																																											
3	*Megophrys leishanensis* sp.nov.	0.004	0.002																																									
4	*Megophrys leishanensis* sp.nov.	0.002	0.000	0.002																																								
5	*Megophrys leishanensis* sp.nov.	0.004	0.002	0.000	0.002																																							
6	*Megophrys leishanensis* sp.nov.	0.002	0.000	0.002	0.000	0.002																																						
7	*Megophrys baolongensis*	0.091	0.093	0.096	0.093	0.096	0.093																																					
8	*Megophrys wushanensis*	0.077	0.074	0.077	0.074	0.077	0.074	0.059																																				
9	*Megophrys tuberogranulata*	0.108	0.106	0.108	0.106	0.108	0.106	0.088	0.071																																			
10	*Megophrys brachykolos*	0.171	0.168	0.171	0.168	0.171	0.168	0.159	0.143	0.136																																		
11	*Megophrys jinggangensis*	0.114	0.114	0.116	0.114	0.116	0.114	0.131	0.091	0.116	0.151																																	
12	*Megophrys huangshanensis*	0.116	0.114	0.116	0.114	0.116	0.114	0.115	0.093	0.108	0.146	0.107																																
13	*Megophrys boettgeri*	0.113	0.111	0.113	0.111	0.113	0.111	0.120	0.093	0.108	0.154	0.115	0.014																															
14	*Megophrys spinata*	0.189	0.186	0.183	0.186	0.183	0.186	0.176	0.157	0.163	0.189	0.178	0.174	0.179																														
15	*Megophrys sangzhiensis*	0.165	0.162	0.160	0.162	0.160	0.162	0.166	0.148	0.154	0.193	0.172	0.148	0.148	0.040																													
16	*Megophrys binlingensis*	0.144	0.142	0.139	0.142	0.139	0.142	0.137	0.120	0.118	0.158	0.153	0.139	0.138	0.075	0.067																												
17	*Megophrys omeimontis*	0.145	0.143	0.145	0.143	0.145	0.143	0.153	0.145	0.137	0.214	0.159	0.181	0.175	0.125	0.115	0.090																												
18	*Megophrys binchuanensis*	0.161	0.158	0.156	0.158	0.156	0.158	0.154	0.126	0.147	0.179	0.154	0.147	0.147	0.099	0.092	0.078	0.100																										
19	*Megophrys palpebralespinosa*	0.176	0.173	0.171	0.173	0.171	0.173	0.167	0.153	0.179	0.188	0.189	0.188	0.182	0.130	0.131	0.105	0.144	0.116																									
20	*Megophrys wuliangshanensis*	0.149	0.146	0.149	0.146	0.149	0.146	0.152	0.121	0.137	0.172	0.155	0.145	0.140	0.107	0.101	0.090	0.108	0.076	0.131																								
21	*Megophrys daweimontis*	0.178	0.175	0.178	0.175	0.178	0.175	0.165	0.136	0.161	0.178	0.165	0.165	0.164	0.133	0.123	0.102	0.128	0.113	0.138	0.105																							
22	*Megophrys jingdongensis*	0.172	0.169	0.172	0.169	0.172	0.169	0.164	0.122	0.143	0.165	0.155	0.151	0.150	0.132	0.135	0.113	0.143	0.108	0.129	0.103	0.117																						
