

# A new species of the Asian leaf litter toad genus *Leptobrachella* Smith, 1925 (Anura, Megophryidae) from northwest Guizhou Province, China

Yan-Lin Cheng<sup>1\*</sup>, Sheng-Chao Shi<sup>2\*</sup>, Jiaqi Li<sup>3</sup>, Jing Liu<sup>1</sup>, Shi-Ze Li<sup>1,2</sup>, Bin Wang<sup>1,2</sup>

**1** Department of Resources and Environment, Moutai Institute, Renhuai 564500, China **2** Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, China **3** Nanjing Institute of Environmental Sciences, Ministry of Ecology and Environment of China, Nanjing 210042, China

Corresponding author: Shi-Ze Li (976722439@qq.com); Bin Wang (wangbin@cib.ac.cn)

Academic editor: A. Ohler | Received 13 November 2020 | Accepted 4 February 2021 | Published 2 March 2021

<http://zoobank.org/0C8921F1-7D8B-417C-B10F-55787C19E501>

**Citation:** Cheng Y-L, Shi S-C, Li J, Liu J, Li S-Z, Wang B (2021) A new species of the Asian leaf litter toad genus *Leptobrachella* Smith, 1925 (Anura, Megophryidae) from northwest Guizhou Province, China. ZooKeys 1021: 81–107. <https://doi.org/10.3897/zookeys.1021.60729>

## Abstract

A new species of the Asian leaf litter toad genus *Leptobrachella* is described from Guizhou Province, China. Molecular phylogenetic analyses support the new species as an independent lineage deeply nested in the *Leptobrachella* clade. The new species is distinguished from its congeners by a combination of the following morphological characters: body size medium (SVL 29.7–31.2 mm in five adult males); dorsal skin shagreened, some of the granules forming longitudinal short skin ridges; tympanum distinctly discernible, slightly concave; supra-axillary, femoral, pectoral and ventrolateral glands distinctly visible; absence of webbing and lateral fringes on fingers; toes with narrow lateral fringes but without webbing; heels overlapping when thighs are positioned at right angles to the body; tibia-tarsal articulation reaching the middle of eye when leg stretched forward. The discovery highlighted the underestimated species diversity in the *Leptobrachella* toads in southwestern China.

## Keywords

*Leptobrachella jinshaensis* sp. nov., molecular phylogenetic analyses, morphology, Taxonomy

\* These authors have contributed equally to this work.

## Introduction

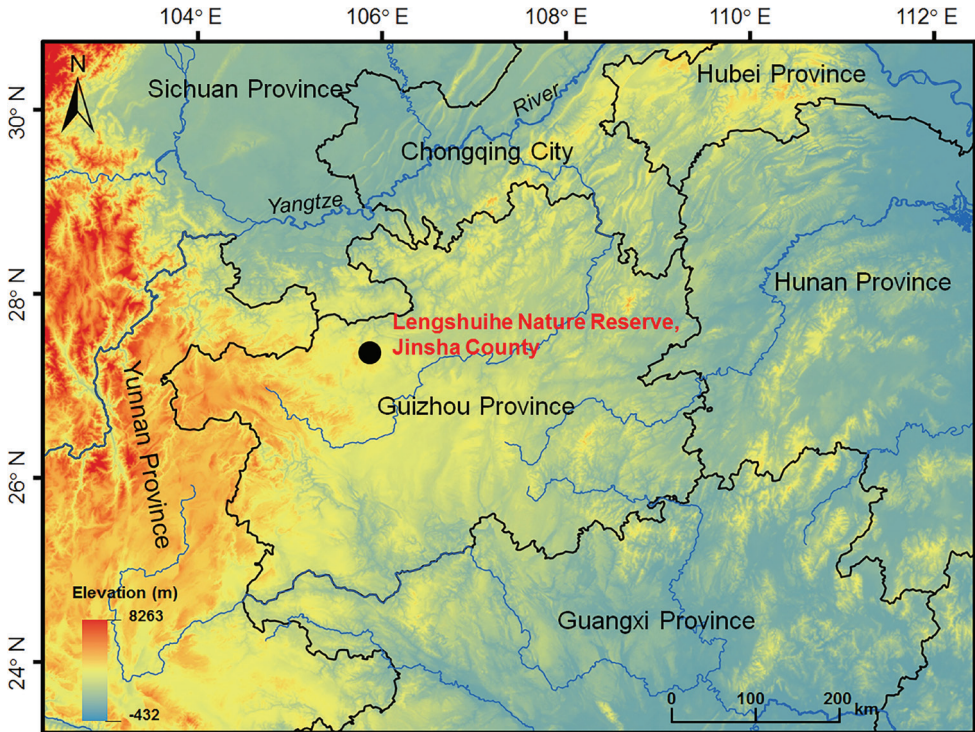
The Asian leaf litter toads of the genus *Leptobrachella* Smith, 1925 (Anura, Megophryidae) are widely distributed from southern China west to northeastern India and Myanmar, through mainland Indochina to peninsular Malaysia and the island of Borneo (Frost 2020). Many species in this genus have been classified into *Leptolalax* Dubois, 1983 (e.g., Fei et al. 2009, 2012), but Chen et al. (2018) placed *Leptolalax* as a junior synonym of *Leptobrachella* based on large-scale molecular analyses. Currently, the genus *Leptobrachella* contains 82 species (Frost 2020) but a series of cryptic species is still suggested by molecular phylogenetic analyses (Chen et al. 2018). To date, 25 species of this genus have been recorded in China, i.e., *L. alpina* (Fei, Ye & Li, 1990) and *L. bourreti* (Dubois, 1983) from Yunnan and Guangxi; *L. eos* (Ohler, Wollenberg, Grosjean, Hendrix, Vences, Ziegler & Dubois, 2011), *L. nyx* (Ohler, Wollenberg, Grosjean, Hendrix, Vences, Ziegler & Dubois, 2011), *L. pelodytoides* (Boulenger, 1893), *L. tengchongensis* (Yang, Wang, Chen & Rao, 2016), *L. yingjiangensis* (Yang, Zeng & Wang, 2018), *L. feii* (Chen, Yuan & Che, 2020), *L. flaviglandulosa* (Chen, Wang & Che, 2020), and *L. niveimontis* (Chen, Poyarkov, Yuan & Che, 2020) from Yunnan; *L. laui* (Sung, Yang & Wang, 2014) and *L. yunkaiensis* Wang, Li, Lyu & Wang, 2018 from Guangdong and Hong Kong; *L. liui* (Fei & Ye, 1990) from Fujian, Jiangxi, Guangdong, Guangxi, Hunan, and Guizhou; *L. oshanensis* (Liu, 1950) from Gansu, Sichuan, Chongqing, Guizhou, and Hubei; *L. purpuraventra* (Wang, Li, Li, Chen & Wang, 2019), *L. bijie* (Wang, Li, Li, Chen & Wang, 2019), *L. chishuiensis* (Li, Liu, Wei & Wang, 2020), and *L. suiyangensis* (Luo, Xiao, Gao & Zhou, 2020) from Guizhou; *L. purpurus* (Yang, Zeng & Wang, 2018), *L. ventripunctata* (Fei, Ye & Li, 1990) from Guizhou and Yunnan; *L. mangshanensis* (Hou, Zhang, Hu, Li, Shi, Chen, Mo & Wang, 2018) from Hunan; and *L. sungi* (Lathrop, Murphy, Orlov & Ho, 1998), *L. maoershanensis* (Yuan, Sun, Chen, Rowley & Che, 2017), *L. shangsiensis* (Chen, Liao, Zhou & Mo, 2019), and *L. wubuangmontis* (Wang, Yang & Wang, 2018) from Guangxi. Among them, ten *Leptobrachella* species occur in Guizhou Province, China, highlighting the high species diversity of the genus in this region.

In recent years, we collected some specimens of *Leptobrachella* from northwest Guizhou Province, China. Molecular phylogenetic analyses, morphological comparisons, and bioacoustics data consistently indicated these specimens as an undescribed species of *Leptobrachella*. We describe it herein as a new species.

## Materials and methods

### Specimens

Five adult males of the new species were collected on 16 May 2020 from Lengshuihe Nature Reserve, Jinsha County, Guizhou Province, China (Fig. 1; Table 1). After taking photographs, toads were euthanised using isoflurane, and then the specimens were fixed in 10% buffered formalin. Tissue samples were taken and preserved separately



**Figure 1.** Location of the type locality of *Leptobrachella jinshaensis* sp. nov., Lengshuihe Nature Reserve, Jinsha County, Guizhou Province, China.

in 95% ethanol prior to fixation. Specimens were deposited in Chengdu Institute of Biology, Chinese Academy of Sciences (**CIB, CAS**).

### Molecular phylogenetic analyses

All five adult male specimens of the new species collected in this work were included in the molecular phylogenetic analyses (Table 1). For phylogenetic analyses, the corresponding gene sequences for all those related species for which comparable sequences were available were also downloaded from GenBank (Table 1). Corresponding sequences of one *Leptobrachium tengchongensis*, one *Leptobrachium huashen*, and one *Megophrys major* were downloaded (Table 1) and used as outgroups based on previous studies (Chen et al. 2018; Li et al. 2020a).

Total DNA was extracted using a standard phenol-chloroform extraction protocol (Sambrook et al. 1989). The mitochondrial 16S rRNA genes were amplified, and the primers P7 (5'-CGCCTGTTTACCAAAAACAT-3') and P8 (5'-CCGGTCT-GAACTCAGATCACGT-3') were used following Simon et al. (1994). 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 sec, annealing at 51 °C for 30 sec and extending at 72 °C for 70 sec. Sequencing was conducted using an ABI3730 automated

