Description of a new horned toad of *Megophrys* Kuhl & Van Hasselt, 1822 (Anura, Megophryidae) from southwest China

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Academic editor: Anthony Herrel  |  Received 1 July 2020  |  Accepted 24 August 2020  |  Published 7 October 2020

Citation: Su H, Shi S, Wu Y, Li G, Yao X, Wang B, Li S (2020) Description of a new horned toad of *Megophrys* Kuhl & Van Hasselt, 1822 (Anura, Megophryidae) from southwest China. ZooKeys 974: 131–159. https://doi.org/10.3897/zookeys.974.56070

Abstract

A new species of the genus *Megophrys* is described from Guizhou Province, China. Molecular phylogenetic analyses supported the new species as an independent clade nested into the *Megophrys*. The new species could be distinguished from its congeners by a combination of the following characters: body size moderate (SVL 49.3–58.2 mm in males); vomerine ridges present distinctly, vomerine teeth present; tongue feebly notched behind; tympanum distinctly visible, oval; two metacarpal tubercles in hand; toes with one-third webbing and wide lateral fringes; heels overlapped when thighs are positioned at right angles to the body; tibiotarsal articulation reaching the level between tympanum and eye when leg stretched forward; an internal single subgular vocal sac present in male; in breeding male, the nuptial pads with large and sparse black nuptial spines present on the dorsal bases of the first two fingers.

Keywords

China, molecular phylogenetic analysis, morphology, new species, taxonomy

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Introduction

The toad genus *Megophrys* Kuhl & Van Hasselt, 1822 (Anura; Megophryidae) is widely distributed in eastern and central China, throughout southeastern Asia, and extending to the islands of the Sunda Shelf and the Philippines (Frost 2020). The taxonomic assignments of the taxa in this group have been debated for a long time (e.g., Tian and Hu 1983; Dubois 1987; Lathrop 1997; Rao and Yang 1997; Jiang et al. 2003; Delorme et al. 2006; Fei et al. 2009; Chen et al. 2016; Fei and Ye 2016; Deuti et al. 2017; Mahony et al. 2017; Frost 2020). Regardless, molecular phylogenetic studies indicate the group as a monophyletic group (Chen et al. 2017; Mahony et al. 2017; Li et al. 2018b; Liu et al. 2018, 2020; Wang et al. 2020), and thus some studies regarded it as a large genus, *Megophrys sensu lato* (Mahony et al. 2017; Li et al. 2018b; Liu et al. 2018, 2020; Wang et al. 2020; Frost 2020) while other studies divided the taxon into different genera and subgenera (Chen et al. 2017; Fei and Ye 2016; Liu et al. 2018).

The genus *Megophrys* currently contains 106 species, of which 50 species have been described in the last decade (Frost 2020). Many cryptic species were indicated in the genus by molecular phylogenetic frameworks (Chen et al. 2017; Liu et al. 2018). In recent years, four species were described from Guizhou Province, China: *Megophrys liboensis* Zhang, Li, Xiao, Li, Pan, Wang, Zhang & Zhou, 2017, *Megophrys leishanensis* Li, Xu, Liu, Jiang, Wei & Wang, 2018, *Megophrys jiangi* Liu, Li, Wei, Xu, Cheng, Wang & Wu, 2020, and *Megophrys chishuiensis* Xu, Li, Liu, Wei, & Wang, 2020. However, many areas have not been well investigated in this province, and it is expected that there are still cryptic species of the toads in the region.

During field surveys in the Huanglian Nature Reserve, Tongzi County, and Kuankuoshui National Nature Reserve, Suiyang County in Guizhou Province, China, we collected a number of *Megophrys* specimens. Molecular phylogenetic analyses, morphological comparisons, and bioacoustics data support it as an undescribed species.

Materials and methods

Sampling

A total of nine molecular samples were collected in this study: five adult males of the undescribed species from two localities of Guizhou Province, China, two *M. sangzhiensis* and two *M. spinata* (Table 1; Fig. 1). In the field, the toads were euthanized using isoflurane, and the specimens were fixed in 75 % ethanol. Tissue samples were taken and preserved separately in 99% ethanol prior to fixation. The specimens were deposited in Chengdu Institute of Biology, Chinese Academy of Sciences (CIB, CAS).
A new species of Megophrys

Total DNA was extracted using a standard phenol-chloroform extraction protocol (Sambrook et al. 1989). Two fragments of the mitochondrial 16S rRNA (16S) and cytochromeoxidase subunit I (COI) genes were amplified. For 16S, the primers P7 (5’-CGCCTGTATTACAAAACAT-3’) and P8 (5’-CCGGTCTGAACTCAGATCACGT-3’) were used following Simon et al. (1994), and for COI, Chmf4 (5’-TYTCWACWAAYCAYAAAGAYATCGG-3’) and Chmr4 (5’-ACYTCRGGRTGRCCRAARAATCA-3’) were used following Che et al. (2012). Gene fragments were amplified under the following conditions: an initial denaturing step at 95 °C for 4 min; 36 cycles of denaturing at 95 °C for 30 s, annealing at 52 °C (for 16S)/47 °C (for COI) for 40 s and extending at 72 °C for 70 s. Sequencing was conducted using an ABI3730 automated DNA sequencer in Shanghai DNA BioTechnologies Co., Ltd. (Shanghai, China). New sequences were deposited in GenBank (for accession numbers see Table 1).

For molecular analyses, the available sequence data for congeners of Megophrys were downloaded from GenBank (Table 1), primarily from previous studies (Chen et al. 2017; Liu et al. 2018). For phylogenetic analyses, corresponding sequences of one Leptobrachella oshanensis (Liu, 1950) and one Leptobrachium boringii (Liu, 1945) were also downloaded (Table 1), and used as outgroups according to Mahony et al. (2017). Sequences were assembled and aligned using the Clustalw module in BioEdit v.7.0.9.0
## Table 1. Information for samples used in molecular phylogenetic analyses in this study.