23	*Megophrys minor*	0.137	0.135	0.137	0.135	0.137	0.135	0.120	0.113	0.129	0.141	0.142	0.129	0.126	0.158	0.148	0.133	0.132	0.127	0.134	0.121	0.116	0.129																						
24	*Megophrys pachyproctus*	0.180	0.180	0.183	0.180	0.183	0.180	0.195	0.172	0.176	0.207	0.198	0.208	0.211	0.204	0.214	0.181	0.183	0.183	0.193	0.177	0.217	0.152	0.166																				
25	*Megophrys gerti*	0.239	0.236	0.239	0.236	0.239	0.236	0.228	0.228	0.246	0.279	0.246	0.231	0.236	0.231	0.239	0.245	0.262	0.241	0.257	0.249	0.267	0.229	0.235	0.259																			
26	*Megophrys hansi*	0.223	0.223	0.226	0.223	0.226	0.223	0.237	0.205	0.229	0.254	0.237	0.219	0.209	0.259	0.242	0.242	0.268	0.251	0.258	0.244	0.244	0.214	0.222	0.253	0.210																		
27	*Megophrys microstoma*	0.209	0.209	0.212	0.209	0.212	0.209	0.205	0.212	0.219	0.224	0.233	0.228	0.236	0.229	0.209	0.207	0.229	0.204	0.224	0.211	0.225	0.202	0.198	0.192	0.229	0.162																	
28	*Megophrys maosonensis*	0.193	0.193	0.196	0.193	0.196	0.193	0.224	0.212	0.211	0.249	0.215	0.226	0.219	0.229	0.231	0.220	0.204	0.190	0.222	0.193	0.211	0.206	0.187	0.190	0.252	0.249	0.180																
29	*Megophrys mangshanensis*	0.219	0.219	0.222	0.219	0.222	0.219	0.226	0.221	0.211	0.253	0.229	0.234	0.227	0.237	0.233	0.208	0.197	0.205	0.232	0.197	0.204	0.202	0.194	0.198	0.273	0.275	0.208	0.042															
30	*Megophrys cf. major*	0.217	0.217	0.220	0.217	0.220	0.217	0.207	0.230	0.202	0.221	0.226	0.243	0.246	0.274	0.253	0.216	0.217	0.221	0.242	0.218	0.211	0.181	0.216	0.216	0.304	0.272	0.199	0.135	0.125														
31	*Megophrys glandulosa*	0.219	0.216	0.219	0.216	0.219	0.216	0.209	0.222	0.191	0.223	0.212	0.198	0.206	0.197	0.204	0.181	0.205	0.193	0.215	0.187	0.177	0.185	0.184	0.206	0.234	0.277	0.201	0.123	0.115	0.126													
32	*Megophrys medogensis*	0.203	0.200	0.203	0.200	0.203	0.200	0.219	0.215	0.207	0.223	0.206	0.211	0.204	0.216	0.218	0.219	0.220	0.198	0.214	0.195	0.188	0.190	0.178	0.203	0.261	0.255	0.197	0.117	0.105	0.136	0.106												
33	*Megophrys zhangi*	0.187	0.187	0.189	0.187	0.189	0.187	0.190	0.215	0.188	0.238	0.214	0.215	0.211	0.221	0.216	0.189	0.190	0.199	0.224	0.208	0.210	0.201	0.200	0.215	0.280	0.272	0.181	0.125	0.117	0.141	0.106	0.125											
34	*Megophrys aurelensis*	0.187	0.187	0.189	0.187	0.189	0.187	0.190	0.215	0.188	0.238	0.214	0.215	0.211	0.221	0.216	0.189	0.190	0.199	0.224	0.208	0.210	0.201	0.200	0.215	0.280	0.272	0.181	0.125	0.117	0.141	0.106	0.125	0.000										
35	*Megophrys cf. parva*	0.219	0.216	0.219	0.216	0.219	0.216	0.229	0.204	0.220	0.230	0.231	0.225	0.218	0.246	0.229	0.205	0.206	0.208	0.223	0.212	0.181	0.186	0.204	0.221	0.247	0.246	0.189	0.145	0.142	0.164	0.150	0.154	0.177	0.177									