**Table 1.** Information for samples used in molecular phylogenetic analyses in this study.

| ID | Species                                     | Voucher                        | Locality  | GenBank accession number |
|----|---|--------------------------------|---|--------------------------|
| 1  | <i>Leptobranchella jinshaensis</i> sp. nov. | CIBJS20200516001               | Lengshuihe Nature Reserve, Jinsha County, Guizhou Province, China           | MT814014                 |
| 2  | <i>Leptobranchella jinshaensis</i> sp. nov. | CIBJS20200516002               | Lengshuihe Nature Reserve, Jinsha County, Guizhou Province, China           | MT814015                 |
| 3  | <i>Leptobranchella jinshaensis</i> sp. nov. | CIBJS20200516003               | Lengshuihe Nature Reserve, Jinsha County, Guizhou Province, China           | MT814016                 |
| 4  | <i>Leptobranchella jinshaensis</i> sp. nov. | CIBJS20200516004               | Lengshuihe Nature Reserve, Jinsha County, Guizhou Province, China           | MT814017                 |
| 5  | <i>Leptobranchella jinshaensis</i> sp. nov. | CIBJS20200516005               | Lengshuihe Nature Reserve, Jinsha County, Guizhou Province, China           | MT814018                 |
| 6  | <i>Leptobranchella chishuiensis</i>         | CIBCS20190518047               | Alsophila National Nature Reserve, Chishui City, Guizhou Province, China    | MT117053                 |
| 7  | <i>Leptobranchella chishuiensis</i>         | CIBCS20190518042               | Alsophila National Nature Reserve, Chishui City, Guizhou Province, China    | MT117054                 |
| 8  | <i>Leptobranchella chishuiensis</i>         | CIBCS20190518043               | Alsophila National Nature Reserve, Chishui City, Guizhou Province, China    | MT117055                 |
| 9  | <i>Leptobranchella bijie</i>                | SYS a007313/<br>CIB110002      | Mt. Zhaozi Nature Reserve, Bijie City, Guizhou Province, China              | MK414532                 |
| 10 | <i>Leptobranchella bijie</i>                | SYS a007314                    | Mt. Zhaozi Nature Reserve, Bijie City, Guizhou Province, China              | MK414533                 |
| 11 | <i>Leptobranchella bijie</i>                | SYS a007315                    | Mt. Zhaozi Nature Reserve, Bijie City, Guizhou Province, China              | MK414534                 |
| 12 | <i>Leptobranchella suiyangensis</i>         | GZNU20180606002                | Huoqiuba Nature Reserve, Suiyang County, Guizhou, China                     | MK829648                 |
| 13 | <i>Leptobranchella suiyangensis</i>         | GZNU20180606006                | Huoqiuba Nature Reserve, Suiyang County, Guizhou, China                     | MK829649                 |
| 14 | <i>Leptobranchella suiyangensis</i>         | GZNU20180606005                | Huoqiuba Nature Reserve, Suiyang County, Guizhou, China                     | MK829650                 |
| 15 | <i>Leptobranchella niveimontis</i>          | KIZ015744                      | Daxueshan Nature Reserve, Yunnan, China                                     | MH055878                 |
| 16 | <i>Leptobranchella purpuraventra</i>        | SYS a007081                    | Wujing Nature Reserve, Bijie City, Guizhou Province, China                  | MK414517                 |
| 17 | <i>Leptobranchella purpuraventra</i>        | SYS a007277/<br>CIB110003      | Wujing Nature Reserve, Bijie City, Guizhou Province, China                  | MK414518                 |
| 18 | <i>Leptobranchella purpuraventra</i>        | SYS a007278                    | Wujing Nature Reserve, Bijie City, Guizhou Province, China                  | MK414519                 |
| 19 | <i>Leptobranchella bourveti</i>             | AMS R 177673                   | Lao Cai Province, Vietnam   | KR018124                 |
| 20 | <i>Leptobranchella purpurus</i>             | SYS a006530                    | Yingjiang County, Yunnan Province, China                                    | MG520354                 |
| 21 | <i>Leptobranchella alpina</i>               | KIZ046816                      | Huangcaoling, Yunnan Province, China  | MH055866                 |
| 22 | <i>Leptobranchella oshanensis</i>           | KIZ025776                      | Emei Shan, Emei Shan City, Sichuan Province, China                          | MH055895                 |
| 23 | <i>Leptobranchella eos</i>                  | MNHN:2004.0278                 | Phongsaly Province, Laos  | JN848450                 |
| 24 | <i>Leptobranchella tengchongensis</i>       | SYS a004598                    | Tengchong County, Yunnan Province, China                                    | KU589209                 |
| 25 | <i>Leptobranchella puboatensis</i>          | AMS:R184852                    | Pu Hoat Nature Reserve, Nghe An Province, Vietnam                           | KY849588                 |
| 26 | <i>Leptobranchella namdongensis</i>         | VNUF A.2017.37                 | Thanh Hoa Provincen, Vietnam  | MK965389                 |
| 27 | <i>Leptobranchella petrops</i>              | AMS:R184826                    | Vietnam   | KY459997                 |
| 28 | <i>Leptobranchella khasiorum</i>            | SDBDU 2009.329                 | East Khasi Hills, Meghalaya, India  | KY022303                 |
| 29 | <i>Leptobranchella yingjiangensis</i>       | SYS a006532                    | Yingjiang County, Yunnan Province, China                                    | MG520351                 |
| 30 | <i>Leptobranchella mangshanensis</i>        | MSZTC201701                    | Mt. Mang, Yizhang County, Hunan Province, China                             | MG132196                 |
| 31 | <i>Leptobranchella liui</i>                 | SYS a001597                    | Mt. Wuyi, Wuyishan City, Fujian Province, China                             | KM014547                 |
| 32 | <i>Leptobranchella laui</i>                 | SYS a001507                    | Mt. Wutong, Shenzhen City, Guangdong Province, China                        | KM014544                 |
| 33 | <i>Leptobranchella yunkaiensis</i>          | SYS a004664 /<br>CIB107272     | Dawuling Forest Station, Maoming City, Guangdong Province, China            | MH605585                 |
| 34 | <i>Leptobranchella maoershanensis</i>       | KIZ019385                      | Mt. Maer Nature Reserve, Ziyuan County, Guangxi Province, China             | KY986930                 |
| 35 | <i>Leptobranchella flaviglandulosa</i>      | KIZ016072                      | Xiaoqiaogou Nature Reserve, Yunnan, China                                   | MH055934                 |
| 36 | <i>Leptobranchella zhangyapingi</i>         | KIZ07258                       | Pang Num Poo, Chiang Mai Province, Thailand                                 | MH055864                 |
| 37 | <i>Leptobranchella sungi</i>                | ROM 20236                      | Tam Dao, Vinh Phuc, Vietnam   | MH055858                 |
| 38 | <i>Leptobranchella isos</i>                 | VNMN A 2015.4/<br>AMS R 176480 | Gia Lai Province, Vietnam   | KT824769                 |
| 39 | <i>Leptobranchella firthi</i>               | AMS R 176524                   | Kon Tum Province, Vietnam   | JQ739206                 |
| 40 | <i>Leptobranchella minimus</i>              | KUHE:19201                     | Thailand  | LC201981                 |
| 41 | <i>Leptobranchella ventripunctata</i>       | SYS a004536                    | Zhushihe, Yunnan Province, China  | MH055831                 |
| 42 | <i>Leptobranchella feii</i>                 | KIZ048893                      | Xiaoqiaogou Nature Reserve, Yunnan, China (E)                               | MH055841                 |
| 43 | <i>Leptobranchella aerea</i>                | ZFMK 86362                     | Quang Binh Province, Vietnam  | JN848409                 |
| 44 | <i>Leptobranchella pluvialis</i>            | MNHN:1999.5675                 | Mt. Fan Si Pan, Lao Cai Province, Vietnam                                   | JN848391                 |
| 45 | <i>Leptobranchella shangsiensis</i>         | NHMG1704003                    | Shangsi County, Guangxi Zhuang minority Autonomous Region, China            | MK095463                 |
| 46 | <i>Leptobranchella wuhuangmontis</i>        | SYS a003500 /<br>CIB107274     | Mt. Wuhuang, Pubei County, Guangxi Zhuang minority Autonomous Region, China | MH605581                 |

| ID | Species                             | Voucher              | Locality   | GenBank accession number |
|----|-------------------------------------|----------------------|--|--------------------------|
| 47 | <i>Leptobrachella nahangensis</i>   | ROM 7035             | Na Hang Nature Reserve, Tuyen Quang, Vietnam                                   | MH055853                 |
| 48 | <i>Leptobrachella nyx</i>           | AMNH A163810         | Ha Giang Province, Vietnam   | DQ283381                 |
| 49 | <i>Leptobrachella tuberosa</i>      | ZMMU-NAP-02275       | Kon Ka Kinh National Park, Gia Lai, Vietnam                                    | MH055959                 |
| 50 | <i>Leptobrachella botsfordi</i>     | VNMN 03682           | Fansipan, Lao Cai, Vietnam   | MH055953                 |
| 51 | <i>Leptobrachella pallida</i>       | UNS00510             | Lam Dong Province, Vietnam   | KR018112                 |
| 52 | <i>Leptobrachella kalonensis</i>    | IEBR A.2015.15       | Binh Thuan Province, Vietnam   | KR018114                 |
| 53 | <i>Leptobrachella bidoupensis</i>   | NAP-01453            | Lam Dong Province, Vietnam   | KP017573                 |
| 54 | <i>Leptobrachella tadungensis</i>   | UNS00515             | Dak Nong Province, Vietnam   | KR018121                 |
| 55 | <i>Leptobrachella maculosa</i>      | AMS R 177660         | Ninh Thuan Province, Vietnam   | KR018119                 |
| 56 | <i>Leptobrachella pyrrhops</i>      | ZMMU ABV-00148       | Loc Bao, Lam Dong Province, Vietnam  | KP017575                 |
| 57 | <i>Leptobrachella macrops</i>       | IEBR A.2017.9        | Hon Den Mt., Phu Yen Province, Vietnam   | MG787990                 |
| 58 | <i>Leptobrachella melica</i>        | MVZ 258197           | Virachey National Park, Ratanakiri Province, Cambodia                          | HM133599                 |
| 59 | <i>Leptobrachella applebyi</i>      | AMS R171704          | Song Thanh, Quang Nam, Vietnam   | HM133598                 |
| 60 | <i>Leptobrachella rowleyae</i>      | ITBCZ 2783           | Son Tra, Da Nang City, Vietnam   | MG682552                 |
| 61 | <i>Leptobrachella ardens</i>        | AMS R 176463         | Gia Lai Province, Vietnam  | KR018110                 |
| 62 | <i>Leptobrachella crocea</i>        | AMS R 173740         | Kon Tum, Vietnam   | MH055954                 |
| 63 | <i>Leptobrachella melanoleuca</i>   | KUHE 23840           | Thailand   | LC201997                 |
| 64 | <i>Leptobrachella fuliginosa</i>    | KUHE:20172           | Thailand   | LC201985                 |
| 65 | <i>Leptobrachella itiokai</i>       | KUHE:55897           | Mulu NP, Sarawak, Borneo, Malaysia   | LC137805                 |
| 66 | <i>Leptobrachella brevicrus</i>     | ZMH A09365           | Sarawak: Gunung Mulu National Park: Small stream of the Sungei Tapin, Malaysia | KJ831302                 |
| 67 | <i>Leptobrachella parva</i>         | KUHE 55308           | Mulu NP, Sarawak, Borneo, Malaysia   | LC056791                 |
| 68 | <i>Leptobrachella baluensis</i>     | SP 21604             | Tambunan, Sabah, Borneo, Malaysia  | LC056792                 |
| 69 | <i>Leptobrachella mjobergi</i>      | KUHE 17064           | Gading NP, Sarawak, Borneo, Malaysia   | LC056785                 |
| 70 | <i>Leptobrachella juliandringi</i>  | SRC 00230/KUHE 49815 | Mulu NP, Sarawak, Borneo, Malaysia   | LC056779                 |
| 71 | <i>Leptobrachella arayai</i>        | BORNEEISIS 22931     | Liwagu, Kinabalu, Borneo, Malaysia   | AB847558                 |
| 72 | <i>Leptobrachella hamidi</i>        | KUHE 17545           | Borneo, Malaysia   | AB969286                 |
| 73 | <i>Leptobrachella marmorata</i>     | KUHE 53227           | Annah Rais, Padawan, Kuching Division, Sarawak, Malaysia                       | AB969289                 |
| 74 | <i>Leptobrachella maura</i>         | SP 21450             | Kinabalu, Sabah, Malaysia  | AB847559                 |
| 75 | <i>Leptobrachella gracilis</i>      | KUHE 55624           | Camp 1, Gunung Mulu, Borneo, Malaysia  | AB847560                 |
| 76 | <i>Leptobrachella sabahmontana</i>  | BORNEENSIS 12632     | Borneo, Malaysia   | AB847551                 |
| 77 | <i>Leptobrachella dringi</i>        | KUHE 55610           | Camp 4 of Gunung Mulu, Malaysia  | AB847553                 |
| 78 | <i>Leptobrachella picta</i>         | UNIMAS 8705          | Borneo, Malaysia   | KJ831295                 |
| 79 | <i>Leptobrachella fritinniensis</i> | KUHE 55371           | Headquarters, Gunung Mulu, Malaysia  | AB847557                 |
| 80 | <i>Leptobrachella sola</i>          | KUHE 23261           | Hala Bala, Thailand  | LC202007                 |
| 81 | <i>Leptobrachella heteropus</i>     | KUHE 15487           | Larut, Peninsular, Malaysia  | AB530453                 |
| 82 | <i>Leptobrachella kecil</i>         | KUHE 52440           | Malaysia   | LC202004                 |
| 83 | <i>Leptobrachella kajangensis</i>   | LSUHC 4439           | Tioman, Malaysia   | LC202002                 |
| 84 | <i>Leptobrachium tengchongense</i>  | SYSa004604d          | Yunnan Province, China   | KX066880                 |
| 85 | <i>Leptobrachium huashen</i>        | KIZ049025            | Yunnan Province, China   | KX811931                 |
| 86 | <i>Megophrys major</i>              | AMS R 173870         | Kon Tum, Vietnam   | KY476333                 |

**Table 2.** Measurements of adult males of *Leptobrachella jinshaensis* sp. nov. Units given in mm. See abbreviations for morphometric characters in Materials and methods section.

| Voucher number   | Sex  | SVL  | HDL  | HDW  | SL  | IND | IOD | UEW | ED  | TYD | LAL  | LW  | ML  | THL  | TW  | TL   | TFL  | FL   |
|------------------|------|------|------|------|-----|-----|-----|-----|-----|-----|------|-----|-----|------|-----|------|------|------|
| CIBCS20200516001 | male | 31.1 | 11.4 | 10.1 | 4.9 | 3.4 | 3.1 | 2.8 | 3.9 | 2.5 | 15.4 | 2.6 | 8.4 | 15.0 | 4.9 | 15.3 | 21.4 | 14.4 |
| CIBCS20200516002 | male | 31.2 | 10.8 | 10.4 | 4.6 | 3.2 | 3.2 | 2.7 | 3.9 | 2.8 | 13.7 | 2.1 | 7.7 | 15.2 | 3.2 | 15.6 | 19.3 | 13.0 |
| CIBCS20200516003 | male | 29.7 | 10.0 | 10.1 | 4.6 | 3.2 | 3.4 | 3.0 | 4.2 | 2.5 | 14.4 | 2.2 | 7.2 | 14.0 | 3.6 | 15.1 | 19.5 | 13.0 |
| CIBCS20200516004 | male | 31.1 | 10.3 | 10.0 | 4.5 | 2.8 | 3.7 | 2.9 | 4.3 | 2.6 | 15.2 | 2.4 | 8.2 | 14.6 | 3.5 | 15.1 | 21.4 | 14.2 |
| CIBCS20200516005 | male | 30.9 | 11.3 | 10.4 | 4.6 | 3.5 | 4.0 | 3.2 | 3.7 | 3.2 | 14.1 | 2.2 | 8.2 | 14.1 | 3.6 | 14.5 | 21.2 | 14.2 |

DNA sequencer in Shanghai DNA BioTechnologies Co., Ltd. (Shanghai, China). New sequences were deposited in GenBank (for GenBank accession numbers see Table 1).

Sequences were assembled and aligned using the Clustalw module in BioEdit v. 7.0.9.0 (Hall 1999) with default settings. Alignments were checked by eye and revised



manually if necessary. 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. We ran Jmodeltest v. 2.1.2 (Darriba et al. 2012) with Akaike and Bayesian information criteria on the alignment, resulting in the best-fitting nucleotide substitution models of GTR + I + G for the data. For the ML tree, branch supports were drawn from 10,000 nonparametric bootstrap replicates. In BI analyses, the parameters for each partition were unlinked, and branch lengths were allowed to vary proportionately across partitions. 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. Finally, genetic distance between *Leptobrachella* species based on uncorrected *p*-distance model was estimated on 16S gene using MEGA v. 6.06 (Tamura et al. 2013).

## Morphological comparisons

All five adult male specimens of the new species were measured (Table 2). The terminology and methods followed Fei et al. (2005), Mahony et al. (2011), and Wang et al. (2019). Measurements were made with a dial caliper to the nearest 0.1 mm (Watters et al. 2016) with digital calipers. Corresponding measurements of *L. bijie* and *L. chishuiensis* were retrieved from Wang et al. (2019) and Li et al. (2020a). Nineteen 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 the fourth toe);
- HDL** head length (distance from the tip of the snout to the articulation of jaw);
- HDW** head width (greatest width between the left and right articulations of jaw);
- HLL** hindlimb length (distance from tip of fourth toe to vent);
- 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);
- ML** manus length (distance from tip of third digit to proximal edge of inner palmar tubercle);
- SL** snout length (distance from the tip of the snout to the anterior corner of the eye);
- SVL** snout-vent length (distance from the tip of the snout to the posterior edge of the vent);

- TEY** tympanum-eye distance (distance from anterior edge of tympanum to posterior corner of 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).