| ID   | Species                      | Voucher number               | Locality                              | GenBank accession number |
|------|------------------------------|------------------------------|---------------------------------------|--------------------------|
| 1    | Megophrys qianheimensis sp. nov. | CIBTZ201906080015            | Huanglian Nature Reserve, Guizhou, China | MT651553                   |
| 2    | Megophrys qianheimensis sp. nov. | CIBTZ201906080017            | Huanglian Nature Reserve, Guizhou, China | MT651554                   |
| 3    | Megophrys qianheimensis sp. nov. | CIBTZ20160715003             | Huanglian Nature Reserve, Guizhou, China | MT651555                   |
| 4    | Megophrys qianheimensis sp. nov. | CIBKKS20180722002            | Huanglian Nature Reserve, Guizhou, China | MT651556                   |
| 5    | Megophrys qianheimensis sp. nov. | CIBKKS20180722001            | Kuanxuoshui Nature Reserve, Guizhou, China | MT651557                   |
| 6    | Megophrys sangzhiensis         | CIBS20120602005              | Badagongshan Nature Reserve, Hunan, China | MT651558                   |
| 7    | Megophrys sangzhiensis         | CIBS20120602008              | Badagongshan Nature Reserve, Hunan, China | MT651559                   |
| 8    | Megophrys sangzhiensis         | SYSa004307                  | Zhangjiajie, Hunan, China              | MH406798                    |
| 9    | Megophrys spinata              | CIBLS20190801001             | Leigong Shan, Guizhou, China          | MT651551                   |
| 10   | Megophrys spinata              | CIBLS20190801002             | Leigong Shan, Guizhou, China          | MT651552                   |
| 11   | Megophrys spinata              | SYSa002227                  | Leigong Shan, Guizhou, China          | MH406676                    |
| 12   | Megophrys tinglingensis        | KZ023507                    | Wawu Shan, Sichuan, China             | KX811852                    |
| 13   | Megophrys tinglingensis        | SYSa005313                  | Wawu Shan, Sichuan, China             | MH406892                    |
| 14   | Megophrys tinglingensis        | SYSa005314                  | Wawu Shan, Sichuan, China             | MH406893                    |
| 15   | Megophrys tinglingensis        | KZ011944                    | Jiuzu Shan, Yunnan, China             | KX811849                    |
| 16   | Megophrys palpebralepina       | KZ0111603                   | Pu Hu Nature Reserve, Thanh Hoa, Vietnam | KX811888                    |
| 17   | Megophrys omemontis           | KZ0225765                   | Emei Shan, Sichuan, China             | KX811884                    |
| 18   | Megophrys angka                | KZ040491                    | Kiew Mac Pae nature trail, Chiang Mai, Thailand | MN508052 –                 |
| 19   | Megophrys walangshanensis      | KZ0404612                   | Huangcaoling, Yunnan, China           | KX811881                    |
| 20   | Megophrys walangshanensis      | SYSa004145                 | Nanke Shan, Guangdong, China          | KX812108                    |
| 21   | Megophrys chimengi             | SYSa001427                  | Jingtang Shan, Jiangxi, China         | KJ603591                    |
| 22   | Megophrys ohsea               | SYSa002272                  | Heishiding Nature Reserve, Guangdong, China | KJ579122 –                  |
| 23   | Megophrys semao               | KRM18                       | Wuyishan, Fujian, China               | KX856404                    |
| 24   | Megophrys semao               | VNMN 2018.01                | Lao Cai, Sa Pa, Vietnam               | MH514886 –                  |
| 25   | Megophrys xingyi               | VNMN 2018.02                | Lao Cai, Sa Pa, Vietnam               | MH514889 –                  |
| 26   | Megophrys minor              | KZ011939                    | Qinzheng Shan, Sichuan, China         | KX811896                    |
| 27   | Megophrys xingyi              | CIBKKS20180722006           | Kuanxuoshui Nature Reserve, Guizhou, China | MN107743                   |
| 28   | Megophrys xingyi              | CIBCS20190518051            | Chihui Nature Reserve, Guizhou, China | MN954070                     |
| 29   | Megophrys xingyi              | SYSa001972                  | Yinping Shan, Guangdong, China        | MK524098                    |
| 30   | Megophrys xingyi              | SYSa004498                  | Nanke Shan, Guangdong, China          | MK524108                    |
| 31   | Megophrys xingyi              | SYSa001579                  | Wawu Shan, Fujian, China              | KJ603576                    |
| 32   | Megophrys xingyi              | KZ017132                    | Chashan Forest Farm, Jiangxi, China   | KX811840                    |
| 33   | Megophrys xingyi              | WYF00169                   | Lishui, Zhejiang, China               | KY021418                    |
| 34   | Megophrys xingyi              | CIBJX3190505                | Xianju, Zhejiang, China               | MN653753                     |
| 35   | Megophrys xingyi              | CIB045469                   | Guangwu, Sichuan, China               | KX811838                    |
| 36   | Megophrys xingyi              | KZ012126                    | Baslong, Chongqing, China             | KX811813                    |
| 37   | Megophrys xingyi              | CIBLS2017110101             | Leigong Shan, Guizhou, China          | MK003510                     |
| 38   | Megophrys xingyi              | SYSa002877                  | Wugongshan Scenic Area, Jiangxi, China | MK524145                    |
| 39   | Megophrys xingyi              | SYSa002370                  | Suichuan, Jiangxi, China              | KJ604012                    |
| 40   | Megophrys xingyi              | SYSa002874                  | Yangming Shan, Hunan, China           | MH406713                     |
| 41   | Megophrys xingyi              | SYSa001959                  | Shimentai Nature Reserve, Guangdong, China | MK524111                   |
| 42   | Megophrys xingyi              | SYSa002107                  | Jiulian Shan, Jiangxi, China          | MK524099                    |
| 43   | Megophrys xingyi              | SYSa001579                  | Shuanhuang Mountain, Yunnan, China    | MK324130                     |
| 44   | Megophrys xingyi              | SYSa001292                  | Huaping Nature Reserve, Guangxi, China | MT651559                   |
| 45   | Megophrys xingyi              | SYSa001292                  | Wugongshan Scenic Area, Jiangxi, China | MT651559                   |
| 46   | Megophrys xingyi              | SYSa001292                  | Wugongshan Scenic Area, Jiangxi, China | MT651559                   |
| 47   | Megophrys xingyi              | SYSa001292                  | Wugongshan Scenic Area, Jiangxi, China | MT651559                   |
| 48   | Megophrys xingyi              | SYSa001292                  | Wugongshan Scenic Area, Jiangxi, China | MT651559                   |
| 49   | Megophrys xingyi              | SYSa001292                  | Wugongshan Scenic Area, Jiangxi, China | MT651559                   |
| 50   | Megophrys xingyi              | SYSa001292                  | Wugongshan Scenic Area, Jiangxi, China | MT651559                   |
| 51   | Megophrys xingyi              | SYSa001292                  | Wugongshan Scenic Area, Jiangxi, China | MT651559                   |
| 52   | Megophrys xingyi              | SYSa001292                  | Wugongshan Scenic Area, Jiangxi, China | MT651559                   |
| 53   | Megophrys xingyi              | SYSa001292                  | Wugongshan Scenic Area, Jiangxi, China | MT651559                   |
| 54   | Megophrys xingyi              | SYSa001292                  | Wugongshan Scenic Area, Jiangxi, China | MT651559                   |
| 55   | Megophrys xingyi              | SYSa001292                  | Wugongshan Scenic Area, Jiangxi, China | MT651559                   |
| 56   | Megophrys xingyi              | SYSa001292                  | Wugongshan Scenic Area, Jiangxi, China | MT651559                   |
| ID  | Species                  | Voucher number | Locality                          | GenBank accession number |
|-----|--------------------------|----------------|-----------------------------------|--------------------------|
| 57  | Megophrys synoria        | FMNH 262778    | O'Reang, Mondolkiri, Cambodia      | KY022198                 |
| 58  | Megophrys hansi          | KIZ010360      | Phong Dien Nature Reserve, Thua Thien Hue, Vietnam | KX811913 KX812155 |
| 59  | Megophrys microstoma     | KIZ048799      | Xiaoyaogou Nature Reserve, Yunnan, China | KX811914 KX812156 |
| 60  | Megophrys pachyproctus   | KIZ010978      | Beibeng, Xizang, China            | KX811908 KX812153 |
| 61  | Megophrys baluensis      | ZMH A13125     | Gunung Kinabalu National Park, Kogopan Trail, Malaysia | KJ831310                 |
| 62  | Megophrys stejnegeri     | KU 314303      | Pasonanca Natural Park, Zambosanga, Philippines | KX811922 KX812052 |
| 63  | Megophrys igyae          | ZMMU NAP-05015 | Palawan, Philippines              | KX811919 KX812051 |
| 64  | Megophrys kobayashii     | UNIMAS 8148    | Gunung Kinabalu National Park, Sabah, Malaysia | KJ831313                 |
| 65  | Megophrys nasuta         | KIZ019419      | Malaysia                          | KX811921 KX812054 |
| 66  | Megophrys eduardinae     | FMNH 273094    | Bintulu, Sarawak, Malaysia        | KX811918 KX812050 |
| 67  | Megophrys aceran         | KIZ025467      | Khao Nan National Park, Nakhon Si Thammarat, Thailand | KX811925 KX812159 |
| 68  | Megophrys diringi        | UNIMAS 8943    | Gunung Mulu National Park, Sarawak, Malaysia | KJ831317                 |
| 69  | Megophrys mawnensis      | KIZ016045      | Xiaoyaogou Nature Reserve, Yunnan, China | KX811780 KX812080 |
| 70  | Megophrys mangshanensis  | KIZ021786      | Narling National Forest Park, Guangdong, China | KX811790 KX812079 |
| 71  | Megophrys flavopunctata  | SDBDU2009.297  | East Khazi Hills dist., Meghalaya  | KY022307 MH647536 |
| 72  | Megophrys glandulosa     | KIZ048439      | Hua, Yunnan, China                | KX811762 KX812075 |
| 73  | Megophrys medogensis      | KIZ06621       | Beibeng, Xizang, China            | KX811767 KX812082 |
| 74  | Megophrys serina         | BNHS 6061      | West Kameng dist., Arunachal Pradesh, IN | KY022309 MH647528 |
| 75  | Megophrys himalayana     | SDBDU2009.75   | East Siang dist., Arunachal Pradesh, IN | KY022351                 |
| 76  | Megophrys sanas          | KSJ987/2SII1393|                                  | KX894679                 |
| 77  | Megophrys zhongi         | KIZ014278      | Zhangmu, Xizang, China            | KX811765 KX812084 |
| 78  | Megophrys katahakho       | ZSA11799       |                                  | KX894669                 |
| 79  | Megophrys major          | SYSa02961      | Zhushuhe, Yunnan, China           | MH406728 MH406180 |
| 80  | Megophrys avrocypta      | BNHS 6046      | West Garo Hills dist., Meghalaya  | KY022306                 |
| 81  | Megophrys awalensis       | NCMS 79599     | Aural, Kampong Speu, Cambodia      | KX811807                 |
| 82  | Megophrys para           | SYSa03042      | Zhushuhe, Yunnan, China           | MH406737 MH406189 |
| 83  | Megophrys nankiangensis  | CIB ZY517      | Nanjiang, Sichuan, China          | KX811900                 |
| 84  | Megophrys wuansui        | KIZ025799      | Wawu Shan, Sichuan, China         | KX811902 KX812062 |
| 85  | Megophrys gigamica       | SYSa03933      | Wuliang Shan, Yunnan, China       | MH406775 MH406235 |
| 86  | Megophrys kaopingensis    | KIZ014512      | Lizining Nature Reserve, Sichuan, China | KX811904 KX812060 |
| 87  | Megophrys montana        | LSUMZ 81916    | Sukabumi, Java, Indonesia         | KX811927 KX812163 |
| 88  | Megophrys lacinip        | MZB:Amp:22233  |                                  | KY679891                 |
| 89  | Megophrys fuse           | KIZ046706      | Huangcailing, Yunnan, China       | KX811810 KX812056 |
| 90  | Megophrys chuanxuanensis | CIB20050881    | Hejiang, Sichuan, China           | KM504261                 |
| 91  | Megophrys carinatus      | Tissue ID: TPA20455 | Dayao Shan, Guangxi, China, China | KX811811 KX812057 |
| 92  | Megophrys popei          | SYS a00589     | Naling Nature Reserve, Guangdong, China | KM504251                 |
| 93  | Megophrys intermediens   | ZFMK 87596     | U Bo, Phong Nha-Ke Bang VN, Vietnam | HGS89590                   |
| 94  | Lepisosteus boreinii     | Tissue ID: TPA37539 | Emei Shan, Sichuan, China         | KX811930 KX812164 |
| 95  | Lepisosteus sphenodontus | KIZ025778      | Emei Shan, Sichuan, China         | KX811928 KX812166 |

