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

Yan-Lin Cheng¹, Sheng-Chao Shi², Jiaqi Li³, Jing Liu¹, Shi-Ze Li¹,², Bin Wang¹,²

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)

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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 jinhaensis* sp. nov., molecular phylogenetic analyses, morphology, Taxonomy

* These authors have contributed equally to this work.

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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. maershanensis* (Yuan, Sun, Chen, Rowley & Che, 2017), *L. shangsiensis* (Chen, Liao, Zhou & Mo, 2019), and *L. wuhuangmontis* (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
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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’-CCGGTCTCTGAACTCAGATCAGGT-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

*Figure 1.* Location of the type locality of *Leptobrachella jinshaensis* sp. nov., Lengshuihe Nature Reserve, Jinsha County, Guizhou Province, China.
Table 1. Information for samples used in molecular phylogenetic analyses in this study.

| ID   | Species                          | Voucher         | Locality                                                                 | GenBank accession number |
|------|----------------------------------|-----------------|---------------------------------------------------------------------------|--------------------------|
| 1    | Leptobrachella jinshaensis sp. nov. | CIBJS20200516001 | Lengshuihe Nature Reserve, Jinsha County, Guizhou Province, China         | MTB14014                 |
| 2    | Leptobrachella jinshaensis sp. nov. | CIBJS20200516002 | Lengshuihe Nature Reserve, Jinsha County, Guizhou Province, China         | MTB14015                 |
| 3    | Leptobrachella jinshaensis sp. nov. | CIBJS20200516003 | Lengshuihe Nature Reserve, Jinsha County, Guizhou Province, China         | MTB14016                 |
| 4    | Leptobrachella jinshaensis sp. nov. | CIBJS20200516004 | Lengshuihe Nature Reserve, Jinsha County, Guizhou Province, China         | MTB14017                 |
| 5    | Leptobrachella jinshaensis sp. nov. | CIBJS20200516005 | Lengshuihe Nature Reserve, Jinsha County, Guizhou Province, China         | MTB14018                 |
| 6    | Leptobrachella chisbuiensis       | CIBC20190518047  | Alsophila National Nature Reserve, Chishui City, Guizhou Province, China | MT17053                  |
| 7    | Leptobrachella chisbuiensis       | CIBC20190518042  | Alsophila National Nature Reserve, Chishui City, Guizhou Province, China | MT17054                  |
| 8    | Leptobrachella chisbuiensis       | CIBC20190518043  | Alsophila National Nature Reserve, Chishui City, Guizhou Province, China | MT17055                  |
| 9    | Leptobrachella bijie              | SYS a007313/     | Mt. Zhaozi Nature Reserve, Bijie City, Guizhou Province, China           | MK14532                  |
| 10   | Leptobrachella bijie              | SYS a007314      | Mt. Zhaozi Nature Reserve, Bijie City, Guizhou Province, China           | MK14533                  |
| 11   | Leptobrachella bijie              | SYS a007315      | Mt. Zhaozi Nature Reserve, Bijie City, Guizhou Province, China           | MK14534                  |
| 12   | Leptobrachella suiyangensis       | SYS a006530      | Yingjiang County, Yunnan Province, China                                 | KG203554                 |
| 13   | Leptobrachella suiyangensis       | SYS a006530      | Yingjiang County, Yunnan Province, China                                 | KG203554                 |
| 14   | Leptobrachella suiyangensis       | SYS a006530      | Yingjiang County, Yunnan Province, China                                 | KG203554                 |
| 15   | Leptobrachella suiyangensis       | SYS a007081      | Wujing Nature Reserve, Bijie City, Guizhou Province, China               | MK14541                  |
| 16   | Leptobrachella purpuraventra      | SYS a007081      | Wujing Nature Reserve, Bijie City, Guizhou Province, China               | MK14541                  |
| 17   | Leptobrachella purpuraventra      | SYS a007277/     | Mt. Zhaozi Nature Reserve, Bijie City, Guizhou Province, China           | MK14542                  |
| 18   | Leptobrachella purpuraventra      | SYS a007278      | Wujing Nature Reserve, Bijie City, Guizhou Province, China               | MK14542                  |
| 19   | Leptobrachella bourreti           | AMS R 177673     | Lao Cai Province, Vietnam                                               | KR180124                 |
| 20   | Leptobrachella pararum            | SYS a006530      | Yingjiang County, Yunnan Province, China                                 | KG203554                 |
| 21   | Leptobrachella alpina             | KIZ046816        | Zhuangzi Nature Reserve, Yunnan Province, China                         | MH059866                 |
| 22   | Leptobrachella shanensis          | KIZ025777        | Emei Shan, Sichuan Province, China                                      | MH055899                 |