In order to reduce the impact of allometry, the correct value from the ratio of each character to SVL was calculated and then all of the data were log-transformed for the following morphometric analyses. Mann-Whitney *U* tests were conducted to test the significance of differences on morphometric characters between *Leptobranchella jinshaensis* sp. nov., *L. bijie* and *L. chishuiensis*. The significance level was set at 0.05. Furthermore, principal component analyses (PCA) were conducted to highlight whether the different species were separated in morphometric space. Due to only the measurements SVL, HDL, HDW, SL, IND, IOD, ED, TYD, TEY, LAL, ML, TL, HLL, and FL of male *L. bijie* being available from Wang et al. (2019), the morphometric analyses were conducted only based on these 14 morphometric characters for male group.

*Leptobranchella jinshaensis* sp. nov. was also compared with all other congeners of *Leptobranchella* based on morphological characters. Comparative morphological data were obtained from literatures (Table 3).

### Bioacoustics data

The advertisement calls of *L. jinshaensis* sp. nov. were recorded from the holotype specimen CIBJS20200516004 in the field on 16 May 2020 in Lengshuihe Nature Reserve, Jinsha County, Guizhou Province, China. The advertisement call of *L. jinshaensis* sp. nov. was recorded in the stream at ambient air temperature of 20 °C and air humidity of 87%. A 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. Calls were recorded and examined as described by Wijayathilaka and Meegaskumbura (2016). Call recordings were visualised and edited with SoundRuler 0.9.6.0 (Gridi-Papp 2003–2007) and Raven Pro 1.5 software (Cornell Laboratory of Ornithology, Ithaca, NY, USA). Ambient temperature of the type locality was taken by a digital hygrothermograph. For comparison, bioacoustics data for the related species *L. bijie* and *L. chishuiensis* were obtained from Li et al. (2020a).

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

| No. | <i>Leptobranchella</i> species  | References                                 |
|-----|---|--|
| 1   | <i>L. aerea</i> (Rowley, Stuart, Richards, Phimmachak & Sivongxay, 2010)                    | Rowley et al. 2010a                        |
| 2   | <i>L. alpina</i> (Fei, Ye & Li, 1990)   | Fei et al. 1990                            |
| 3   | <i>L. applebyi</i> (Rowley & Cao, 2009)   | Rowley and Cao 2009                        |
| 4   | <i>L. arayai</i> (Matsui, 1997)   | Matsui 1997                                |
| 5   | <i>L. ardens</i> (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016)     | Rowley et al. 2016                         |
| 6   | <i>L. baluensis</i> (Smith, 1931)   | Dring 1983; Eto et al. 2016, 2018          |
| 7   | <i>L. bidoupensis</i> (Rowley, Le, Tran & Hoang, 2011)                                      | Rowley et al. 2011                         |
| 8   | <i>L. bijie</i> (Wang, Li, Li, Chen & Wang, 2019)   | Wang et al. 2019                           |
| 9   | <i>L. bondangensis</i> (Eto, Matsui, Hamidy, Munir & Iskandar, 2018)                        | Eto et al. 2018                            |
| 10  | <i>L. borsfordi</i> (Rowley, Dau & Nguyen, 2013)  | Rowley et al. 2013                         |
| 11  | <i>L. bourreti</i> (Dubois, 1983)   | Ohler et al. 2011                          |
| 12  | <i>L. brevicrus</i> (Dring, 1983)   | Dring 1983; Eto et al. 2015                |
| 13  | <i>L. chishuiensis</i> Li, Liu, Wei & Wang, 2020  | Li et al. 2020a                            |
| 14  | <i>L. crocea</i> (Rowley, Hoang, Le, Dau & Cao, 2010)                                       | Rowley et al. 2010b                        |
| 15  | <i>L. dringi</i> (Dubois, 1987)   | Inger et al. 1995; Matsui and Dehling 2013 |
| 16  | <i>L. eos</i> (Ohler, Wollenberg, Grosjean, Hendrix, Vences, Ziegler & Dubois, 2011)        | Ohler et al. 2011                          |
| 17  | <i>L. feii</i> (Chen, Yuan & Che, 2020)   | Chen et al. 2020                           |
| 18  | <i>L. firthi</i> (Rowley, Hoang, Dau, Le & Cao, 2012)                                       | Rowley et al. 2012                         |
| 19  | <i>L. flaviglandulosa</i> (Chen, Yuan & Che, 2020)  | Chen et al. 2020                           |
| 20  | <i>L. frittmieni</i> (Dehling & Matsui, 2013)   | Dehling and Matsui 2013                    |
| 21  | <i>L. fuliginosa</i> (Matsui, 2006)   | Matsui 2006                                |
| 22  | <i>L. fusca</i> (Eto, Matsui, Hamidy, Munir & Iskandar, 2018)                               | Eto et al. 2018                            |
| 23  | <i>L. gracilis</i> (Günther, 1872)  | Günther 1872; Dehling 2012a                |
| 24  | <i>L. hamidi</i> (Matsui, 1997)   | Matsui 1997                                |
| 25  | <i>L. heteropus</i> (Boulenger, 1900)   | Boulenger 1900                             |
| 26  | <i>L. isos</i> (Rowley, Stuart, Neang, Hoang, Dau, Nguyen & Emmett, 2015)                   | Rowley et al. 2015                         |
| 27  | <i>L. itiokai</i> Eto, Matsui & Nishikawa, 2016   | Eto et al. 2016                            |
| 28  | <i>L. juliandringi</i> Eto, Matsui & Nishikawa, 2015  | Eto et al. 2015                            |
| 29  | <i>L. kajangensis</i> (Grismer, Grismer & Youmans, 2004)                                    | Grismer et al. 2004                        |
| 30  | <i>L. kalonensis</i> (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016) | Rowley et al. 2016                         |
| 31  | <i>L. kecil</i> (Matsui, Belabut, Ahmad & Yong, 2009)                                       | Matsui et al. 2009                         |
| 32  | <i>L. khasiorum</i> (Das, Tron, Rangad & Hooroo, 2010)                                      | Das et al. 2010                            |
| 33  | <i>L. lateralis</i> (Anderson, 1871)  | Anderson 1871; Humtsoe et al. 2008         |
| 34  | <i>L. laui</i> (Sung, Yang & Wang, 2014)  | Sung et al. 2014                           |
| 35  | <i>L. liui</i> (Fei & Ye, 1990)   | Fei et al. 2009; Sung et al. 2014          |
| 36  | <i>L. macrops</i> (Duong, Do, Ngo, Nguyen & Poyarkov, 2018)                                 | Duong et al. 2018                          |
| 37  | <i>L. maculosa</i> (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016)   | Rowley et al. 2016                         |
| 38  | <i>L. mangshanensis</i> (Hou, Zhang, Hu, Li, Shi, Chen, Mo & Wang, 2018)                    | Hou et al. 2018                            |
| 39  | <i>L. maoershanensis</i> (Yuan, Sun, Chen, Rowley & Che, 2017)                              | Yuan et al. 2017                           |
| 40  | <i>L. marmorata</i> (Matsui, Zainudin & Nishikawa, 2014)                                    | Matsui et al. 2014a                        |
| 41  | <i>L. maura</i> (Inger, Lakim, Biun & Yambun, 1997)   | Inger et al. 1997                          |
| 42  | <i>L. melanoleuca</i> (Matsui, 2006)  | Matsui 2006                                |
| 43  | <i>L. melica</i> (Rowley, Stuart, Neang & Emmett, 2010)                                     | Rowley et al. 2010c                        |
| 44  | <i>L. minima</i> (Taylor, 1962)   | Taylor 1962; Ohler et al. 2011             |
| 45  | <i>L. mjobergi</i> (Smith, 1925)  | Eto et al. 2015, 2018                      |
| 46  | <i>L. nabangensis</i> (Lathrop, Murphy, Orlov & Ho, 1998)                                   | Lathrop et al. 1998                        |
| 47  | <i>L. namdongensis</i> (Hoang, Nguyen, Luu, Nguyen & Jiang, 2019)                           | Hoang et al. 2019                          |
| 48  | <i>L. natunae</i> (Günther, 1895)   | Günther 1895                               |
| 49  | <i>L. neangi</i> (Stuart & Rowley, 2020)  | Stuart and Rowley 2020                     |
| 50  | <i>L. niveimontis</i> (Chen, Yuan & Che, 2020)  | Chen et al. 2020                           |
| 51  | <i>L. nokrekensis</i> (Mathew & Sen, 2010)  | Mathew and Sen 2010                        |
| 52  | <i>L. nyx</i> (Ohler, Wollenberg, Grosjean, Hendrix, Vences, Ziegler & Dubois, 2011)        | Ohler et al. 2011                          |
| 53  | <i>L. oshanensis</i> (Liu, 1950)  | Liu 1950, 1961; This paper                 |
| 54  | <i>L. pallida</i> (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016)    | Rowley et al. 2016                         |
| 55  | <i>L. palmata</i> Inger & Stuebing, 1992  | Inger and Stuebing 1992                    |
| 56  | <i>L. parva</i> Dring, 1983   | Dring 1983                                 |
| 57  | <i>L. pelodytoides</i> (Boulenger, 1893)  | Boulenger 1893; Ohler et al. 2011          |
| 58  | <i>L. petrops</i> (Rowley, Dau, Hoang, Le, Cutajar & Nguyen, 2017)                          | Rowley et al. 2017a                        |
| 59  | <i>L. picta</i> (Malkmus, 1992)   | Malkmus 1992                               |
| 60  | <i>L. platycephala</i> (Dehling, 2012)  | Dehling 2012b                              |