(Hall 1999) with default settings. Alignments were checked by eye and revised manually if necessary. For phylogenetic analyses of mitochondrial DNA, the dataset concatenated with 16S and COI gene sequences. 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 v. 1.1.1 (Robert et al. 2012). In this analysis, 16S gene and each codon position of COI gene were defined, and Bayesian Inference Criteria was used. As a result, the analysis suggested that the best partition scheme is 16S gene/each codon position of COI gene, and selected GTR + G + I model as the best model for each partition. Phylogenetic analyses were conducted using maximum likelihood (ML)
and Bayesian Inference (BI) methods, implemented in PhyML v. 3.0 (Guindon et al. 2010) and MrBayes v. 3.12 (Ronquist and Huelsenbeck 2003), respectively. For the ML tree, branch supports were drawn from 10,000 nonparametric bootstrap replicates. In BI, two runs each with four Markov chains were simultaneously run for 50 million generations with sampling every 1,000 generations. The first 25% trees were removed as the “burn-in” stage followed by calculations of Bayesian posterior probabilities and the 50% majority-rule consensus of the post burn-in trees sampled at stationarity.

**Morphological comparisons**

In total, 16 specimens including six males of the undescribed species, five males of *M. sangzhiensis*, and five males of *M. spinata* were measured (for voucher information see Table 2). The terminology and methods followed Fei et al. (2009). Measurements were taken with a dial caliper to 0.1 mm. Twenty-one morphometric characters of adult specimens were measured:

- **ED** eye diameter (distance from the anterior corner to the posterior corner of the eye);
- **FL** foot length (distance from tarsus to the tip of fourth toe);
- **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);
- **HLL** hindlimb length (maximum length from the vent to the distal tip of the Toe IV);
- **IAE** distance between posterior corner of eyes;
- **IFE** distance between anterior corner of eyes;
- **IND** internasal distance (minimum distance between the inner margins of the external nares);
- **IOD** interorbital distance (minimum distance between the inner edges of the upper eyelids);
- **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);
- **NED** nasal to eye distance (distance between the nasal and the anterior corner of the eye);
- **NSD** nasal to snout distance (distance between the nasal the posterior edge of the vent);
- **SVL** snout-vent length (distance from the tip of the snout to the posterior edge of the vent);
- **SL** snout length (distance from the tip of the snout to the anterior corner of the eye);
- **TFL** length of foot and tarsus (distance from the tibiotarsal articulation to the distal end of the Toe IV);
- **THL** thigh length (distance from vent to knee);
- **TL** tibia length (distance from knee to tarsus);
- **TW** maximal tibia width;
- **TYD** maximal tympanum diameter;
- **UEW** upper eyelid width (greatest width of the upper eyelid margins measured perpendicular to the anterior-posterior axis).
Table 2. Measurements of the adult specimens of *Megophrys qianbeiensis* sp. nov., *M. spinata*, and *M. sangzhiensis*. Units are given in mm. See abbreviations for the morphological characters in Materials and methods section.

| Species                        | Voucher number | Sex | SVL  | HDL  | HDW  | SL   | NED  | NSD  | IND  | IOD  | ED   | UEW  | LAL  | LW   | HLL  | THL  | TL   | TW   | TFL  | FL   | TYD  | IFE  | IAE  |
|-------------------------------|----------------|-----|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| *Megophrys qianbeiensis* sp. nov. | CIBTZ20190608016 | male | 58.2 | 16.5 | 21.0 | 6.7  | 2.7  | 3.9  | 7.5  | 5.1  | 6.9  | 6.2  | 25.0 | 6.7  | 89.3 | 27.9 | 32.4 | 8.2  | 40.7 | 28.2 | 4.3  | 10.1 | 16.4 |
| *Megophrys qianbeiensis* sp. nov. | CIBTZ20190608018 | male | 55.1 | 14.9 | 20.6 | 6.9  | 3.0  | 4.0  | 7.0  | 4.2  | 6.5  | 5.1  | 25.0 | 6.5  | 93.0 | 28.4 | 30.6 | 8.7  | 44.0 | 29.1 | 3.5  | 9.0  | 16.0 |
| *Megophrys qianbeiensis* sp. nov. | CIBTZ20190608017 | male | 56.3 | 14.6 | 19.2 | 7.2  | 3.2  | 3.6  | 6.7  | 4.3  | 6.5  | 5.3  | 24.0 | 6.9  | 87.0 | 27.6 | 28.0 | 7.8  | 38.3 | 25.7 | 3.2  | 10.9 | 15.0 |
| *Megophrys qianbeiensis* sp. nov. | CIBTZ20160715003 | male | 54.1 | 17.0 | 20.8 | 6.9  | 3.4  | 3.7  | 6.3  | 4.5  | 6.9  | 6.0  | 25.4 | 5.7  | 93.7 | 27.0 | 30.8 | 7.8  | 43.7 | 29.4 | 3.5  | 10.4 | 15.8 |
| *Megophrys qianbeiensis* sp. nov. | CIBTZ20190608015 | male | 52.6 | 15.3 | 19.4 | 6.8  | 2.4  | 4.3  | 6.9  | 3.7  | 5.9  | 5.5  | 24.1 | 7.4  | 86.9 | 24.1 | 28.3 | 8.3  | 41.4 | 28.0 | 3.3  | 10.0 | 15.7 |
| *Megophrys qianbeiensis* sp. nov. | CIBKKS20180722001 | male | 49.3 | 15.5 | 18.3 | 6.8  | 3.0  | 3.5  | 5.7  | 5.3  | 5.4  | 5.5  | 20.4 | 6.6  | 76.9 | 24.5 | 25.0 | 7.0  | 34.5 | 24.5 | 3.4  | 7.8  | 14.0 |
| *M. spinata*                  | CIBLS20190801002 | male | 56.2 | 14.9 | 18.4 | 6.0  | 3.0  | 3.4  | 5.8  | 4.2  | 5.1  | 6.1  | 24.2 | 5.5  | 93.7 | 27.4 | 29.9 | 6.1  | 40.8 | 28.6 | 2.7  | 9.1  | 14.0 |
| *M. spinata*                  | CIBLS20190801004 | male | 53.5 | 14.5 | 19.1 | 7.1  | 2.8  | 4.1  | 6.0  | 5.0  | 5.7  | 5.0  | 24.1 | 5.9  | 99.0 | 29.8 | 30.4 | 8.0  | 43.1 | 28.1 | 2.8  | 9.6  | 14.4 |
| *M. spinata*                  | CIBLS20190801001 | male | 54.8 | 14.6 | 18.6 | 6.7  | 2.8  | 3.9  | 6.0  | 4.8  | 5.8  | 4.5  | 24.3 | 6.1  | 87.7 | 27.6 | 28.9 | 7.0  | 39.7 | 26.4 | 2.8  | 9.2  | 14.2 |
| *M. spinata*                  | CIBLS20190801003 | male | 51.2 | 14.3 | 18.8 | 6.6  | 2.9  | 3.6  | 6.1  | 5.2  | 6.0  | 5.1  | 25.1 | 6.5  | 93.0 | 26.1 | 29.6 | 7.7  | 41.6 | 29.6 | 2.5  | 9.1  | 14.0 |
| *M. spinata*                  | CIBLS20160610008 | male | 53.8 | 15.8 | 18.4 | 5.7  | 2.7  | 3.8  | 6.3  | 5.3  | 5.5  | 4.5  | 24.0 | 7.4  | 85.7 | 26.9 | 29.0 | 6.0  | 39.2 | 27.9 | 2.9  | 8.5  | 14.3 |
| *M. sangzhiensis*             | CIBSZ20120620005 | male | 59.8 | 17.8 | 20.6 | 7.2  | 3.1  | 4.1  | 7.3  | 4.8  | 7.1  | 5.7  | 26.6 | 6.6  | 105.0 | 31.6 | 32.6 | 7.8  | 46.1 | 29.4 | 3.1  | 10.4 | 16.1 |
| *M. sangzhiensis*             | CIBSZ20120620008 | male | 58.8 | 17.8 | 21.5 | 7.6  | 3.0  | 4.4  | 7.3  | 4.7  | 7.4  | 6.1  | 26.8 | 6.2  | 97.3 | 30.3 | 31.6 | 7.8  | 42.9 | 26.7 | 3.4  | 10.9 | 17.3 |
| *M. sangzhiensis*             | CIBSZ20120620006 | male | 59.5 | 16.1 | 21.0 | 8.2  | 3.5  | 4.6  | 7.7  | 5.0  | 6.7  | 6.1  | 26.6 | 6.3  | 99.8 | 27.9 | 32.3 | 7.3  | 43.8 | 29.2 | 3.4  | 10.1 | 17.3 |
| *M. sangzhiensis*             | CIBSZ2012062019  | male | 57.4 | 18.0 | 20.9 | 7.3  | 3.5  | 3.7  | 6.8  | 5.0  | 6.4  | 5.1  | 26.2 | 6.1  | 99.5 | 30.2 | 32.6 | 8.1  | 43.2 | 29.9 | 3.7  | 10.4 | 17.3 |
| *M. sangzhiensis*             | CIBSZ20120620007 | male | 56.1 | 16.1 | 20.0 | 6.6  | 3.9  | 4.1  | 6.9  | 5.8  | 6.1  | 5.7  | 28.2 | 6.7  | 100.0 | 28.0 | 32.0 | 7.5  | 44.9 | 29.4 | 3.4  | 9.4  | 16.2 |
In order to reduce the impact of allometry, the correct value from the ratio of each character to SVL was calculated, and then was log-transformed for subsequent morphometric analyses. One-way analysis of variance (ANOVA) was used to test the significance of differences on morphometric characters between different species. The significance level was set at 0.05. To show the spatial distribution of different species on the morphometric characters, principal component analyses (PCA) were performed. These analyses were carried out in the R (R Development Core Team 2008). The new species was also compared with all other *Megophrys* species on morphology. Comparative data were obtained from related species as described in literature (Table 3).