36	*Megophrys acerus*	0.222	0.218	0.222	0.218	0.222	0.218	0.205	0.202	0.220	0.261	0.234	0.247	0.240	0.268	0.267	0.243	0.223	0.218	0.261	0.215	0.241	0.215	0.207	0.195	0.230	0.218	0.198	0.228	0.239	0.261	0.244	0.236	0.262	0.262	0.224								
37	*Megophrys wuwuensis*	0.177	0.174	0.177	0.174	0.177	0.174	0.181	0.162	0.177	0.201	0.188	0.205	0.201	0.222	0.215	0.192	0.185	0.186	0.199	0.178	0.217	0.164	0.168	0.182	0.227	0.203	0.169	0.157	0.169	0.188	0.197	0.173	0.168	0.168	0.157	0.201							
38	*Megophrys gigantica*	0.219	0.216	0.219	0.216	0.219	0.216	0.211	0.188	0.189	0.235	0.215	0.221	0.212	0.241	0.231	0.178	0.210	0.206	0.214	0.209	0.212	0.172	0.188	0.232	0.231	0.225	0.210	0.174	0.183	0.199	0.200	0.188	0.206	0.206	0.180	0.219	0.103						
39	*Megophrys shapingensis*	0.173	0.170	0.173	0.170	0.173	0.170	0.173	0.168	0.168	0.216	0.200	0.202	0.193	0.231	0.221	0.183	0.199	0.200	0.194	0.196	0.205	0.180	0.160	0.202	0.228	0.225	0.181	0.144	0.166	0.197	0.178	0.168	0.151	0.151	0.169	0.208	0.074	0.076					
40	*Megophrys feae*	0.236	0.239	0.242	0.239	0.242	0.239	0.234	0.207	0.239	0.246	0.226	0.217	0.213	0.241	0.239	0.228	0.248	0.235	0.273	0.245	0.224	0.230	0.222	0.238	0.278	0.259	0.232	0.227	0.237	0.243	0.202	0.193	0.218	0.218	0.224	0.239	0.188	0.196	0.199				
41	*Megophrys carinense*	0.231	0.234	0.237	0.234	0.237	0.234	0.246	0.227	0.275	0.216	0.244	0.253	0.249	0.257	0.259	0.247	0.254	0.253	0.265	0.245	0.259	0.249	0.224	0.236	0.273	0.265	0.208	0.236	0.249	0.239	0.236	0.222	0.215	0.215	0.236	0.245	0.192	0.226	0.225	0.100			
42	*Megophrys montana*	0.322	0.325	0.329	0.325	0.329	0.325	0.311	0.322	0.311	0.306	0.330	0.315	0.307	0.297	0.281	0.265	0.282	0.310	0.287	0.313	0.275	0.277	0.283	0.314	0.300	0.301	0.288	0.300	0.283	0.277	0.271	0.289	0.282	0.282	0.244	0.282	0.286	0.285	0.297	0.268	0.240		
43	*Megophrys stejnegeri*	0.251	0.248	0.251	0.248	0.251	0.248	0.270	0.230	0.254	0.285	0.241	0.258	0.247	0.256	0.256	0.239	0.251	0.252	0.310	0.257	0.244	0.240	0.248	0.270	0.281	0.259	0.268	0.273	0.281	0.299	0.255	0.293	0.262	0.262	0.273	0.226	0.250	0.271	0.250	0.236	0.251	0.270	
44	*Megophrys ligayae*	0.273	0.273	0.277	0.273	0.277	0.273	0.278	0.270	0.284	0.298	0.286	0.270	0.258	0.292	0.285	0.262	0.280	0.273	0.320	0.260	0.270	0.274	0.259	0.285	0.317	0.237	0.248	0.300	0.306	0.329	0.328	0.310	0.276	0.276	0.277	0.283	0.252	0.276	0.265	0.274	0.284	0.290	0.215	
45	*Megophrys nasuta*	0.275	0.272	0.275	0.272	0.275	0.272	0.292	0.276	0.302	0.311	0.257	0.313	0.301	0.277	0.277	0.257	0.270	0.243	0.297	0.250	0.256	0.270	0.290	0.244	0.310	0.282	0.220	0.241	0.255	0.260	0.260	0.237	0.240	0.240	0.254	0.239	0.237	0.265	0.236	0.252	0.280	0.326	0.209	0.233