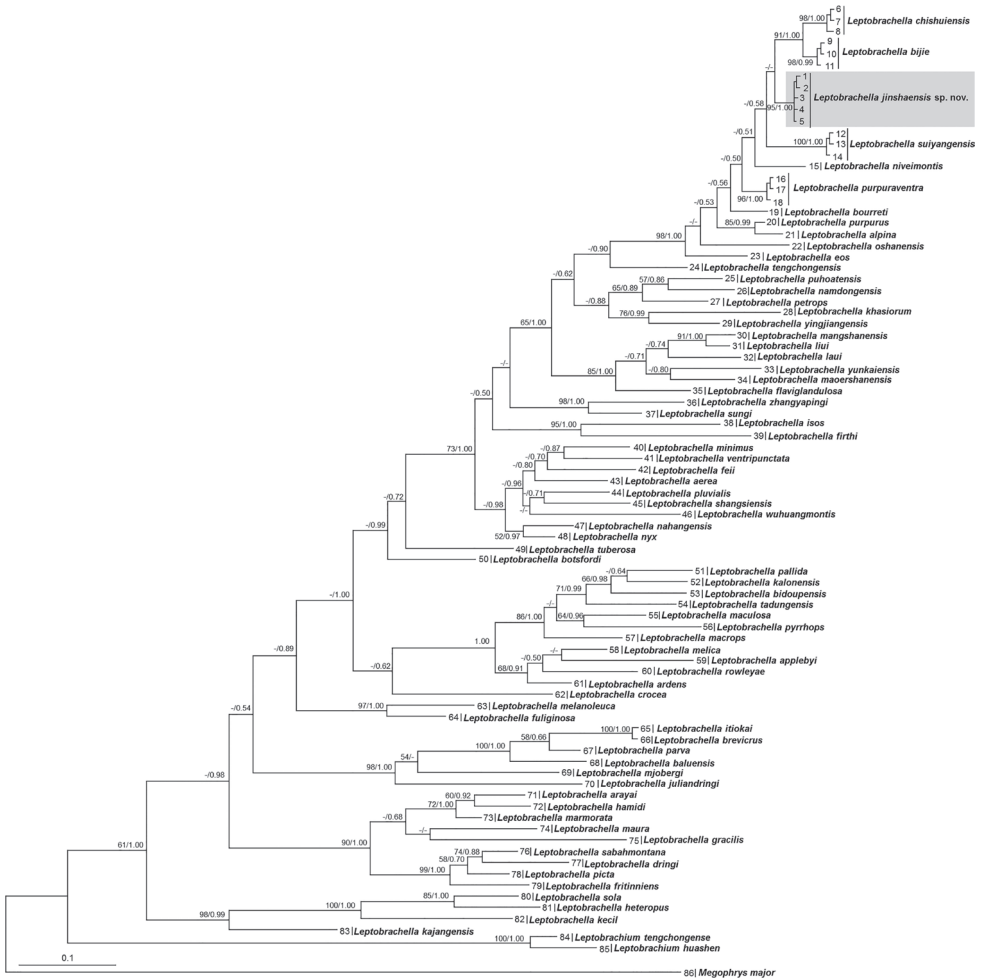


| No. | <i>Leptobrachella</i> species  | References              |
|-----|--|-------------------------|
| 61  | <i>L. pluviialis</i> (Ohler, Marquis, Swan & Grosjean, 2000)                                 | Ohler et al. 2000, 2011 |
| 62  | <i>L. puhoatensis</i> (Rowley, Dau & Cao, 2017)  | Rowley et al. 2017b     |
| 63  | <i>L. purpuraventra</i> Wang, Li, Li, Chen & Wang, 2019                                      | Wang et al. 2019        |
| 64  | <i>L. purpurus</i> (Yang, Zeng & Wang, 2018)   | Yang et al. 2018        |
| 65  | <i>L. pyrhaps</i> (Poyarkov, Rowley, Gogoleva, Vassilieva, Galoyan & Orlov, 2015)            | Poyarkov et al. 2015    |
| 66  | <i>L. rowleyae</i> (Nguyen, Poyarkov, Le, Vo, Ninh, Duong, Murphy & Sang, 2018)              | Nguyen et al. 2018      |
| 67  | <i>L. sabahmontana</i> (Matsui, Nishikawa & Yambun, 2014)                                    | Matsui et al. 2014b     |
| 68  | <i>L. serasanae</i> Dring, 1983  | Dring 1983              |
| 69  | <i>L. shangsiensis</i> Chen, Liao, Zhou & Mo, 2019   | Chen et al. 2019        |
| 70  | <i>L. sola</i> (Matsui, 2006)  | Matsui 2006             |
| 71  | <i>L. suiyangensis</i> (Luo, Xiao, Gao & Zhou, 2020)   | Luo et al. 2020         |
| 72  | <i>L. sungi</i> (Lathrop, Murphy, Orlov & Ho, 1998)  | Lathrop et al. 1998     |
| 73  | <i>L. tadungensis</i> (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016) | Rowley et al. 2016      |
| 74  | <i>L. tamdil</i> (Sengupta, Sailo, Lalremanga, Das & Das, 2010)                              | Sengupta et al. 2010    |
| 75  | <i>L. tengchongensis</i> (Yang, Wang, Chen & Rao, 2016)                                      | Yang et al. 2016        |
| 76  | <i>L. tuberosa</i> (Inger, Orlov & Darevsky, 1999)   | Inger et al. 1999       |
| 77  | <i>L. ventripunctata</i> (Fei, Ye & Li, 1990)  | Fei et al. 2009         |
| 78  | <i>L. wubuangmontis</i> Wang, Yang & Wang, 2018  | Wang et al. 2018        |
| 79  | <i>L. wulingensis</i> Qian, Xiao, Cao, Xiao & Yang, 2020                                     | Qian et al. 2020        |
| 80  | <i>L. yingjiangensis</i> (Yang, Zeng & Wang, 2018)   | Yang et al. 2018        |
| 81  | <i>L. yunkaiensis</i> Wang, Li, Lyu & Wang, 2018   | Wang et al. 2018        |
| 82  | <i>L. zhangyapingi</i> (Jiang, Yan, Suwannapoom, Chomdej & Che, 2013)                        | Jiang et al. 2013       |

## Results

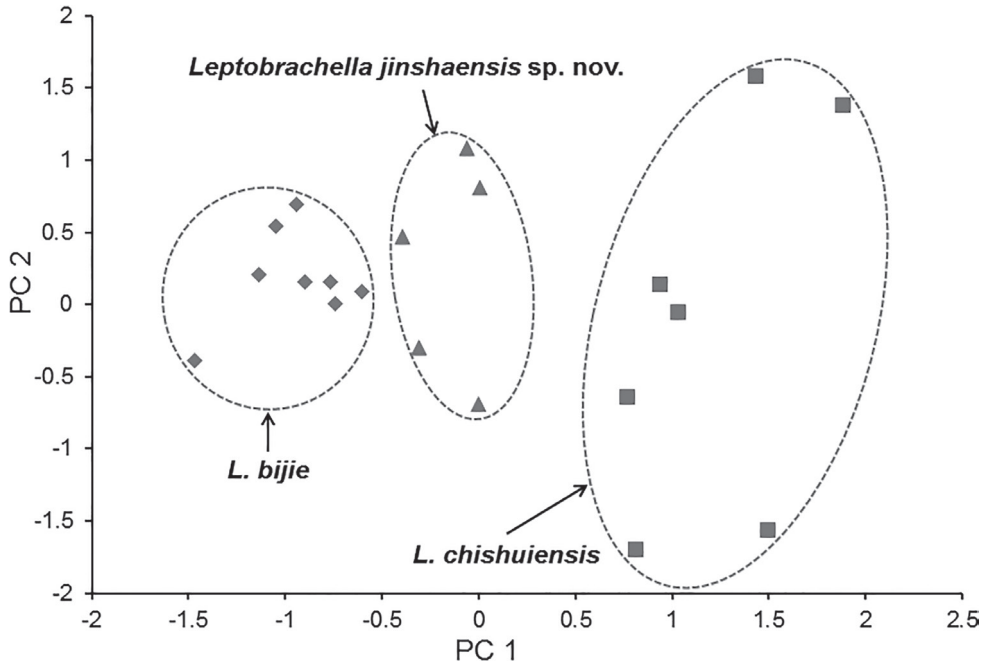
Aligned sequence matrix of 16S rRNA gene contained 537 bps. ML and BI analyses resulted in essentially identical topologies (Fig. 2). All samples of *L. jinshaensis* sp. nov. were clustered into one independent clade nested into the *Leptobrachella* clade. The relationships between *L. jinshaensis* sp. nov. and its congeners are not resolved though it is likely sister to a clade in comprising of *L. bijie* and *L. chishuiensis* (Fig. 2). The smallest pairwise genetic divergence between *L. jinshaensis* sp. nov. and all other species of the genus *Leptobrachella* is 2.6% (vs. *L. niveimontis* or vs. *L. purpurus*), being at the same level with or higher than that between some pairs of substantial species, such as *L. bijie* vs. *L. chishuiensis* (2.1%), and *L. chishuiensis* vs. *L. alpina* (2.6%; Suppl. material 1: Table S1).

For the male group, PCA extracted five principal component axes with eigenvalues greater than one, and the percentage of variance of the first five principal components are 37.7%, 15.7%, 13.0%, 9.0% and 8.1%, with percentage of cumulative is 83.5% (Suppl. material 2: Table S2). There were 14 morphological features with major contributions in the first five principal components, and these morphological features were distributed in the anterior, middle, and posterior parts of the body (Suppl. material 2: Table S2). The total variation of the first two principal components was 53.4% (Suppl. material 2: Table S2). On the PCA plot (PC1 vs. PC2), the first principal component axis could separate *L. jinshaensis* sp. nov. from *L. bijie* and *L. chishuiensis* (Fig. 3) mainly based on SVL, HDL, HDW, SL, ED, IND, TEY, and FL, and the second component axis mainly based on ML, FL, and LAL. Mann-Whitney *U* tests indicated that *L. jinshaensis* sp. nov. was significantly different from *L. bijie* on HDW, SL, IOD, TYD, TEY, LW, and FL, and from *L. chishuiensis* on SVL, TYD, and TL (*p*-values < 0.05; Table 4).



**Figure 2.** Bayesian Inference (BI) tree based on the mitochondrial 16S rRNA sequences. Bootstrap supports from Maximum Likelihood analyses/Bayesian posterior probabilities from BI analyses are labelled beside nodes. Information of samples 1–86 refer to Table 1.

In total, 109 advertisement calls of *Leptobranchella jinshaensis* sp. nov. were recorded in Lengshuihe Nature Reserve, Jinsa County, Guizhou Province, China on 16 May 2020 between 21:00–22:00. The call description is based on recordings of the holotype CIB-JS20200516004 under a stone nearby a stream, and the ambient air temperature was 20 °C. The call characters of *L. jinshaensis* sp. nov. were demonstrated in the following section for describing it. There were some differences in sonograms and waveforms of calls between *L. jinshaensis* sp. nov., *L. bijie*, and *L. chishuiensis* (Suppl. material 3: Table S3). *Leptobranchella jinshaensis* sp. nov. has longer call interval ( $132.7 \pm 8.6$ ,  $N = 109$ ) than *L. bijie* ( $101.9 \pm 6.4$ ,  $N = 33$ ), and has lower dominant frequency ( $4525 \pm 0.065$  Hz) than *L. bijie* ( $4780.4 \pm 76.5$  Hz) and *L. chishuiensis* ( $6064\text{--}6284$  H). Each call of *L. jinshaensis* sp. nov. has two kinds of notes, while each call of *L. chishuiensis* only has one kind of note.



**Figure 3.** Plots of the first principal component (PC1) versus the second (PC2) for *Leptobrachella jinshaensis* sp. nov., *L. bijie*, and *L. chishuiensis* in males from a principal component analysis based on morphometric data.

***Leptobrachella jinshaensis* sp. nov.**

<http://zoobank.org/C2982600-D9EF-46C1-A539-CC1151444B18>

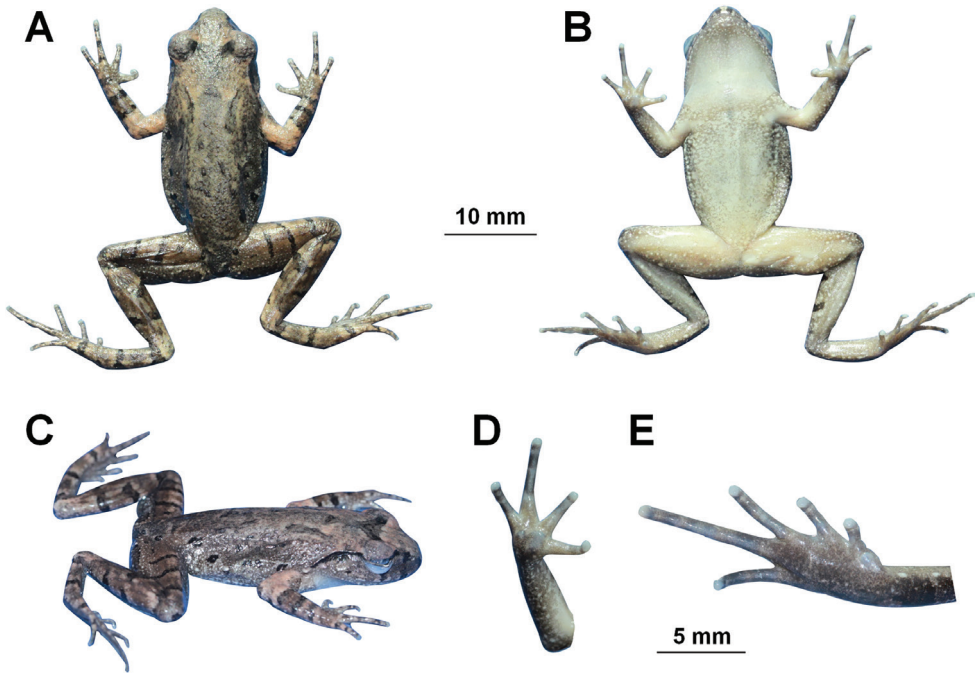
Figs 3–6; Tables 1, 2, 4, Suppl. material 1: Table S1, Suppl. material 2: Table S2

**Holotype.** CIBJS20200516004, adult male (Figs 4, 5), collected from Lengshuihe Nature Reserve, Jinsha County (27.536944°N, 105.999166°E, ca. 770 m a. s. l.), Guizhou Province, China by Shi-Ze Li on 16 May 2020.

**Paratypes.** Four adult males from the same place as holotype. Two adult males CIBJS20200516001 and CIBJS20200516002 collected by Shi-Ze LI, and two adult males CIBJS20200516003 and CIBJS20200516005 collected by Jing LIU, all of them were collected on 16 May 2020.

**Diagnosis.** *Leptobrachella jinshaensis* sp. nov. is assigned to the genus *Leptobrachella* based on molecular phylogenetic analyses and the following morphological characters: medium size, rounded finger tips, the presence of an elevated inner palmar tubercle not continuous to the thumb, the presence of macroglands on body (including supra-axillary, pectoral, and femoral glands), vomerine teeth absent, tubercles on eyelids, and the anterior tip of snout with a vertical white bar.

*Leptobrachella jinshaensis* sp. nov. can be distinguished from its congeners by a combination of the following characters: body of medium size (SVL 29.7–31.2 mm in five adult males); dorsal skin shagreened, some of the granules forming longitudi-



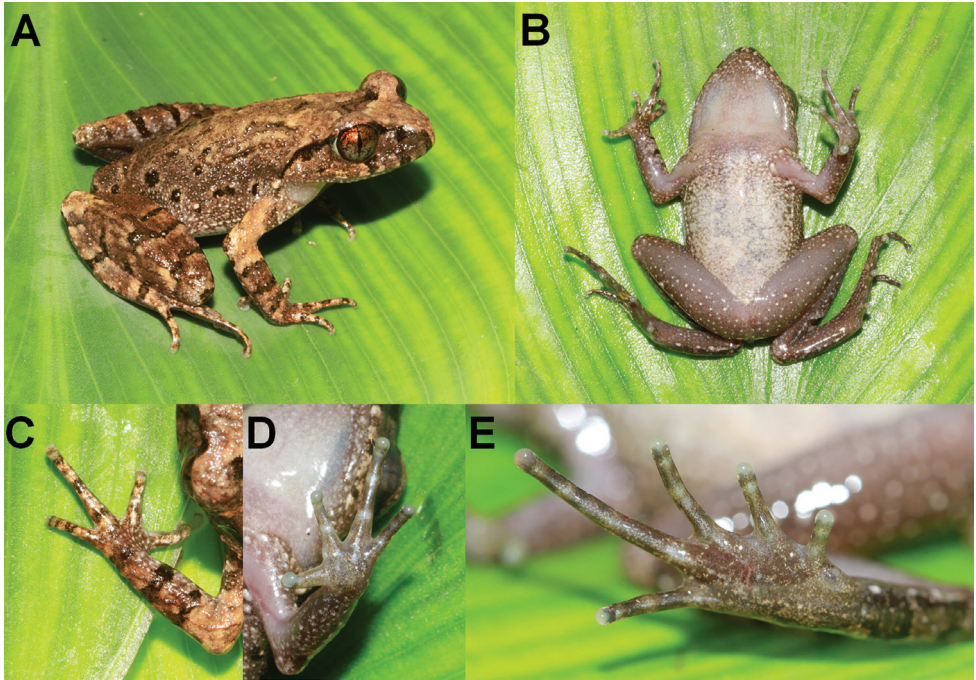
**Figure 4.** Photos of the holotype specimen CIBCS20200516004 of *Leptobrachella jinshaensis* sp. nov. **A** dorsal view **B** ventral view **C** lateral view **D** ventral view of hand **E** ventral view of foot.

nal short skin ridges; tympanum distinctly discernible, slightly concave; supra-axillary, femoral, pectoral, and ventrolateral glands distinctly visible; absence of webbing and lateral fringes on fingers; toes with narrow lateral fringes and without webbing; heels overlapping when thighs positioned at right angles to the body; tibia-tarsal articulation reaching the middle eye when leg stretched forward.