**Table 3.** References for morphological characters for congeners of the genus *Megophrys*.

| Species                          | Literature                  |
|---------------------------------|-----------------------------|
| *M. aceras* Boulenger, 1903      | Boulenger 1903              |
| *M. acuta* Wang, Li & Jin, 2014 | Li et al. 2014              |
| *M. acuta* Mahony, Teeling & Biju, 2013 | Mahony et al. 2013       |
| *M. angka* Wu, Sowannapoom, Poyarkov, Chen, Pwawanghanant, Xu, Jin, Murphy & Che, 2019 | Wu et al. 2019            |
| *M. awasaleus* Ohler, Swan & Daltry, 2002 | Ohler et al. 2002         |
| *M. balensis* (Boulenger, 1899) | Boulenger 1899a             |
| *M. buolongensis* Ye, Fei & Xie, 2007 | Ye et al. 2007            |
| *M. chinshunensis* Ye & Fei, 1995 | Ye and Fei 1995           |
| *M. chinlingensis* Jiang, Fei & Ye, 2009 | Fei et al. 2009           |
| *M. boettgeri* (Boulenger, 1899) | Boulenger 1899b             |
| *M. brachykolos* Inger & Romer, 1961 | Inger and Romer 1961     |
| *M. carinense* (Boulenger, 1889) | Boulenger 1889              |
| *M. caobangensis* Nguyen, Pham, Nguyen, Luong, & Zieglet, 2020 | Nguyen et al. 2020        |
| *M. catesbryi* Shen, 1994       | Shen. 1994                 |
| *M. chenii* (Wang & Liu, 2014)  | Wang et al. 2014            |
| *M. chisuaensis* Xu, Li, Wei & Wang, 2020 | Xu et al. 2020            |
| *M. chuannanensis* (Fei, Ye & Huang, 2001) | Fei et al. 2001           |
| *M. dameri* Mahony, 2011        | Mahony 2011                 |
| *M. dawimensis* Rao & Yang, 1997 | Rao and Yang 1997         |
| *M. dongguanensis* Wang & Wang, 2019 | Wang et al. 2019b         |
| *M. dringi* Inger, Stuebing & Tan, 1995 | Inger et al. 1995        |
| *M. edwardinae* Inger, 1989     | Inger 1989                  |
| *M. elfina* Poyarkov, Orlov, Gogoleva, Vassilieva, Nguyen, Nguyen, Che & Mahony, 2017 | Poyarkov et al. 2017     |
| *M. feminalensis* Tapley, Cutajar, Mahony, Nguyen, Dau, Luong, Le, Nguyen, Nguyen, Portway, Luong & Rowley, 2018 | Tapley et al. 2018 |
| *M. feae* Boulenger, 1887       | Boulenger 1887              |
| *M. feii* Yang, Wang & Wang, 2018 | Yang et al. 2018          |
| *M. flavipunctata* Mahony, Kamei, Teeling & Biju, 2018 | Mahony et al. 2018        |
| *M. gerti* (Ohler, 2003)        | Ohler 2003                  |
| *M. gigantica* Liu, Hu & Yang, 1960 | Liu et al. 1960         |
| *M. glandulosa* Fei, Ye & Huang, 1990 | Fei et al. 1990           |
| *M. hassi* (Ohler, 2003)        | Ohler 2003                  |
| *M. himalayana* Mahony, Kamei, Teeling & Biju, 2018 | Mahony et al. 2018        |
| *M. huangshanensis* Tapley, Cutajar, Mahony, Nguyen, Dau, Luong, Le, Nguyen, Nguyen, Portway, Luong & Rowley, 2018 | Tapley et al. 2018 |
| *M. insularis* (Wang, Liu, Lyu, Zeng & Wang, 2017) | Wang et al. 2017a         |
| *M. intermedia* Smith, 1921     | Smith 1921                  |
| *M. jiugongi* Liu, Li, Wei, Xu, Cheng, Wang & Wu, 2020 | Liu et al. 2020           |
| *M. jinggangensis* Fei & Ye, 1983 | Fei et al. 1983           |
| *M. jinggangensis* (Wang, 2012) | Wang et al. 2012            |
| *M. jiulianensis* Wang, Zeng, Lyu & Wang, 2019 | Wang et al. 2019b         |
| *M. kalimantanensis* Munir, Hamidy, Matsui, Iskandar, Sidik & Shimada, 2019 | Munir et al. 2019        |
| *M. kobayashii* Malkmus & Matsui, 1997 | Malkmus and Matsui 1997    |
| Species                      | Literature                  |
|------------------------------|-----------------------------|
| M. kouei Mahony, Foley, Biju & Teeling, 2017 | Mahony et al. 2017          |
| M. kuatanensis Pope, 1929     | Pope 1929                   |
| M. lancep Munir, Hamidy, Farajallah & Smith, 2018 | Munir et al. 2018          |
| M. leishanensis Li, Xi, Liu, Jiang, Wei & Wang, 2018 | Li et al. 2018b          |
| M. lekaguli Stuurt, Chuanerynkm, Chan-ard & Inger, 2006 | Stuart et al. 2006         |
| M. lebenses Zhang, Li, Xiao, Li, Pan, Wang, Zhang & Zhou, 2017 | Zhang et al. 2017          |
| M. leiger Taylor, 1920        | Taylor 1920                 |
| M. lini (Wang & Yang, 2014)   | Wang et al. 2014            |
| M. lismanensis (Wang, Liu & Jiang, 2017) | Wang et al. 2017b         |
| M. longipes Boulenger, 1886   | Boulenger 1886              |
| M. major Boulenger, 1908      | Boulenger 1908              |
| M. manghanensis Fei & Ye, 1990 | Fei et al. 2012            |
| M. massonensis Bourret, 1937  | Bourret 1937                |
| M. medogensis Fei, Ye & Huang, 1983 | Fei et al. 1983        |
| M. megacephala Mahony, Sengupta, Kamei & Biju, 2011 | Mahony et al. 2011        |
| M. microstoma (Boulenger, 1903) | Boulenger 1903          |
| M. minor Stejneger, 1926      | Stejneger 1926              |
| M. mirabilis Lyu, Wang & Zhao, 2020 | Lyu et al. 2020         |
| M. montana Kuhl & Van Hasselt, 1822 | Kuhl and Van Hasselt 1822  |
| M. monticola (Günther, 1864)  | Günther 1864                |
| M. mutumontaista Wang, Lyu & Wang, 2019 | Wang et al. 2019b        |
| M. nankiangensis Liu & Hu, 1966 | Hu and Liu 1966           |
| M. nankunensis Wang, Zeng & Wang, 2019 | Wang et al. 2019b        |
| M. nanlingensus Lyu, Wang & Wang, 2019 | Wang et al. 2019b        |
| M. nanua (Schlegel, 1858)    | Schlegel 1858               |
| M. noei Wang, Li & Zhao, 2014 | Wang et al. 2014            |
| M. ombrophila Messenger & Dahn, 2019 | Messenger et al. 2019 |
| M. omeinonous Liu, 1950       | Liu 1950                    |
| M. orcepyra Mahony, Kamei, Teeling & Biju, 2018 | Mahony et al. 2018       |
| M. orpedion Mahony, Teeling & Biju, 2013 | Mahony et al. 2013       |
| M. orientalis Lyu, Wang & Wang, 2020 | Li et al. 2020              |
| M. pacynuscius Huang, 1981    | Huang and Fei 1981          |
| M. palpebralEPinnos Bourret, 1957 | Bourret 1957                |
| M. parallelina Inger & Iskandar, 2005 | Inger and Iskandar 2005    |
| M. parva (Boulenger, 1893)    | Boulenger 1893              |
| M. perina Mahony, Kamei, Teeling & Biju, 2018 | Mahony et al. 2018       |
| M. peripatetic Zhao, Yang, Chen, Chen & Wang, 2014 | Zhao et al. 2014          |
| M. robusta Boulenger, 1908    | Boulenger 1908              |
| M. rubritent Tapley, Cutajar, Mahony, Chung, Dau, Nguyen, Luong & Rowley, 2017 | Tapley et al. 2017       |
| M. ruchebeni Jiang, Ye & Fei, 2008 | Jiang et al. 2008          |
| M. archibiata (Mathew & Sen, 2007) | Mathew and Sen 2007         |
| M. shapingensis Liu, 1950     | Liu 1950                    |
| M. shimenxina Lyu, Liu & Wang, 2020 | Lyu et al. 2020            |
| M. shuihegenuis Tian & Sun, 1995 | Tian and Sun 1995        |
| M. shunhuanensis Wang, Deng, Liu, Wu & Liu, 2019 | Wang et al. 2019a        |
| M. spinata Liu & Hu, 1973     | Hu et al. 1973              |
| M. stegneri Taylor, 1920      | Taylor 1920                 |
| M. suparia (Stuart, Sok & Neang, 2006) | Stuart et al. 2006         |
| M. takaenii Mahony, 2011      | Mahony 2011                 |
| M. tabergranulata Shen, Mo & Li, 2010 | Mo et al. 2010             |
| M. vegandis Mahony, Teeling, Biju, 2013 | Mahony et al. 2013       |
| M. wuwuuenis Fei, Jiang & Zheng, 2001 | Fei et al. 2012            |
| M. wugongensis Wang, Lyu & Wang, 2019 | Wang et al. 2019b        |
| M. wuluanghehenui Fei & Fei, 1995 | Ye and Fei 1995           |
| M. wuwuuenis Ye & Fei, 1995   | Ye and Fei 1995             |
| M. xianfuensis Wang, Wu, Peng, Shi, Lu & Wu, 2020 | Wang et al. 2020        |
| M. xiangnuensis Lyu, Zeng & Wang, 2020 | Lyu et al. 2020           |
| M. yangmingensis Lyu, Zeng & Wang, 2020 | Lyu et al. 2020           |
| M. zhangi Ye & Fei, 1992      | Ye and Fei 1992             |
| M. zufitolutinis (Mathew & Sen, 2007) | Mathew and Sen 2007        |
Bioacoustics analyses