**Description of holotype (Figs 4, 5).** Adult male. SVL in 31.1 mm. **Head** length slightly longer than head width (HDL/HDW 1.02); snout slightly protruding, projecting slightly beyond margin of the lower jaw; nostril closer to snout than eye; canthus rostralis gently rounded; loreal region slightly concave; interorbital space flat, interorbital distance slightly longer than internarial distance; pineal ocellus absent; vertical pupil; eye diameter slightly shorter than snout length; tympanum distinct, rounded, and slightly concave, diameter smaller than that of the eye (TMP/ED 0.61); upper margin of tympanum in contact with supratympanic ridge; vomerine teeth absent; tongue notched behind; supratympanic ridge distinct, extending from posterior corner of eye to supra-axillary gland.

**Forelimbs** slender, 48.9% of snout-vent length; tips of fingers rounded, slightly swollen; relative finger lengths I < II <= IV < III; absence of webbing; nuptial pad and subarticular tubercles absent; inner palmar tubercle large, rounded separated from the smaller, round outer palmar tubercle.

**Hindlimbs** slender, tibia slightly longer than thigh length and 48.4% of snout-vent length; heels overlapping when thighs are positioned at right angles to the body,



**Figure 5.** Photos of the holotype CIBCS20200516004 of *Leptobrachella jinshaensis* sp. nov. in life **A** dorsal view **B** ventral view **C** dorsal view of hand **D** ventral view of hand **E** ventral view of foot.

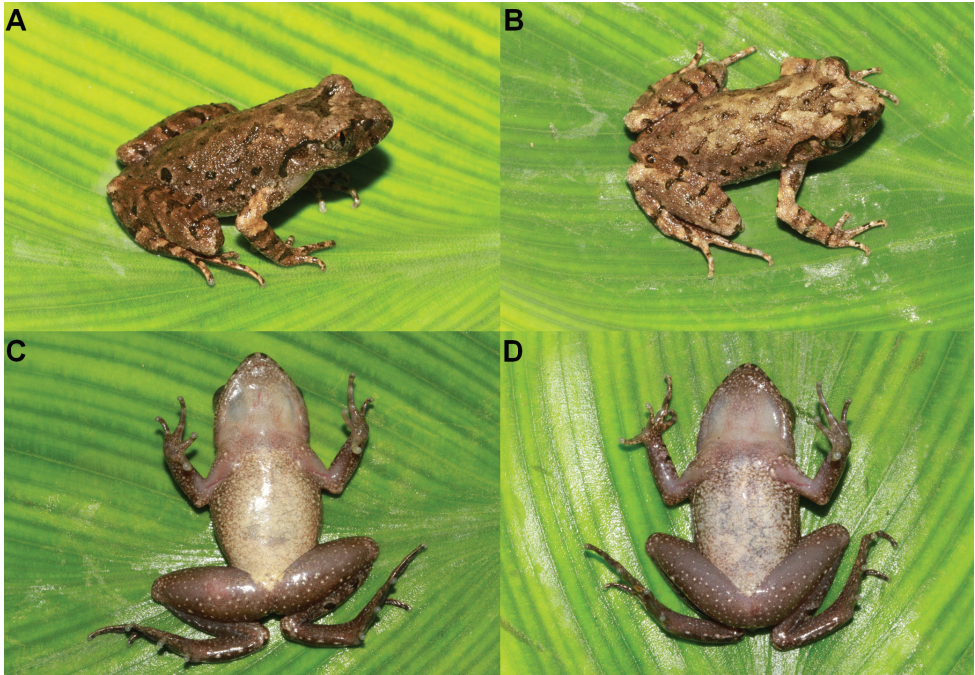
tibiotarsal articulation reaching middle eye when leg stretched forward; relative toe lengths  $I < II < V < III < IV$ ; tips of toes round, slightly dilated; subarticular tubercle at the articulations of the toes absent; toes without webbing; lateral fringes narrow on all toes; inner metatarsal tubercle present, large, oval, outer metatarsal tubercle absent.

Dorsal surface shagreened and granular, some of the granules forming short longitudinal folds dorsally on the flank; ventral skin smooth; dense tiny granules present on ventral surface of thigh and tibia; pectoral gland and femoral gland white, oval, distinctly visible. Ventrolateral gland distinctly visible and forming an incomplete line.

**Colouration of holotype in life.** Dorsum brown, with small, distinct darker brown markings and spots, and irregularly dispersed light orange speckles. A dark brown inverted triangular pattern between anterior corners of eyes. Tympanum brown, a dark brown bar above tympanum, and a dark brown bar under the eye, distinct black supratympanic line present; transverse dark brown bars on dorsal surface of limbs; distinct dark brown blotches on flanks from groin to axilla, longitudinally in two rows; elbow and upper arms with dark bars and distinct coppery orange colouration; fingers and toes with distinct dark bars. Ventral surface of throat cream white, chest, and belly cream yellow with purple speckling, and on flanks presence of distinct nebulous greyish speckling; ventral surface of limbs grey purple. Supra-axillary gland, femoral, pectoral, and ventrolateral glands white (Fig. 5).

**Colouration of holotype in preservation.** Dorsum of body and limbs fade to brown copper; transverse bars on limbs become more distinct. Ventral surface of body



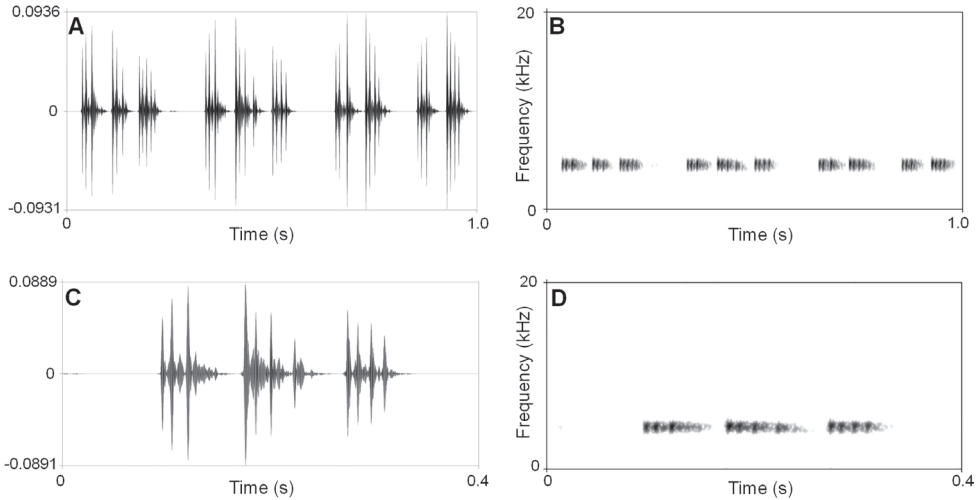


**Figure 6.** Colour variation in *Leptobrachella jinshaensis* sp. nov. **A** dorsal view of the male specimen CIBJS20200516002 **B** dorsal view of the male specimen CIBJS20200516005 **C** ventral view of the male specimen CIBJS20200516005 **D** ventral view of the male specimen CIBJS20200516003.

and limbs fade to cream white. Supra-axillary, femoral, and pectoral glands fade to creamy yellow (Fig. 4).

**Variation.** Measurements of adult specimens were presented in Tables 2 and 4. All specimens were similar but some individuals different from the holotype in colour pattern. In CIBJS20200516002, the tympana are dark brown (Fig. 6A); in CIBJS20200516005, the dorsum is olive grey (Fig. 6B) and the pectoral glands on the left side not obviously (Fig. 6D); in CIBJS20200516003 ventrolateral glands scattered and unlined (Fig. 6C).

**Advertisement call.** In total, 109 advertisement calls of *Leptobrachella jinshaensis* sp. nov. were recorded in Lengshuihe Nature Reserve, Jinsa County, Guizhou Province, China on 16 May 2020 between 21:00–22:00. The call description is based on recordings of the holotype CIBJS20200516004 under a stone nearby a stream, and the ambient air temperature was 20 °C. The sonograms and waveforms of the new species are shown in Fig. 7 and Suppl. material 2: Table S2. The call has two kinds of notes, and each call contains two or three notes (mean  $2.12 \pm 0.33$ ,  $n = 109$ ). Call duration was 117–156 ms (mean  $132.7 \pm 8.6$ ,  $n = 109$ ). Call interval was 62–106 ms (mean  $84.3 \pm 10.4$ ,  $n = 108$ ), and each consists of two types of note. The first type of note is the start note in each call and beginning with lowest energy pulses, then increasing to the peak; in the second type, the amplitude begins with highest pulses and then decreasing



**Figure 7.** Advertisement calls of the holotype CIBCS20200516004 of *Leptobrachella jinshaensis* sp. nov. **A** waveform showing one second contains 4 calls **B** sonogram showing one second contains 4 calls **C** waveform showing 0.4 second contains a call **D** sonogram showing 0.4 second contains a call.

towards the end of each note. The duration of first type of note with 35–71 ms (mean  $48.77 \pm 7.90$ ,  $n = 109$ ), the duration of the second type of note with 39–78 ms (mean  $52.93 \pm 8.85$ ,  $n = 122$ ), the duration between notes 18–40 ms (mean  $23 \pm 5.68$ ,  $n = 122$ ). The dominant frequency of calls is 4500–4688 Hz (mean  $4525 \pm 0.065$  Hz).

**Secondary sexual characteristics.** Adult males with a comparatively large single subgular vocal sac and nuptial pads and spines absent.

**Comparisons.** Measurements were given in mm. In male, by body size moderate in male (SVL 29.7–31.2,  $n = 5$ ), *Leptobrachella jinshaensis* sp. nov. is larger than *L. aerea* (25.1–28.9), *L. alpina* (24.0–26.4), *L. applebyi* (19.6–22.3), *L. ardens* (21.3–24.7), *L. baluensis* (14.9–15.9), *L. bidoupensis* (18.5–25.4), *L. bondangensis* (17.8), *L. brevicrus* (17.1–17.8), *L. crocea* (22.2–27.3), *L. feii* (21.5–22.8), *L. flaviglandulosa* (23.0–27.0), *L. fusca* (16.3), *L. isos* (23.7–27.9), *L. itiokai* (15.2–16.7), *L. juliandringi* (17.0–17.2), *L. khasiorum* (24.5–27.3), *L. laui* (24.8–26.7), *L. maculosa* (24.2–26.6), *L. mangshanensis* (22.22–27.76), *L. maura* (26.1), *L. melica* (19.5–22.8), *L. mjobergi* (15.7–19.0), *L. natunae* (17.6), *L. niveimontis* (22.5–23.6), *L. parva* (15.0–16.9), *L. palmata* (14.4–16.8), *L. pallida* (24.5–27.7), *L. petrops* (23.6–27.6), *L. pluvialis* (21.3–22.3), *L. purpurus* (25.0–27.5), *L. rowleyae* (23.4–25.4), *L. serasanae* (16.9), *L. tengchongensis* (23.9–26.0), *L. ventripunctata* (25.5–28.0), and *L. yingjiangensis* (25.7–27.6); and smaller than *L. eos* (33.1–34.7), *L. gracilis* (34.3–39.0), *L. marmorata* (32.3–38.0), *L. nahangensis* (40.8), *L. platycephala* (35.1), *L. sungi* (48.3–52.7), *L. tamdil* (32.0), and *L. zhangyapingi* (45.8–52.5).

By the presence of supra-axillary and ventrolateral glands, *Leptobrachella jinshaensis* sp. nov. can be easily distinguished from *L. arayai*, *L. dringi*, *L. fritinniensis*, *L. gracilis*, *L. hamidi*, *L. heteropus*, *L. kajangensis*, *L. kecil*, *L. marmorata*, *L. melanoleuca*, *L. maura*,

*L. picta*, *L. platycephala*, *L. sabahmontana*, and *L. sola* (vs. lacking supra-axillary and ventrolateral glands in the latter).

By tympanum distinctly visible, *Leptobrachella jinshaensis* sp. nov. differs from *L. crocea* and *L. tuberosa* (vs. invisible in the latter).

By having black spots on flanks, *Leptobrachella jinshaensis* sp. nov. differs from *L. aerea*, *L. botsfordi*, *L. firthi*, *L. crocea*, *L. isos*, *L. pallida*, *L. petrops*, and *L. tuberosa* (vs. lacking in the latter).

By toes without webbing, *Leptobrachella jinshaensis* sp. nov. differs from *L. aerea*, *L. alpina*, *L. applebyi*, *L. bidoupensis*, *L. bijie*, *L. botsfordi*, *L. bourreti*, *L. chishuiensis*, *L. crocea*, *L. eos*, *L. feii*, *L. firthi*, *L. fuliginosa*, *L. isos*, *L. khasiorum*, *L. lateralis*, *L. laui*, *L. liui*, *L. macrops*, *L. mangshanensis*, *L. maershanensis*, *L. marmorata*, *L. melica*, *L. minima*, *L. nahangensis*, *L. namdongensis*, *L. niveimontis*, *L. nokrekensis*, *L. nyx*, *L. pluviialis*, *L. pluviialis*, *L. puhoatensis*, *L. purpurus*, *L. purpuraventra*, *L. pyrrhops*, *L. sabahmontana*, *L. shangsiensis*, *L. suiyangensis*, *L. tengchongensis*, *L. tuberosa*, *L. ventripunctata*, *L. wuhuangmontis*, *L. yingjiangensis*, *L. yunkaiensis*, and *L. zhangyapingi* (vs. webbing rudimentary in the latter); and differs from *L. flaviglandulosa* and *L. pelodytoides* (vs. webbing present in the latter).

By toes with narrow lateral fringes, *Leptobrachella jinshaensis* sp. nov. differs from *L. aerea*, *L. alpina*, *L. firthi*, *L. laui*, *L. liui*, *L. khasiorum*, and *L. yunkaiensis* (vs. wide in the latter); and differs from *L. kalonensis*, *L. macrops*, *L. minima*, *L. marmorata*, *L. namdongensis*, *L. nyx*, *L. oshanensis*, *L. pyrrhops*, *L. rowleyae*, and *L. tuberosa* (vs. lacking in the latter).

By dorsal surface shagreened and granular, lacking enlarge tubercles or warts, *Leptobrachella jinshaensis* sp. nov. differs from *L. applebyi*, *L. bidoupensis*, *L. kalonensis*, *L. melica*, *L. minima*, *L. nahangensis*, *L. shangsiensis*, and *L. tadungensis* (all of which have the dorsum smooth), and *L. bourreti* (dorsum smooth with small warts), *L. fuliginosa* (dorsum smooth with fine tubercles), *L. liui* (dorsum with round tubercles), *L. macrops* (dorsum roughly granular with large tubercles), *L. maershanensis* (dorsum shagreened with tubercles), *L. minima* (dorsum smooth), *L. nyx* (dorsum with round tubercles), *L. nokrekensis* (dorsum tubercles and longitudinal folds), *L. pelodytoides* (dorsum with small, smooth warts), *L. tamdil* (dorsum weakly tuberculate, with low, oval tubercles), *L. tuberosa* (dorsum very tuberculate), *L. yunkaiensis* (dorsum with raised warts), and *L. wuhuangmontis* (dorsum rough with conical tubercles).

By having higher dominant frequency (4.5–4.7 kHz, 20 °C), *Leptobrachella jinshaensis* sp. nov. differs from *L. applebyi* (3.9–4.3 kHz, 21.5 °C), *L. ardens* (3.1–3.4 kHz, 23.6 °C), *L. bidoupensis* (1.9–2.3 kHz, 19.9 °C), *L. botsfordi* (2.6–3.2 kHz, 14 °C), *L. crocea* (2.6–3.0 kHz, 21.6–25.1 °C), *L. fuliginosa* (2.3–2.4 kHz, 19.3–19.6 °C), *L. heteropus* (2.8 kHz, 21 °C), *L. maculosa* (2.7 kHz, 23.3–24.1 °C), *L. melanoleuca* (3.1–3.3 kHz, 23.9 °C), *L. melica* (2.9–3.8 kHz, 26.1 °C), *L. pallida* (2.4–2.7 kHz, 18.9 °C), *L. pyrrhops* (1.9–2.2 kHz, 25 °C), *L. rowleyae* (2.6–3.0 kHz, 21.6–25.1 °C), *L. sola* (3.1–3.2 kHz, 24.2–24.3 °C), *L. tadungensis* (2.6–3.1 kHz, 12.9–22.3 °C) and *L. tuberosa* (2.6–2.8 kHz, 22.5–24.5 °C). The call of the new species appears to have lower frequency compared to the calls attributed to *L. aerea* (6.2–6.4 kHz, 22.4 °C), *L. isos* (7.83–8.55 kHz, 26.4 °C), *L. marmorata* (6.0–6.2 kHz, 22.8 °C), *L. pelodytoides* (6.4–6.6 kHz, 22.7 °C), *L. ventripunctata* (6.1–6.4 kHz, 15 °C) and *L. yingjiangensis* (5.7–5.9 kHz, 19 °C).

By call duration 117–156 ms, *Leptobrachella jinshaensis* sp. nov. differs from *L. aerea* (16–28 ms), *L. bidoupensis* (308–400), *L. botsfordi* (239–303 ms), *L. firthi* (18–24 ms), *L. fuliginosa* (51–80 ms), *L. isos* (31–38 ms), *L. maculosa* (889–907 ms), *L. marmorata* (1900–6700 ms), *L. melanoleuca* (40–63 ms), *L. pallida* (627–729 ms), *L. petrops* (44–57 ms), *L. puhoatensis* 6–14 ms, *L. shangsiensis* (64–69 ms), *L. tadungensis* (248–353 ms) and *L. yingjiangensis* (28–42 ms).

Seven species (*L. liui*, *L. oshanensis*, *L. purpuraventra*, *L. bijie*, *L. suiyangensis*, *L. chishuiensis*, and *L. ventripunctata*) of the genus occur in Guizhou Province, China (Fei et al. 2012; Wang et al. 2019; Luo et al. 2020; Li et al. 2020a). The new species differs from *L. liui* by having narrow lateral fringes on toes (vs. wide in the latter), dorsal surface shagreened with small granules, and lacking enlarge tubercles or warts (vs. dorsum with round tubercles in the latter); differs from *L. oshanensis* by having narrow lateral fringes on toes (vs. lacking in the latter); differs from *L. purpuraventra* and *L. suiyangensis* by heels overlapping when thighs are positioned at right angles to the body (vs. just meeting in the latter); differs from *L. purpuraventra* by tibia-tarsal articulation reaches the middle eye when leg stretched forward (vs. only reaches the level between tympanum to eye in the latter).

In mitochondrial DNA trees, *Leptobrachella jinshaensis* sp. nov. clustered as an independent clade and appears to be sister to a clade in comprising of *L. bijie* and *L. chishuiensis*. The latter two species also occur near the type locality of the new species. The new species differs from *L. bijie* by the following characters: webbing on toes absent (vs. webbing rudimentary in the latter), heels overlapping when thighs are positioned at right angles to the body (vs. just meeting in the latter), having longer call interval ( $132.7 \pm 8.6$ ,  $N = 109$  in the new species vs.  $101.9 \pm 6.4$ ,  $N = 33$  in the latter),

**Table 4.** Morphometric comparisons between *Leptobrachella jinshaensis* sp. nov. and its relatives. Units given in mm. See abbreviations for morphometric characters in Materials and methods section. *P*-value was resulted from Mann-Whitney *U* test. Significant level at 0.05. Abbreviations for species name: *LJ*, *Leptobrachella jinshaensis* sp. nov.; *LC*, *L. chishuiensis*; *LB*, *L. bijie*.

| Character | <i>Leptobrachella jinshaensis</i> sp. nov. |                | <i>L. chishuiensis</i> |                | <i>L. bijie</i> |                | <i>P</i> -value         |                         |
|-----------|--|----------------|------------------------|----------------|-----------------|----------------|-------------------------|-------------------------|
|           | Male (n = 5)                               |                | Male (n = 7)           |                | Male (n = 8)    |                | <i>LJ</i> vs. <i>LC</i> | <i>LJ</i> vs. <i>LB</i> |
|           | Ranging                                    | Mean $\pm$ SD  | Ranging                | Mean $\pm$ SD  | Ranging         | Mean $\pm$ SD  |                         |                         |
| SVL       | 29.7–31.2                                  | 30.8 $\pm$ 0.6 | 30.8–33.4              | 32.1 $\pm$ 1.0 | 29.0–30.4       | 29.7 $\pm$ 0.6 | 0.088                   | 0.019                   |
| HDL       | 10.0–11.4                                  | 10.7 $\pm$ 0.6 | 11.1–12.3              | 11.8 $\pm$ 0.4 | 10.0–10.6       | 10.2 $\pm$ 0.2 | 0.123                   | 0.661                   |
| HDW       | 10.0–10.4                                  | 10.2 $\pm$ 0.2 | 10.6–11.9              | 11.4 $\pm$ 0.5 | 9.5–10.2        | 9.8 $\pm$ 0.3  | 0.012                   | 0.463                   |
| SL        | 4.5–4.9                                    | 4.6 $\pm$ 0.1  | 4.8–5.8                | 5.2 $\pm$ 0.3  | 4.0–4.7         | 4.2 $\pm$ 0.2  | 0.019                   | 0.057                   |
| IND       | 2.8–3.5                                    | 3.2 $\pm$ 0.3  | 3.5–3.8                | 3.7 $\pm$ 0.1  | 2.8–3.4         | 3.1 $\pm$ 0.2  | 0.062                   | 0.464                   |
| IOD       | 3.1–4.0                                    | 3.5 $\pm$ 0.4  | 2.7–3.1                | 3.0 $\pm$ 0.2  | 2.8–3.4         | 3.1 $\pm$ 0.2  | 0.004                   | 0.242                   |
| UEW       | 2.7–3.2                                    | 2.9 $\pm$ 0.2  | 3.0–3.3                | 3.2 $\pm$ 0.1  | /               | /              | 0.223                   | /                       |
| ED        | 3.7–4.3                                    | 4.0 $\pm$ 0.2  | 4.0–5.0                | 4.4 $\pm$ 0.4  | 3.6–4.1         | 3.8 $\pm$ 0.2  | 0.064                   | 0.558                   |
| TYD       | 2.5–3.2                                    | 2.7 $\pm$ 0.3  | 2.0–2.6                | 2.3 $\pm$ 0.2  | 1.9–2.2         | 2.0 $\pm$ 0.1  | 0.019                   | 0.003                   |
| TEY       | 0.9–1.4                                    | 1.0 $\pm$ 0.2  | 1.2–1.6                | 1.4 $\pm$ 0.2  | 0.9–1.1         | 1.0 $\pm$ 0.1  | 0.042                   | 0.464                   |
| LAL       | 13.7–15.4                                  | 14.6 $\pm$ 0.7 | 14.7–17.0              | 15.6 $\pm$ 0.8 | 14.0–14.8       | 14.3 $\pm$ 0.3 | 0.570                   | 0.661                   |
| LW        | 2.1–2.6                                    | 2.3 $\pm$ 0.2  | 2.6–3.2                | 3.0 $\pm$ 0.2  | /               | /              | 0.004                   | /                       |
| ML        | 7.2–8.4                                    | 7.9 $\pm$ 0.5  | 7.9–8.8                | 8.2 $\pm$ 0.39 | 7.4–8.3         | 7.8 $\pm$ 0.3  | 0.935                   | 0.770                   |
| HLL       | 41.3–46.4                                  | 44.4 $\pm$ 2.0 | 43.3–49.7              | 49.7 $\pm$ 2.7 | 43.0–45.5       | 43.7 $\pm$ 0.8 | 0.291                   | 0.464                   |
| THL       | 14.0–15.2                                  | 14.6 $\pm$ 0.5 | 13.7–17.1              | 15.1 $\pm$ 1.2 | /               | /              | 0.465                   | /                       |
| TW        | 3.2–4.9                                    | 3.8 $\pm$ 0.7  | 3.3–4.3                | 3.8 $\pm$ 0.4  | /               | /              | 0.935                   | /                       |
| TL        | 14.5–15.6                                  | 15.1 $\pm$ 0.4 | 14.9–16.8              | 15.6 $\pm$ 0.6 | 13.5–14.4       | 13. $\pm$ 0.3  | 0.685                   | 0.008                   |
| TFL       | 19.3–21.4                                  | 20.6 $\pm$ 1.0 | 20.9–22.3              | 21.7 $\pm$ 0.6 | /               | /              | 0.962                   | /                       |
| FL        | 13.0–14.4                                  | 13.7 $\pm$ 0.7 | 14.4–15.9              | 15.1 $\pm$ 0.5 | 13.0–13.8       | 13.3 $\pm$ 0.2 | 0.019                   | 0.558                   |





**Figure 8.** Habitats of *Leptobrachella jinshaensis* sp. nov. in the type locality Lengshuihe Nature Reserve, Jinsha County, Guizhou Province, China. Forest and a mountain stream in the type locality (insert holotype CIBCS20200516004 in life in the field).

having lower dominant frequency of  $4525 \pm 0.065$  Hz vs.  $4780.4 \pm 76.5$  Hz in the latter, having significantly higher value of SVL in males, and having significantly higher value of TYD and TL to SVL in males. *Leptobrachella jinshaensis* sp. nov. differs from *L. chishuiensis* by webbing on toes absent (vs. webbing rudimentary in the latter), tibiotarsal articulation reaches the middle of eye when leg stretched forward (vs. reaches the tympanum or the level between tympanum to eye in the latter), the lower dominant frequency of calls 4500–4688 Hz (mean  $4525 \pm 0.065$ , 20 °C) vs. 6064–6284 Hz ( $6140.15 \pm 69.35$ , 20 °C) in the latter, each call with two kinds of notes vs. only one kind of note in the latter, and having significantly higher value of HDW, SL, IOD, TYD, TEY and FL to SVL in males (all  $p$ -values < 0.05; Table 4).

**Ecology.** *Leptobrachella jinshaensis* sp. nov. is known from the type locality, Lengshuihe Nature Reserve, Jinsha County, Guizhou Province, China. Specimens of the new species are frequently found from stream covered with reeds, and under the rocks (Fig. 8).