The advertisement calls of the undescribed species were recorded from the holotype specimen CIBTZ20190608017 in a stream at ambient air temperature of 20.5 °C and air humidity of 87 % in the field on 8 June 2019 in Huanglian Nature Reserve, Tongzi County, Guizhou Province, China. The advertisement calls of *M. sangzhiensis* were recorded from the specimen CIBSZ2012062005 in a stream at ambient air temperature of 18.5 °C and air humidity of 85 % in the field on 20 June 2012 in Sangzhi County, Hunan Province, China. The advertisement calls of *M. spinata* were recorded from the specimen CIBLS20190801001 in a stream at ambient air temperature of 19.0 °C and air humidity of 85 % in the field on 1 August 2019 in Leishan County, Guizhou Province, China. SONY PCM-D50 digital sound recorder was used to record within 20 cm of the calling individual. 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.

Results

Phylogenetic analyses

Aligned sequence matrix of 16S+COI contains 1104 bp. ML and BI trees had almost consistent topology though relationships of some lineages were unresolved (Fig. 2). In trees, the undescribed species was clustered as an independent clade and sister to a clade in comprising of *M. sangzhiensis* and *M. spinata* (Fig. 2).

Genetic distances on COI gene with uncorrected *p*-distance model between all samples of the undescribed species were below 0.2%. The genetic distance between the undescribed species and its closest related species *M. sangzhiensis* was 4.3 % on COI gene, which was higher or at the same level with those among many pairs of congeners, for example, 3.6 % between *M. spinata* and *M. sangzhiensis*, 1.8% between *M. huangshanensis* and *M. boettgeri*, and 4.3 % between *M. maosonensis* and *M. mangshanensis* (Suppl. material 1: Table S1).

Morphological comparisons

In PCA for males, the total variation of the first two principal components was 63.2 %. In males on the two-dimensional plots of PC1 vs. PC2, the undescribed species could be distinctly separated from *M. sangzhiensis* and *M. spinata* (Fig. 3). The results of one-way ANOVA indicated that in males, the undescribed species was significantly different from *M. sangzhiensis* and *M. spinata* on many morphometric characters (all *p*-values < 0.05; Table 4). More detailed descriptions of results from morphological comparisons between the undescribed species and its congeners were presented in the following sections for describing the new species.
A new species of *Megophrys*

Figure 2. Bayesian Inference (BI) tree of the genus *Megophrys* reconstructed based on the 16S rRNA and COI gene sequences. Bayesian posterior probability resulted from BI analyses/ML bootstrap supports from Maximum Likelihood analyses were denoted beside each node. Samples 1–90 refer to Table 1.

**Bioacoustics comparisons**

There were many differences in sonograms and waveforms of calls between the undescribed species, *M. sangzhiensis*, and *M. spinata* (Fig. 4; Table 5). Firstly, in the note interval, the undescribed species were shorter than those of both *M. sangzhiensis* and *M. spinata*. Secondly, the dominant frequency of call in the undescribed species was lower than both of *M. sangzhiensis* and *M. spinata*. Thirdly, the amplitude of the undescribed species beginning with moderately high energy pulses, increasing slightly to a maximum by approximately mid note, and then decreasing towards the end of each note, in *M. sangzhiensis* beginning with maximum energy pulses and then decreasing towards the end of note, and in *M. spinata* beginning with lower energy pulses, then
**Table 4.** Morphometric comparisons between *Megophrys qianbeiensis* sp. nov., *M. sangzhiensis*, and *M. spinata*. Units given in mm. Abbreviations for the species name: MQ, *Megophrys qianbeiensis* sp. nov.; MSZ, *M. sangzhiensis*; MSP, *M. spinata*. See abbreviations for morphometric characters in Materials and methods section.

| Character | *Megophrys qianbeiensis* sp. nov. | *M. sangzhiensis* | *M. spinata* | p-value from ANOVA in male |
|-----------|-----------------------------------|-------------------|---------------|--------------------------|
|           | males (N=6)                        | males (N=5)       | males (N=5)   | MQ vs. MSZ | MQ vs. MSP |
| SVL       | 49.3–58.2 54.5 ± 3.09              | 56.1–59.8 58.3 ± 1.56 | 51.2–56.2 55.9 ± 1.84 | 0.029       | 0.851      |
| HDL       | 14.6–17.0 15.6 ± 0.93               | 16.1–18.0 17.2 ± 0.96 | 14.3–15.8 14.8 ± 0.59 | 0.027       | 0.124      |
| HDW       | 18.3–21.0 19.9 ± 1.08               | 20.0–21.5 20.8 ± 0.55 | 18.4–19.1 18.7 ± 0.30 | 0.123       | 0.037      |
| SL        | 6.7–7.2 6.9 ± 0.17                  | 6.6–8.2 7.4 ± 0.58  | 5.7–7.1 6.4 ± 0.56  | 0.067       | 0.085      |
| TYD       | 3.2–4.3 3.5 ± 0.39                  | 3.1–3.7 3.4 ± 0.21  | 2.5–2.9 2.7 ± 0.15  | 0.639       | 0.001      |
| IFE       | 7.8–10.9 9.7 ± 1.13                 | 9.4–10.9 10.2 ± 0.54 | 8.5–9.6 9.1 ± 0.39  | 0.340       | 0.348      |
| IAE       | 14.0–16.4 15.5 ± 0.86               | 16.1–17.3 16.8 ± 0.62 | 14.0–14.4 14.2 ± 0.18 | 0.019       | 0.009      |
| NED       | 2.4–3.4 2.9 ± 0.36                  | 3.0–3.9 3.4 ± 0.36  | 2.7–3.0 2.8 ± 0.11  | 0.060       | 0.618      |
| NSD       | 3.5–4.3 3.8 ± 0.29                  | 3.7–4.6 4.2 ± 0.34  | 3.4–4.1 3.8 ± 0.27  | 0.101       | 0.683      |
| IOD       | 5.7–7.5 6.7 ± 0.62                  | 6.8–7.7 7.2 ± 0.54  | 5.8–6.3 6.0 ± 0.18  | 0.150       | 0.056      |
| ED        | 3.7–5.3 4.5 ± 0.59                  | 4.7–5.8 5.1 ± 0.45  | 4.2–5.3 4.9 ± 0.44  | 0.117       | 0.257      |
| UEW       | 5.1–6.2 5.6 ± 0.43                  | 5.1–6.1 5.7 ± 0.42  | 4.5–6.1 5.0 ± 0.65  | 0.484       | 0.126      |
| LAL       | 20.4–25.4 24.0 ± 1.84               | 26.2–28.2 26.9 ± 0.78| 24.0–25.1 24.3 ± 0.44 | 0.014       | 0.654      |
| LW        | 5.7–7.4 6.6 ± 0.55                  | 6.1–6.7 6.4 ± 0.24  | 5.5–7.4 6.3 ± 0.72  | 0.394       | 0.364      |
| HLL       | 76.9–93.7 87.8 ± 6.07               | 97.3–105.0 100.3 ± 2.84 | 85.7–99.0 91.8 ± 5.27 | 0.003       | 0.280      |
| THL       | 24.1–28.4 26.6 ± 1.82               | 27.9–31.6 29.6 ± 1.59 | 26.1–29.8 27.6 ± 1.38 | 0.019       | 0.343      |
| TL        | 25.0–32.4 29.2 ± 2.63               | 31.6–32.6 32.2 ± 0.42 | 28.9–30.4 29.6 ± 0.63 | 0.038       | 0.713      |
| TW        | 7.0–8.7 8.0 ± 0.58                  | 7.3–8.1 7.7 ± 0.32  | 6.0–8.0 7.0 ± 0.91  | 0.404       | 0.053      |
| TFL       | 34.5–44.0 40.4 ± 3.58               | 42.9–46.1 44.2 ± 1.32 | 39.2–43.1 40.9 ± 1.55 | 0.062       | 0.761      |
| FL        | 24.5–29.4 27.5 ± 1.95               | 26.7–29.9 28.9 ± 1.28 | 26.4–29.6 28.1 ± 1.16 | 0.201       | 0.531      |