**Etymology.** The specific name *jinshaensis* refers to the distribution of this species, Jinsha County, Guizhou Province, China. We suggest its English common name “Jinsha leaf litter toads” and Chinese name “Jin Sha Zhang Tu Chan (金沙掌突蟾)”.



## Discussion

Molecular phylogenetic analyses, detailed morphological comparisons, and advertisement call data all supported the new species distinctly separated from its congeners especially the superficially-morphological-similar species, *L. bijie* and *L. chishuiensis*. Although the relationships between the new species and other closely related species were not resolved, the new species appears to be phylogenetically closer to *L. bijie* and *L. chishuiensis*, corresponding to their high similarity on morphology. However, the new species appears to have lower dominant frequency on calling than the two closely related species. Moreover, they could be separated by morphometric analyses on contributions of some characters, for example, on PC1 of PCA, several characters of head, SVL and FL, which might be associated the calling behaviours, breeding behaviours, and jumping behaviours. We need future work to detect the function of the characters of these species to explore the ecological differences between them.

The large-scale molecular phylogenetic analyses in Chen et al. (2018) revealed many cryptic species in the genus *Leptobrachella* but did not included samples of *Leptobrachella jinshaensis* sp. nov. Similarly, this large phylogenetic framework likely included a few population samples in Guizhou Province, China. However, the phylogenetic framework indicated that Guizhou Province might be the biogeographical zone of transition for western-to-eastern or southwestern-to-northeastern clades (Chen et al. 2018). The findings of series of new species (*Leptobrachella jinshaensis* sp. nov., *L. chishuiensis*, *L. suiyangensis*, *L. bijie*, and *L. purpuraventra*) obviously supply important supplemental materials for detecting detailed evolutionary and biogeographical models of the genus. Moreover, the findings of the new species also indicated a high degree of localised diversification and micro-endemism for the species in the genus *Leptobrachella* because in Guizhou Province, China, the five recent-described *Leptobrachella* species are just known only from their type localities or nearby areas. In addition, in recent years, large number of discoveries have been made from Guizhou, dramatically raising the number of frog species known from the region (Zhang et al. 2017; Li et al. 2018a, b, 2019a, b, 2020a, b, c; Lyu et al. 2019; Wang et al. 2019; Luo et al. 2020; Su et al. 2020; Xu et al. 2020; Wei et al. 2020). This further indicated that more investigations should be conducted in Guizhou Province to define more precisely distribution area of the new species and detect more cryptic species especially in the poorly-investigated areas.

## Acknowledgements

This work was supported by Project supported by the National Natural Science Foundation of China (Nos. 32070426 and 31960099), Biodiversity Conservation Key Laboratory of Guizhou Province Education Department, Guiyang College, Basic research project of science and technology department of Guizhou Province (Nos. [2020]1Y083), Guizhou Provincial Science and Technology Project (No. [2020]4Y029), Guizhou

Provincial Department of Education Youth Science and Technology Talents Growth Project (Nos. KY[2018]455 and KY[2018]468), and China Biodiversity Observation Networks (Sino BON–Amphibian & Reptile).

## References

- Anderson J (1871) A list of the reptilian accession to the Indian Museum, Calcutta from 1865 to 1870, with a description of some new species. *Journal of the Asiatic Society of Bengal* 40: 12–39.
- Boulenger GA (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* 13: 304–347.
- Boulenger GA (1900) Descriptions of new batrachians and reptiles from the Larut Hills, Perak. *Annals and Magazine of Natural History* 6: 186–194. <https://doi.org/10.1080/00222930008678356>
- Chen JM, Poyarkov NJ, Suwannapoom C, Lathrop A, Wu YH, Zhou WW, Yuan ZY, Jin JQ, Chen HM, Liu HQ, Nguyen TQ, Nguyen SN, Duong TV, Eto K, Nishikawa K, Matsui M, Orlov NL, Stuart BL, Brown RM, Rowley J, Murphy RW, Wang YY, Che J (2018) Large-scale phylogenetic analyses provide insights into unrecognized diversity and historical biogeography of Asian leaf-litter frogs, genus *Leptolalax* (Anura: Megophryidae). *Molecular Phylogenetics and Evolution* 124: 162–171. <https://doi.org/10.1016/j.ympev.2018.02.020>
- Chen WC, Liao X, Zhou SC, Mo YM (2019) A new species of *Leptobranchella* (Anura: Megophryidae) from southern Guangxi, China. *Zootaxa* 4563: 67–82. <https://doi.org/10.11646/zootaxa.4563.1.3>
- Chen JM, Xu K, Poyarkov NA, Wang K, Yuan ZY, Hou M, Suwannapoom C, Wang J, Che J (2020) How little is known about “the little brown frogs”: description of three new species of the genus *Leptobranchella* (Anura: Megophryidae) from Yunnan Zoological Research 41(3): 292–313. <https://doi.org/10.24272/j.issn.2095-8137.2020.036>
- Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9(8): 772–772. <https://doi.org/10.1038/nmeth.2109>
- Das I, Tron RKL, Rangad D, Hooroo RN (2010) A new species of *Leptolalax* (Anura: Megophryidae) from the sacred groves of Mawphlang, Meghalaya, north-eastern India. *Zootaxa* 2339: 44–56. <https://doi.org/10.11646/zootaxa.2339.1.2>
- Dehling JM (2012a) Eine neue Art der Gattung *Leptolalax* (Anura: Megophryidae) vom Gunung Benom, Westmalaysia/A new species of the genus *Leptolalax* (Anura: Megophryidae) from Gunung Benom, Peninsular Malaysia. *Sauria* 34: 9–21.
- Dehling JM (2012b) Redescription of *Leptolalax gracilis* (Günther, 1872) from Borneo and taxonomic status of two populations of *Leptolalax* (Anura: Megophryidae) from Peninsular Malaysia. *Zootaxa* 3328: 20–34. <https://doi.org/10.11646/zootaxa.3328.1.2>
- Dehling JM, Matsui M (2013) A new species of *Leptolalax* (Anura: Megophryidae) from Gunung Mulu National Park, Sarawak, East Malaysia (Borneo). *Zootaxa* 3670: 33–44.

- Dring J (1983) Frogs of the genus *Leptobrachella* (Pelobatidae). *Amphibia-Reptilia* 4: 89–102. <https://doi.org/10.1163/156853883X00012>
- Dubois A (1983) Note preliminaire sur le genre *Leptolalax* Dubois, 1980 (Amphibiens, Anoures), avec diagnose d'une espece nouvelle du Vietnam. *Alytes* 2: 147–153.
- Duong TV, Do DT, Ngo CD, Nguyen TQ, Poyarkov Jr NA (2018) A new species of the genus *Leptolalax* (Anura: Megophryidae) from southern Vietnam. *Zoological Research* 39: 181–196. <https://doi.org/10.24272/j.issn.2095-8137.2018.009>
- Eto K, Matsui M, Nishikawa K (2015) Description of a new species of the genus *Leptobrachella* (Amphibia, Anura, Megophryidae) from Borneo. *Current Herpetology* 34(2): 128–139. <https://doi.org/10.5358/hsj.34.128>
- Eto K, Matsui M, Nishikawa K (2016) A new highland species of dwarf litter frog genus *Leptobrachella* (Amphibia, Anura, Megophryidae) from Sarawak. *Raffles Bulletin of Zoology*. Singapore 64: 194–203.
- Eto K, Matsui M, Hamidy A, Munir M, Iskandar DT (2018) Two new species of the genus *Leptobrachella* (Amphibia: Anura: Megophryidae) from Kalimantan, Indonesia. *Current Herpetology* 37(2): 95–105. <https://doi.org/10.5358/hsj.37.95>
- Fei L, Ye CY, Huang YZ (1990) Key to Chinese Amphibians. Publishing House for Scientific and Technological Literature, Chongqing, 364 pp.
- Fei L, Ye CY (2005) The key and illustration of Chinese. Sichuan Publishing House of Science and Technology, Chongqing, 253–255.
- Fei L, Hu SQ, Ye CY, Huang YZ (2009) Fauna Sinica. Amphibia Vol. 2 Anura. Science Press, Beijing, 957 pp.
- Fei L, Ye CY, Jiang JP (2012) Colored atlas of Chinese amphibians and their distributions. Sichuan Publishing House of Science and Technology, Chengdu, 619 pp.
- Fouquet A, Gilles A, Vences M, Marty C, Blanc M, Gemmel NJ (2007) Underestimation of species richness in Neotropical frogs revealed by mtDNA analyses. *PLoS ONE* 2(10): e1109. <https://doi.org/10.1371/journal.pone.0001109>
- Frost DR (2020) Amphibian species of the world. Version 6.0. New York: American Museum of Natural History. <http://research.amnh.org/vz/herpetology/amphibia/index.html> [accessed 22 Sep 2020]
- Gosner KL (1960) A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16(3): 183–190.
- Grismer LL, Grismer JL, Youmans TM (2004) A new species of *Leptolalax* (Anura: Megophryidae) from Pulau Tioman, West Malaysia. *Asiatic Herpetological Research* 10: 8–11.
- Guindon S, Dufayard JF, 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. *Systematic Biology* 59(3): 307–321. <https://doi.org/10.1093/sysbio/syq010>
- Günther A (1872) On the reptiles and amphibians of Borneo. *Proceedings of the Scientific Meetings of the Zoological Society of London* 1872: 586–600.
- Günther A (1895) The reptiles and batrachians of the Natuna Islands. *Novitates Zoologicae* 2: 499–502.
- Hall TA (1999) BIOEDIT: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41(41): 95–98. <https://doi.org/10.1021/bk-1999-0734.ch008>

- Hoang CV, Nguyen TT, Luu VQ, Nguyen TQ, Jiang JP (2019) A new species of *Leptobranchella* Smith 1925 (Anura: Megophryidae) from Thanh Hoa Province, Vietnam. Raffles Bulletin of Zoology. Singapore 67: 536–556. <https://doi.org/10.26107/RBZ-2019-0042>
- Hou YM, Zhang MF, Hu F, Li SY, Shi SC, Chen J, Mo XY, Wang B (2018) A new species of the genus *Leptolalax* (Anura, Megophryidae) from Hunan, China. Zootaxa 4444(3): 247–266. <https://doi.org/10.11646/zootaxa.4444.3.2>
- Humtsoe LN, Bordoloi S, Ohler A, Dubois A (2008) Rediscovery of a long known species, *Ixalus lateralis* Anderson, 1871. Zootaxa 1921: 24–34. <https://doi.org/10.11646/zootaxa.1921.1.2>
- Inger RE, Stuebing RB (1992 [“1991”]) A new species of frog of the genus *Leptobranchella* Smith (Anura: Pelobatidae), with a key to the species from Borneo. Raffles Bulletin of Zoology. Singapore 39: 99–103.
- Inger RE, Stuebing RB, Tan F (1995) New species and new records of anurans from Borneo. Raffles Bulletin of Zoology. Singapore 43: 115–132.
- Inger RE, Lakim M, Biun A, Yambun P (1997) A new species of *Leptolalax* (Anura: Megophryidae) from Borneo. Asiatic Herpetological Research 7: 48–50. <https://doi.org/10.5962/bhl.part.18855>
- Inger RE, Orlov N, Darevsky I (1999) Frogs of Vietnam: a report on new collections. Fieldiana Zoology 92: 1–46.
- Jiang K, Yan F, Suwannapoom C, Chomdej S, Che J (2013) A new species of the genus *Leptolalax* (Anura: Megophryidae) from northern Thailand. Asian Herpetological Research 4: 100–108. <https://doi.org/10.3724/SPJ.1245.2013.00100>
- Lathrop A, Murphy RW, Orlov N, Ho CT (1998) Two new species of *Leptolalax* (Anura: Megophryidae) from northern Vietnam. Amphibia-Reptilia 19: 253–267. <https://doi.org/10.1163/156853898X00160>
- Li SZ, Xu N, Lv JC, Jiang JP, Wei G, Wang B (2018a) A new species of the odorous frog genus *Odorrana* (Amphibia, Anura, Ranidae) from southwestern China. PeerJ 6(e5695): 1–28. <https://doi.org/10.7717/peerj.5695>
- Li SZ, Xu N, Liu J, Jiang JP, Wei G, Wang B (2018b) A new species of the Asian Toad genus *Megophrys sensu lato* (Amphibia: Anura: Megophryidae) from Guizhou Province, China. Asian Herpetological Research 9: 224–239. <https://doi.org/10.16373/j.cnki.ahr.180072>
- Li SZ, Wei G, Xu N, Cui JG, Fei L, Jiang JP, Liu J, Wang B (2019a) A new species of the Asian music frog genus *Nidirana* (Amphibia, Anura, Ranidae) from Southwestern China. PeerJ 7: e7157. <https://doi.org/10.7717/peerj.7157>
- Li SZ, Zhang MH, Xu N, Lv JC, Jiang JP, Liu J, Wei G, Wang B (2019b) A new species of the genus *Microhyla* (Amphibia: Anura: Microhylidae) from Guizhou Province, China. Zootaxa 4624: 551–575. <https://doi.org/10.11646/zootaxa.4624.4.7>
- Li SZ, Liu J, Wei G, Wang B (2020a) A new species of the Asian leaf litter toad genus *Leptobranchella* (Amphibia, Anura, Megophryidae) from southwest China. ZooKeys 943: 91–118. <https://doi.org/10.3897/zookeys.943.51572>
- Li SZ, Lu NN, Liu J, Wang B (2020b) Description of a new *Megophrys* Kuhl & Van Hasselt, 1822 (Anura, Megophryidae) from Guizhou Province, China. ZooKeys 986: 101–126. <https://doi.org/10.3897/zookeys.986.57119>

- Li SZ, Wei G, Cheng YL, Zhang BW, Wang B (2020c) Description of a new species of the Asian newt genus *Tylototriton sensu lato* (Amphibia: Urodela: Salamandridae) from South-west China. *Asian Herpetological Research* 11(4): 282–296. <https://doi.org/10.16373/j.cnki.ahr.200026>
- Liu CC (1950) Amphibians of western China. *Fieldiana Zoology Memoires* 2: 1–397. [+ 10 pl.] <https://doi.org/10.5962/bhl.part.4737>
- Luo T, Xiao N, Gao K, Zhou J (2020) A new species of *Leptobrachella* (Anura, Megophryidae) from Guizhou Province, China. *ZooKeys* 923: 115–140. <https://doi.org/10.3897/zookeys.923.47172>
- Lyu ZT, Zeng ZC, Wan H, Yang JH, Li YL, Pang H, Wang YY (2019) A new species of *Amolops* (Anura: Ranidae) from China, with taxonomic comments on *A. liangshanensis* and Chinese populations of *A. marmoratus*. *Zootaxa* 4609: 247–268. <https://doi.org/10.11646/zootaxa.4609.2.3>
- Mahony S, Sengupta S, Kamei RG, Biju SD (2011) A new low altitude species of *Megophrys* Kuhl and van Hasselt (Amphibia: Megophryidae), from Assam, Northeast India. *Zootaxa* 3059: 36–46. <https://doi.org/10.11646/zootaxa.3059.1.2>
- Malkmus R (1992) *Leptolalax pictus* sp. nov. (Anura: Pelobatidae) vom Mount Kinabalu/Nord Borneo. *Sauria* 14: 3–6.
- Mathew R, Sen N (2010 [“2009”]) Description of a new species of *Leptobrachium* Tschudi, 1838 (Amphibia: Anura: Megophryidae) from Meghalaya, India. *Records of the Zoological Survey of India* 109: 91–108.
- Matsui M (1997) Call characteristics of Malaysian *Leptolalax* with a description of two new species (Anura: Pelobatidae). *Copeia* 1997: 158–165. <https://doi.org/10.2307/1447851>
- Matsui M (2006) Three new species of *Leptolalax* from Thailand (Amphibia, Anura, Megophryidae). *Zoological Science* 23 (9): 821–830. <https://doi.org/10.2108/zsj.23.821>
- Matsui M, Belabut DM, Ahmad N, Yong HS (2009) A new species of *Leptolalax* (Amphibia, Anura, Megophryidae) from Peninsular Malaysia. *Zoological Science* 26(3): 243–247. <https://doi.org/10.2108/zsj.26.243>
- Matsui M, Dehling JM (2012) Notes on an enigmatic Bornean megophryid, *Leptolalax dringi* Dubois, 1987 (Amphibia: Anura). *Zootaxa* 3317(1): 49–58. <https://doi.org/10.11646/zootaxa.3317.1.4>
- Matsui M, Zainudin R, Nishikawa K (2014a) A new species of *Leptolalax* from Sarawak, Western Borneo (Anura: Megophryidae). *Zoological Science* 31(11): 773–779. <https://doi.org/10.2108/zs140137>
- Matsui M, Nishikawa K, Yambun P (2014b) A new *Leptolalax* from the mountains of Sabah, Borneo (Amphibia, Anura, Megophryidae). *Zootaxa* 3753(3): 440–452. <https://doi.org/10.11646/zootaxa.3753.5.3>
- Nguyen LT, Poyarkov Jr NA, Le DT, Vo BD, Ninh HT, Duong TV, Murphy RW, Sang NV (2018) A new species of *Leptolalax* (Anura: Megophryidae) from Son Tra Peninsula, central Vietnam. *Zootaxa* 4388: 1–21. <https://doi.org/10.11646/zootaxa.4388.1.1>
- Ohler A, Marquis O, Swan S, Grosjean S (2000) Amphibian biodiversity of Hoang Lien Nature Reserve (Lao Cai Province, northern Vietnam) with description of two new species. *Herpetozoa* 13(1/2): 71–87.



- Ohler A, Wollenberg KC, Grosjean S, Hendrix R, Vences M, Ziegler T, Dubois A (2011) Sorting out *Lalos*: description of new species and additional taxonomic data on megophryid frogs from northern Indochina (genus *Leptolalax*, Megophryidae, Anura). *Zootaxa* 3147: 1–83. <https://doi.org/10.11646/zootaxa.3147.1.1>
- Poyarkov NJ, Rowley JJ, Gogoleva SI, Vassilieva AB, Galoyan EA, Orlov NL (2015) A new species of *Leptolalax* (Anura: Megophryidae) from the western Langbian Plateau, southern Vietnam. *Zootaxa* 3931(2): 221–252. <https://doi.org/10.11646/zootaxa.3931.2.3>
- Qian TY, Xiao X, Cao Y, Xiao NW, Yang DD (2020) A new species of *Leptobranchella* (Anura: Megophryidae) Smith, 1925 from Wuling Mountains in Hunan Province, China. *Zootaxa* 4816: 491–526. <https://doi.org/10.11646/zootaxa.4816.4.4>
- Ronquist FR, Huelsenbeck JP (2003) MrBayes3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19(12): 1572–1574. <https://doi.org/10.1093/bioinformatics/btg180>
- Rowley JJ, Cao TT (2009) A new species of *Leptolalax* (Anura: Megophryidae) from central Vietnam. *Zootaxa* 2198: 51–60. <https://doi.org/10.11646/zootaxa.2198.1.5>
- Rowley JJ, Hoang DH, Le TTD, Dau QV, Cao TT (2010a) A new species of *Leptolalax* (Anura: Megophryidae) from Vietnam and further information on *Leptolalax tuberosus*. *Zootaxa* 2660: 33–45.
- Rowley JJ, Stuart BL, Neang T, Emmett DA (2010b) A new species of *Leptolalax* (Anura: Megophryidae) from northeastern Cambodia. *Zootaxa* 2567: 57–68. <https://doi.org/10.11646/zootaxa.2567.1.3>
- Rowley JJ, Stuart BL, Richards SJ, Phimmachak S, Sivongxay N (2010c) A new species of *Leptolalax* (Anura: Megophryidae) from Laos. *Zootaxa* 2681: 35–46. <https://doi.org/10.11646/zootaxa.2681.1.3>
- Rowley JJ, Le DTT, Tran DTA, Hoang DH (2011) A new species of *Leptobranchella* (Anura: Megophryidae) from southern Vietnam. *Zootaxa* 2796: 15–28. <https://doi.org/10.11646/zootaxa.4563.1.3>
- Rowley JJ, Hoang HD, Dau VQ, Le TTD, Cao TT (2012) A new species of *Leptolalax* (Anura: Megophryidae) from central Vietnam. *Zootaxa* 3321: 56–68. <https://doi.org/10.11646/zootaxa.3321.1.4>
- Rowley JJ, Dau VQ, Nguyen TT (2013) A new species of *Leptolalax* (Anura: Megophryidae) from the highest mountain in Indochina. *Zootaxa* 3737(4): 415–428. <https://doi.org/10.11646/zootaxa.3737.4.5>
- Rowley JJ, Stuart BL, Neang T, Hoang HD, Dau VQ, Nguyen TT, Emmett DA (2015) A new species of *Leptolalax* (Anura: Megophryidae) from Vietnam and Cambodia. *Zootaxa* 4039: 401–417. <https://doi.org/10.11646/zootaxa.4039.3.1>
- Rowley JJ, Tran DTA, Le DTT, Dau VQ, Peloso PLV, Nguyen TQ, Hoang HD, Nguyen TT, Ziegler T (2016) Five new, microendemic Asian Leaf-litter Frogs (*Leptolalax*) from the southern Annamite mountains, Vietnam. *Zootaxa* 4085: 63–102. <https://doi.org/10.11646/zootaxa.4085.1.3>
- Rowley JJ, Dau VQ, Hoang HD, Le DTT, Cutajar TP, Nguyen TT (2017a) A new species of *Leptolalax* (Anura: Megophryidae) from northern Vietnam. *Zootaxa* 4243: 544–564. <https://doi.org/10.11646/zootaxa.4243.3.7>

- Rowley JJ, Dau VQ, Cao TT (2017b) A new species of *Leptolalax* (Anura: Megophryidae) from Vietnam. *Zootaxa* 4273(1): 61–79. <https://doi.org/10.11646/zootaxa.4273.1.5>
- Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, New York.
- Sengupta S, Sailo S, Lalremsanga HT, Das A, Das I (2010) A new species of *Leptolalax* (Anura: Megophryidae) from Mizoram, north-eastern India. *Zootaxa* 2406: 56–68. <https://doi.org/10.11646/zootaxa.2406.1.3>
- Stuart BL, Rowley JJJ (2020) A new *Leptobranchella* (Anura: Megophryidae) from the Cardamom Mountains of Cambodia. *Zootaxa* 4834: 556–572. <https://doi.org/10.11646/zootaxa.4834.4.4>
- 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. *Annals of the Entomological Society of America* 87(6): 651–701. <https://doi.org/10.1093/aesa/87.6.651>
- 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>
- Sung YH, Yang JH, Wang YY (2014) A new species of *Leptolalax* (Anura: Megophryidae) from southern China. *Asian Herpetological Research* 5(2): 80–90. <https://doi.org/10.3724/SPJ.1245.2014.00080>
- Tamura K, Stecher G, Peterson D, Fiipiski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Phylogenetics and Evolution* 28: 2725–2729. <https://doi.org/10.1093/molbev/mst197>
- Taylor EH (1962) The amphibian fauna of Thailand. *University of Kansas Science Bulletin* 43: 265–599. <https://doi.org/10.5962/bhl.part.13347>
- Wang J, Yang JH, Li Y, Lyu ZT, Zeng ZC, Liu ZY, Ye YH, Wang YY (2018) Morphology and molecular genetics reveal two new *Leptobranchella* species in southern China (Anura, Megophryidae). *ZooKeys* 776: 105–137. <https://doi.org/10.3897/zookeys.776.22925>
- Wang J, Li YL, Li Y, Chen HH, Zeng YJ, Shen JM, Wang YY (2019) Morphology, molecular genetics, and acoustics reveal two new species of the genus *Leptobranchella* from northwestern Guizhou Province, China (Anura, Megophryidae). *ZooKeys* 848: 119–154. <https://doi.org/10.3897/zookeys.848.29181>
- Watters JL, Cummings ST, Flanagan RL, Siler CD (2016) Review of morphometric measurements used in anuran species descriptions and recommendations for a standardized approach. *Zootaxa* 4072(4): 477–495. <https://doi.org/10.11646/zootaxa.4072.4.6>
- Wei G, Li SZ, Liu J, Cheng YL, Xu N, Wang B (2020) A new species of the Music frog *Nidirana* (Anura, Ranidae) from Guizhou Province, China. *ZooKeys* 904: 63–87. <https://doi.org/10.3897/zookeys.904.39161>
- Wijayathilaka N, Meegaskumbura M (2016) An acoustic analysis of the genus *Microhyla* (Anura: Microhylidae) of Sri Lanka. *PLoS ONE* 11: e0159003. <https://doi.org/10.1371/journal.pone.0159003>
- Xu N, Li S-Z, Liu J, Wei G, Wang B (2020) A new species of the horned toad *Megophrys* Kuhl & Van Hasselt, 1822 (Anura, Megophryidae) from southwest China. *ZooKeys* 943: 119–144. <https://doi.org/10.3897/zookeys.943.50343>

- Yang JH, Wang YY, Chen GL, Rao DQ (2016) A new species of the genus *Leptolalax* (Anura: Megophryidae) from Mt. Gaoligongshan of western Yunnan Province, China. *Zootaxa* 4088: 379–394. <https://doi.org/10.11646/zootaxa.4088.3.4>
- Yang JH, Zeng ZC, Wang YY (2018) Description of two new sympatric species of the genus *Leptolalax* (Anura: Megophryidae) from western Yunnan of China. *PeerJ* 6(e4586): 1–32. <https://doi.org/10.7717/peerj.4586>
- Yuan ZY, Sun RD, Chen JM, Rowley JJ, Wu ZJ, Hou SB, Wang SN, Che J (2017) A new species of the genus *Leptolalax* (Anura: Megophryidae) from Guangxi, China. *Zootaxa* 4300: 551–570. <https://doi.org/10.11646/zootaxa.4300.4.5>
- Zhang Y, Li G, Xiao N, Li J, Pan T, Wang H, Zhang B, Zhou J (2017) A new species of the genus *Xenophrys* (Amphibia: Anura: Megophryidae) from Libo County, Guizhou, China. *Asian Herpetological Research* 8: 75–85.

## Supplementary material 1

### Table S1. Uncorrected *p*-distance between *Leptobranchella* species on the 16S rRNA gene

Authors: Bin Wang

Data type: molecular data

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

Link: <https://doi.org/10.3897/zookeys.1021.60729.suppl1>

## Supplementary material 2

### Table S2. Variable loadings for principal components with Eigenvalue greater than 1, from morphometric characters corrected by SVL

Authors: Yan-Lin Cheng, Sheng-Chao Shi, Jiaqi Li, Jing Liu, Shi-Ze Li, Bin Wang

Data type: species data

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

Link: <https://doi.org/10.3897/zookeys.1021.60729.suppl2>

### Supplementary material 3

**Table S3. Advertisement call comparisons between *Leptobrachella jinshaensis* sp. nov. and its congeners**

Authors: Yan-Lin Cheng, Sheng-Chao Shi, Jiaqi Li, Jing Liu, Shi-Ze Li, Bin Wang

Data type: statistical data

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

Link: <https://doi.org/10.3897/zookeys.1021.60729.suppl3>