**Figure 3.** Plots of the first principal component (PCA1) versus the second (PCA2) for *Megophrys qianbeiensis* sp. nov., *M. sangzhiensis*, and *M. spinata* from principal component analyses.
A new species of *Megophrys*

**Figure 4.** Visualization of advertisement calls of *Megophrys qianbeiensis* sp. nov., *M. sangzhiensis*, and *M. spinata*. **A1** waveform showing one note of *Megophrys qianbeiensis* sp. nov. **A2** sonogram showing one note of *Megophrys qianbeiensis* sp. nov. **A3** waveform showing 25 notes of one call of *Megophrys qianbeiensis* sp. nov. **A4** sonogram showing 25 notes of one call of *Megophrys qianbeiensis* sp. nov. **B1** waveform showing one note of *M. sangzhiensis*. **B2** sonogram showing one note of *M. sangzhiensis*. **B3** waveform showing 38 notes of one call of *M. sangzhiensis*. **B4** sonogram showing 38 notes of one call of *M. sangzhiensis*. **C1** waveform showing one note of *M. spinata*. **C2** sonogram showing one note of *M. spinata*. **C3** waveform showing 20 notes of one call of *M. spinata*. **C4** sonogram showing 20 notes of one call of *M. spinata*.

**Table 5.** Comparisons of characteristics of advertisement calls of *Megophrys qianbeiensis* sp. nov., *M. sangzhiensis*, and *M. spinata*.

| Call character            | *Megophrys qianbeiensis* sp. nov. | *M. sangzhiensis* | *M. spinata* |
|---------------------------|-----------------------------------|-------------------|--------------|
|                           | Range    | Mean ± SD | Range    | Mean ± SD | Range    | Mean ± SD |
| Number of notes in a call | 14–26    | 22.5 ± 4.4 | 38      | /        | 7–28     | 17 ± 7.92 |
| Call duration (ms)        | 2832–5621| 4413 ± 972| 8152    | /        | 1500–6623| 3905 ± 2010|
| Call interval (ms)        | 6812–14387| 10878 ± 2701| /      | /        | 592–5770| 2708 ± 1863.33|
| Note duration (ms)        | 129–211  | 167 ± 0.02| 107–155 | 120.3 ± 8.73| 107–123 | 114 ± 3.79 |
| Note interval (ms)        | 34–94    | 57 ± 0.01 | 72–132  | 95.6 ± 13.89| 113–232 | 147 ± 33.12 |
| Dominant frequency (Hz)   | 2250–3000| 2469 ± 197.47| 10380–13200| 11795 ± 670.58| 4260–4589| 4416 ± 130.04|
| Temperature (°C)          | 20.5     | 18.5     |         |           | 19.0     |           |
increasing to the maximum by approximately one-fourth note and then decreasing to the mid note then increasing to the second highest energy pulses and then decreasing towards the end of note.

Taxonomic accounts

**Megophrys qianbeiensis** sp. nov.

http://zoobank.org/C6C89A51-8178-4C7B-A100-80C0D2D42AD3

Figures 4A1–C1, 5, 6A1–A6, 7; Tables 1, 2, 4, 5, Suppl. material 2: Table S2

**Type material.** *Holotype*. CIBTZ20190606017 (Figs 5, 6), adult male, from Huan-glian Nature Reserve, Tongzi County, Guizhou Province, China (28.498056°N, 107.046944°E, ca. 1500 m a.s.l.), collected by Shi-Ze Li 8 June 2019.

*Paratype*. Four adult males from the same place as holotype, and one from Kuankuoshui National Nature Reserve (28.21835°N, 107.166388°E, ca.1520 m a.s.l.) collected by Shi-Ze Li. CIBKKS20180722001 collected 22 July 2018 from Kuankuoshui National Nature Reserve and CIBTZ20160715003 collected 15 July 2016, CIBTZ20190608015, CIBTZ20190608016 and CIBTZ20190608018 collected 8 June 2019 from Kuankuoshui National Nature Reserve.

**Diagnosis.** *Megophrys qianbeiensis* sp. nov. is assigned to the genus *Megophrys* based on molecular phylogenetic analyses and the following generic diagnostic characters: snout shield-like; projecting beyond the lower jaw; canthus rostralis distinct; chest glands small and round, closer to the axilla than to midventral line; femoral glands on rear part of thigh; vertical pupils.

*Megophrys qianbeiensis* sp. nov. could be distinguished from its congeners by a combination of the following morphological characters: body size moderate (SVL 49.3–58.2 mm in males); vomerine ridges present distinctly, vomerine teeth present; tongue feebly notched behind; tympanum distinctly visible, oval; two metacarpal tubercles in hand; toes with one-third webbing and wide lateral fringes; heels overlapped when thighs are positioned at right angles to the body; tibiotarsal articulation reaching the level between tympanum and eye when leg stretched forward; an internal single subgular vocal sac present in male; in breeding male, the nuptial pads with large and sparse black nuptial spines present on the dorsal bases of the first two fingers.

**Description of holotype.** (Figs 5, 6). SVL 56.3 mm; head width larger than head length (HDW/HDL ratio ca. 1.3); snout obtusely pointed, protruding well beyond the margin of the lower jaw in ventral view; loreal region vertical and concave; canthus rostralis well-developed; top of head flat in dorsal view; eye large, eye diameter 44.5 % of head length; pupils vertical; nostril orientated laterally, closer to snout than eye; tympanum distinct, TYP/EYE ratio 0.49; vomerine ridges present distinctly as V-shape, vomerine teeth present; margin of tongue smooth, feebly notched behind.

Forelimbs slender, the length of lower arm and hand 42.6 % of SVL; fingers burly, relative finger lengths: II < I < V < III; tips of digits globular, without lateral fringes; subarticular tubercle distinct at the base of each finger; two metacarpal tubercles, prominent, oval-shaped, the inner one bigger than the outer one.
Hindlimbs slender, 1.54 times of SVL; heels overlapping when thighs are positioned at right angles to the body, tibiotarsal articulation reaching 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 tubercles present on the base of each toes; toes with one-third webbing and relative wide lateral fringe; inner metatarsal tubercle oval-shaped; outer metatarsal tubercle absent.

Dorsal skin rough, with numerous granules with black spins; several large warts scattered on flanks; tubercles on the dorsum forming a weak V-shaped ridge; two discontinuous dorsolateral parallel ridges on either side of the V-shaped ridges; an inverted triangular brown speckle between two upper eyelids; several tubercles on the flanks and dorsal surface of thighs and tibias; supratympanic fold distinct.

Ventral surface smooth with numerous white granules; glands on chest indistinct; femoral glands on rear of thighs, numerous white granules on outer thighs; posterior end of the body distinctly protruding and forming an arc-shaped swelling above the anal region.

**Coloration of holotype in life** (Fig. 5). An inverted triangular brown speckle between the eyes; V-shaped ridges on the dorsum with brown speckle, on transverse bands on the dorsal surface of the thigh and shank; several dark brown and white vertical bars on the lower and upper lip; belly whitish grey with dark brown marbling; ventral surface of posterior limb orange with numerous granules; palms, soles and tip of digits uniform purple grey; femoral glands white.

**Coloration of holotype in preservation** (Fig. 6). Color of dorsal surface fades to brownness; the inverted triangular brown speckle between the eyes and V-shaped ridges on dorsum indistinct; ventral surface greyish white; creamy-white substitutes...
the purple grey on tip of digits; the posterior of ventral surface of body, inner of thigh and upper of tibia fades to creamy-white.

Variation. In CIBTZ20160715003 the dorsolateral parallel ridges are short, just a little bit above the shoulder (Fig. 7A); in CIBTZ20190608015 the X-shaped marking on back of trunk consists of a ridge with brown spots (Fig. 7B) and the posterior belly are orange with black spots on the flank belly (Fig. 7C); in CIBKKS20180722001 the belly is grey brown with some white spots (Fig. 7D).

Advertisement call. The call description is based on recordings of the holotype CIBTZ20190608017 (Fig. 4) from the shrub leaf near the streamlet, and the ambient air temperature was 20.5 °C. Each call consists of 14–26 (mean 22.5 ± 4.4, \( N = 6 \)) notes. Call duration was 2832–5621 ms (mean 4413 ± 972, \( N = 6 \)). Call interval was 6812–14387 ms (mean 10878 ± 2701, \( N = 5 \)). Each note had a duration of 129–211 ms (mean 167 ± 0.02, \( N = 135 \)) and the intervals between notes 34–94 ms (mean 57 ± 0.01, \( N = 128 \)). Amplitude modulation within note was apparent, beginning with moderately high energy pulses, increasing slightly to a maximum by approximately mid note, and then decreasing towards the end of each note. The average dominant frequency was 2469 ± 197.47 (2250–3000 Hz, \( N = 6 \)).
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Secondary sexual characters. Adult males have a single subgular vocal sac. In breeding males, brownish red nuptial pads are present on the dorsal bases of the first two fingers with big and sparse black nuptial spines (Fig. 5A).

Comparisons. By having moderate body size (minimum SVL > 49.8 mm in males), Megophrys qianbeiensis sp. nov. differs from M. aceras, M. acuta, M. angka, M. ancras, M. baluensis, M. baolongensis, M. binchuanensis, M. binlingensis, M. boettgeri, M. brachykolos, M. caobangensis, M. cheni, M. daweimontis, M. dongguanensis, M. dringi, M. edwardinae, M. elfina, M. fansipanensis, M. feii, M. gerti, M. hansi, M. hoanglieniensis, M. huangshanensis, M. insularis, M. jiangi, M. jinggangensis, M. jiulianensis, M. kuatunensis, M. lancip, M. leishanensis, M. lini, M. lishuiensis, M. longipes, M. major, M. microstoma, M. minor, M. monticola, M. mufumontana, M. nankunensis, M. nanlingensis, M. obesa, M. ombrophila, M. oropedion, M. pachyproctus, M. palpebralispinosa, M. parallela, M. parva, M. rubrimera, M. serchhipii, M. shimentaina, M. shunhuangensis, M. tuberogranulata, M. vegrandi, M. wawuensis, M. wugongensis, M. wuliangshanensis, M. wushanensis, M. xianjuensis, M. zhangi, M. zunhebotoensis, M. xiangnanensis, and M. yangmingensis (vs. minimum SVL < 48.0 mm).
By having moderate body size (minimum SVL < 59.0 mm in males), *Megophrys qianbeiensis* sp. nov. differs from *M. auralensis*, *M. carinense*, *M. caudoprotca*, *M. chuananensis*, *M. feae*, *M. gigantica*, *M. glandulosa*, *M. himalayana*, *M. kalimantanensis*, *M. kobayashii*, *M. ligayae*, *M. mangshanensis*, *M. orientalis*, *M. periosa*, *M. platyparietus*, *M. popei*, *M. shapingensis*, and *M. shuichengensis* (vs. minimum SVL > 60.0 mm).

By having vomerine teeth, *Megophrys qianbeiensis* sp. nov. differs from *M. aceras*, *M. aucta*, *M. angka*, *M. auralensis*, *M. baolongensis*, *M. biinchuanensis*, *M. binlingensis*, *M. boettgeri*, *M. brachykolos*, *M. caobangensis*, *M. cheni*, *M. chishuiensis*, *M. dringi*, *M. feae*, *M. leishanensis*, *M. lini*, *M. lishuiensis*, *M. major*, *M. microstoma*, *M. minor*, *M. mirabilis*, *M. mufumontana*, *M. nankiangensis*, *M. obesa*, *M. ombrophila*, *M. shapingensis*, *M. shuichengensis*, *M. shunhuangensis*, *M. tuberogranulata*, *M. vegrandis*, *M. wawuensis*, *M. wugongensis*, *M. wuliangshanensis*, *M. wushanensis*, *M. xianjuensis*, *M. xiangnanensis*, and *M. yangmingensis* (vs. absent).

By the absence of horn-like tubercle at the edge of each upper eyelid, *Megophrys qianbeiensis* sp. nov. differs from *M. aceras*, *M. aucta*, *M. angka*, *M. auralensis*, *M. baolongensis*, *M. biinchuanensis*, *M. binlingensis*, *M. boettgeri*, *M. brachykolos*, *M. caobangensis*, *M. carinense*, *M. caudoprotca*, *M. cheni*, *M. chishuiensis*, *M. chuananensis*, *M. da-wei-montis*, *M. dongguanensis*, *M. dringi*, *M. edwardinae*, *M. elfina*, *M. fansipanensis*, *M. feae*, *M. feii*, *M. flavipunctata*, *M. gerti*, *M. glandulosa*, *M. hansii*, *M. himalayana*, *M. hoanglienensis*, *M. huangshanensis*, *M. insularis*, *M. intermedia*, *M. jangi*, *M. jing-dongensis*, *M. jinggangensis*, *M. jiulianensis*, *M. kalimantanensis*, *M. kouri*, *M. kua-tunensis*, *M. lancip*, *M. leishanensis*, *M. lekguli*, *M. liboensis*, *M. ligayae*, *M. lini*, *M. lishuiensis*, *M. longipes*, *M. mangshanensis*, *M. medogensis*, *M. microstoma*, *M. mirabilis*, *M. montana*, *M. mufumontana*, *M. nankunensis*, *M. nanlingensis*, *M. nasuta*, *M. obesa*, *M. ombrophila*, *M. omeimontis*, *M. oreocrypta*, *M. orientalis*, *M. palpebralespinosa*, *M. parallelala*, *M. parva*, *M. periosa*, *M. platyparietus*, *M. popei*, *M. rubrmera*, *M. shimentaina*, *M. shuichengensis*, *M. shunhuangensis*, *M. stejnegeri*, *M. synoria*, *M. vegrandis*, *M. wugongensis*, *M. xianjuensis*, *M. xiangnanensis*, and *M. yangmingensis* (vs. present).

With the tongue feebly notched behind, *Megophrys qianbeiensis* sp. nov. differs from *M. aceras*, *M. aucta*, *M. angka*, *M. auralensis*, *M. brachykolos*, *M. caobangensis*, *M. caudoprotca*, *M. dongguanensis*, *M. elfina*, *M. hansii*, *M. jangi*, *M. jinggangensis*, *M. lancip*, *M. leishanensis*, *M. lekguli*, *M. lini*, *M. lishuiensis*, *M. megacephala*, *M. mufumontana*, *M. nankunensis*, *M. obesa*, *M. ombrophila*, *M. orientalis*, *M. palpebralespinosa*, *M. parallelala*, *M. parva*, *M. shunhuangensis*, *M. takensis*, *M. wushanensis*, and *M. xianjuensis* (vs. tongue notched behind in the latter), and differs from *M. cheni*, *M. damrei*, *M. dringi*, *M. flavipunctata*, *M. gigantica*, and *M. popei* (vs. tongue noted behind).

By having lateral wide fringes on toes, *Megophrys qianbeiensis* sp. nov. differs from *M. angka*, *M. baolongensis*, *M. brachykolos*, *M. caobangensis*, *M. damrei*, *M. dawei-montis*, *M. dongguanensis*, *M. fansipanensis*, *M. feae*, *M. himalayana*, *M. hoanglienensis*, *M. huangshanensis*, *M. insularis*, *M. jangi*, *M. jiulianensis*, *M. kalimantanensis*, *M. kouri*, *M. leishanensis*, *M. lekguli*, *M. lishuiensis*, *M. major*, *M. mangshanensis*, *M. medogensis*, *M. megacephala*, *M. microstoma*, *M. minor*, *M. nankunensis*, *M. obesa*, *M. ombrophila*, *M. oreocrypta*, *M. oropedion*, *M. pachyproctus*, *M. parva*, *M. periosa*, *M. shunhuangensis*,
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*M. takensis*, *M. tuberogranulata*, *M. wawuensis*, *M. wugongensis*, *M. wuliangshanensis*, and *M. xianjuensis* (vs. lacking lateral fringes on toes).

By toes with one-third webs, *Megophrys qianbeiensis* sp. nov. differs from *M. aceras*, *M. acuta*, *M. angka*, *M. aurataliensis*, *M. balaensis*, *M. baolongensis*, *M. binchuanensis*, *M. binlingensis*, *M. boettgeri*, *M. brachykolos*, *M. caobangensis*, *M. caudoprocta*, *M. cheni*, *M. chunnanensis*, *M. damrei*, *M. dauwetmonits*, *M. dongguanensis*, *M. dringi*, *M. elfina*, *M. fansipanensis*, *M. feae*, *M. feii*, *M. flavipunctata*, *M. gerti*, *M. gigantica*, *M. glandulosa*, *M. hansi*, *M. hoanglieniensis*, *M. huangshanensis*, *M. insularis*, *M. jiangi*, *M. jinggangensis*, *M. jiulianensis*, *M. kalanitans*, *M. kou*, *M. kuatunensis*, *M. lancip*, *M. letshanensis*, *M. lekaguli*, *M. liboensis*, *M. lini*, *M. lishuiensis*, *M. longipes*, *M. major*, *M. mangshanensis*, *M. medogensis*, *M. megacephala*, *M. microstoma*, *M. minor*, *M. mufumontana*, *M. nankiangensis*, *M. nankunensis*, *M. nanlingensis*, *M. obesa*, *M. ombrophila*, *M. omeimontis*, *M. operdion*, *M. pachyproctus*, *M. parva*, *M. periosa*, *M. robusta*, *M. rubrimeria*, *M. serchhipii*, *M. shunhuangensis*, *M. takensis*, *M. tuberogranulata*, *M. vegrandis*, *M. wawuensis*, *M. wugongensis*, *M. wuliangshanensis*, *M. wushanensis*, *M. xianjuensis*, and *M. zhangi* (vs. with rudimentary or without webs).

By heels overlapping when thighs are positioned at right angles to the body, *Megophrys qianbeiensis* sp. nov. differs from *M. acuta*, *M. brachykolos*, *M. dongguanensis*, *M. Huangshanensis*, *M. kuatunensis*, *M. nankunensis*, *M. obesa*, *M. ombrophila*, and *M. wugongensis* (vs. not meeting).

By the tibiotarsal articulation reaching to the level between tympanum and eye when leg stretched forward, *Megophrys qianbeiensis* sp. nov. differs from *M. dauwetmonits*, *M. glandulosa*, *M. lini*, *M. major*, *M. medogensis*, and *M. obesa* (vs. reaching the anterior corner of the eye or beyond eye or nostril and tip of snout).

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

The congeners *M. carinense* and *M. jiangi* have sympatric distribution with *Megophrys qianbeiensis* sp. nov. (Fei et al. 2012). The new species can be distinguished from these species by a series of morphological characters as follows. The new species differs from *M. carinense* in the smaller body size in the new species (adult males with 49.3–58.2 mm vs. adult males with 92–123 mm in the latter), a horn-like tubercle at the edge of each upper eyelid absent (vs. prominent in the latter), the tongue feebly notched behind (vs. notched behind in the latter). The new species differs from *M. jiangi* by a larger body size (49.3–58.2 mm in males in the new species vs. 34.4–39.2 mm in the latter), a horn-like tubercle at the edge of each upper eyelid absent (vs. present in the latter), the tongue feebly notched behind (vs. notched behind in the latter), presence of wide lateral fringes on the toes (vs. lacking in the latter), and toes with one-third webbing (vs. rudimentary webbing in the latter).

*Megophrys qianbeiensis* sp. nov. is phylogenetically closest to *M. sangzhiensis* and *M. spinata*. The new species differs from *M. sangzhiensis* by the following characters: horn-like tubercle absent at the edge of each upper eyelid (vs. present in the latter), toes with one-third webs (vs. with rudimentary webbing in the latter), vomerine ridges present distinctly as V-shape and vomerine teeth present (vs. vomerine ridges separated and weak, vomerine teeth absent in the latter), tibiotarsal articulation reaching to the
level between tympanum and eye when leg stretched forward (vs. reaching the anterior corner of eye in the latter), spines on nuptial pads on the first two fingers larger and sparser (vs. finer and thicker in the latter), and having significantly higher ratios of HDL, LAL, HLL, TL, and IAE to SVL. On bioacoustics, the new species differs from *M. sangzhiensis* in the following characters: lower dominant frequency (2250–3000 Hz in the new species vs. 10380 – 13200 Hz in the latter), the amplitude beginning with moderately high energy pulses, increasing slightly to a maximum by approximately mid note, and then decreasing towards the end of each note (vs. beginning with maximum energy pulses and then decreasing towards the end of note in the latter).

The new species differs from *M. spinata* by the following characters: tibiotarsal articulation reaching the level between tympanum to eye when leg stretched forward (vs. reaching the anterior corner of eye in the latter), present distinctly as V-shape and vomerine teeth present (vs. vomerine ridges separated and weak, vomerine teeth absent in the latter), spines on nuptial pads on the first two fingers little weaker (vs. spines larger in the latter), and having significantly higher ratios of HDW, ED, LAL, TYD and IAE to SVL. On bioacoustics, the new species differs from *M. spinata* in the following characters: lower dominant frequency (2250–3000 Hz in the new species vs. 4260–4589 Hz in the latter), the amplitude beginning with moderately high energy pulses, increasing slightly to a maximum by approximately mid note, and then decreasing towards the end of each note (vs. beginning with lower energy pulses, then increasing to the maximum by approximately one-four note and then decreasing to the mid note then increasing to the second highest energy pulses and then decreasing towards the end of note in the latter).

**Distribution and habitats.** *Megophrys qianbeiensis* sp. nov. is known from Huannglian Nature Reserve, Tongzi County and Kuankuoshui National Nature Reserve, Suiyang County, Guizhou Province, China at elevations between 1400–1600 m. The individuals of the new species were frequently found on stone in the streams surrounded by evergreen broadleaved forests (Fig. 8), and three sympatric amphibian species were found, i.e., *Megophrys jiangi*, *Odorrana margaratae* (Liu, 1950), and *Zhangixalus omeimontis* (Stejneger, 1924).

![Figure 8. Habitats of Megophrys qianbeiensis sp. nov. in the type locality, Huanglian Nature Reserve, Tongzi County, Guizhou Province, China](image)

A landscape of montane forests in the type locality. B a mountain stream where toads of the new species live (insert the holotype CIBTZ20190608017 standing on the stone).
**Etymology.** The specific epithet *qianbeiensis* refers to northern part of Guizhou, also called “黔”, the province where the type locality of the species belongs to. We propose the common English name “Qianbei horned toad” and Chinese name “Qian Bei Jiao Chan (黔北角蟾)”.

**Discussion**

The new species *Megophrys qianbeiensis* sp. nov. resembles *M. spinata* and *M. sangzhienensis*, and detailed comparisons with different data are important for recognizing them. Our molecular phylogenetic data on mitochondrial DNA and morphological comparisons both separated the new species from the two closely related species. *Megophrys spinata* were reported to be distributed widely through the provinces of Sichuan, Guizhou, Hunan, Chongqing, Yunnan, and Guangxi (Fei et al. 2012), but detailed investigations with multiple data suggested that several populations of the species should contain cryptic species (including *Megophrys qianbeiensis* sp. nov. and *M. sangzhienensis*). In recent years, many new species of the genus *Megophrys* have been gradually described, of which a large part of number was found in China (Frost 2020). To date, among the 106 species of *Megophrys*, 56 species were discovered in China. Even so, many cryptic species still need to be described just in southern China (Chen et al. 2017; Liu et al. 2018).

South-western China has long been proposed as biodiversity hotspot (Myers et al. 2000). Guizhou Province is an important part of southwest China, especially with the particular environments of karst rocky desertification, and knowledge of biodiversity levels and/or patterns are still seriously lacking. Recently, a series of new amphibian species were described from this province (Zhang et al. 2017; Li et al. 2018a, b, 2019a, b; Lyu et al. 2019; Wang et al. 2019c; Luo et al. 2020; Liu et al. 2020; Wei et al. 2020; Xu et al. 2020), indicating that species diversity of amphibians in this region is highly underestimated. It is urgent for herpetologists to conduct comprehensive and in-depth surveys to explore the level of amphibian species diversity in this region under accelerating climate changes. Obviously, more work should be conducted on detecting the differentiation of the populations and further describe the cryptic species in this region.

**Acknowledgements**

We are grateful to the editors and reviewers for their working on the manuscript. This work was supported by National Natural Sciences Foundation of China (NSFC31960099), Key project for bio-diversity conservation, Ministry of Ecology and Environment of People’s Republic of China. (Code: 2018-02-06-M2019-49/50), Basic research project of science and technology department of Guizhou Province (Nos. [2020] 1Y083), Science and technology support project of science and technology department of Guizhou Provincial (No. [2020] 4Y029) and Guizhou Provincial Department of Education Youth Science and Technology Talents Growth Project (Nos. KY[2018]455 and KY[2018]468).
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Supplementary material 1

Table S1. Uncorrected p-distances between the *Megophrys* species based on COI gene sequences
Authors: Haijun Su, Shengchao Shi, Yanqing Wu, Guangrong Li, Xiaogang Yao, Bin Wang, Shize Li
Data type: molecular data
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Link: https://doi.org/10.3897/zookeys.974.56070.suppl1

Supplementary material 2

Table S2. Diagnostic characters separating the new species described in this study from other species of *Megophrys*
Authors: Bin Wang
Data type: morphological data
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