| 23   | Leptobrachella nos                | MNHN:2004.0278   | Phongsaly Province, Laos                                                | JN484560                 |
| 24   | Leptobrachella tchengegensis      | SYS a004598      | Tengchong County, Yunnan Province, China                                 | KUS89209                 |
| 25   | Leptobrachella pabataensis        | AMSR184852       | Pu Hoat Nature Reserve, Nghe An Province, Vietnam                      | KY489588                 |
| 26   | Leptobrachella nambengensis       | VNUF A.2017.37   | Thanh Hoa Province, Vietnam                                             | MK655389                 |
| 27   | Leptobrachella petropi            | AMSR184826       | Vietnam                                                                  | KY549997                 |
| 28   | Leptobrachella khaicisum          | SDRDU 2009.329   | East Khasi Hills, Meghalaya, India                                       | KY223303                 |
| 29   | Leptobrachella yingxiangensis     | SYS a006532      | Yuanjiang County, Yunnan Province, China                                 | KG203554                 |
| 30   | Leptobrachella mangshanensis      | MSZT201701       | Mt. Mang, Yingxiang County, Hunan Province, China                       | MG132196                 |
| 31   | Leptobrachella kui                | SYS a001597      | Mt. Wuji, Yiwu Xian City, Fujian Province, China                       | KM014547                 |
| 32   | Leptobrachella kui                | SYS a001507      | Mt. Wuji, Shenzhen City, Guangdong Province, China                     | KM014544                 |
| 33   | Leptobrachella yunkaiensis        | SYS a004664/     | Dawuling Forest Station, Maoming City, Guangdong Province, China        | MH055858                 |
| 34   | Leptobrachella mauershanensis     | KIZ019385        | Mt. Miaoer Nature Reserve, Zuyuan County, Guangxi Province, China       | KY980930                 |
| 35   | Leptobrachella flaviglandulina    | KIZ016072        | Xiaojiaogou Nature Reserve, Yunnan Province, China                      | MH055934                 |
| 36   | Leptobrachella zhanggapingi       | KIZ07258         | Pang Num Poo, Chiang Mai Province, Thailand                            | MH055864                 |
| 37   | Leptobrachella suiyi             | ROM 20236        | Tam Dao, Vinh Phuc, Vietnam                                             | MH055858                 |
| 38   | Leptobrachella iusi              | VNMIN A 2015.4/  | Gia Lai Province, Vietnam                                               | KB247690                 |
| 39   | Leptobrachella flabri             | AMS R 176524     | Kon Tum Province, Vietnam                                              | JQ739206                 |
| 40   | Leptobrachella fangminia          | KUHE:19201       | Thanh Hoa Province, Vietnam                                             | LC201981                 |
| 41   | Leptobrachella crenipunctata      | SYS a004536      | Zhusihue, Yunnan Province, China                                        | MH055831                 |
| 42   | Leptobrachella fei                | KIZ048893        | Xiaojiaogou Nature Reserve, Yunnan Province, China (E)                 | MH055841                 |
| 43   | Leptobrachella aerata             | ZFMK 86366       | Quang Binh Province, Vietnam                                            | JN484609                 |
| 44   | Leptobrachella flavidulosa        | MNHN:1999.5675   | Mt. Fan Si Pan, Lao Cai Province, Vietnam                               | JN484931                 |
| 45   | Leptobrachella shuangensis        | NHMG1704003      | Shangui County, Guangxi Zhuang minority, Autonomous Region, China      | MH059463                 |
| 46   | Leptobrachella wuhanwangnanensis  | SYS a003500/     | Mt. Wuhuang, Pubei County, Guangxi Zhuang minority                     | MH059581                 |
|      |                                 | CIB107274        | Autonomous Region, China                                                | **                      **  |
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 | HSI | HDW | SL | IND | IOD | UEW | ED | TYP | LAL | LW | ML | THL | TW | TL | FL |
|----------------|-----|-----|-----|-----|----|-----|-----|-----|----|-----|-----|----|----|-----|----|----|----|
| CIBCS20200516001 | male | 31.1 | 11.4 | 10.1 | 4.9 | 3.4 | 5.1 | 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);
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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 *Leptobrachella 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.

*Leptobrachella jinshaensis* sp. nov. was also compared with all other congeners of *Leptobrachella* 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 *Leptobrachella*.

| No. | *Leptobrachella* species | References |
|-----|--------------------------|------------|
| 1   | *L. aerea* (Rowley, Stuart, Richards, Phimmachak & Sivongxay, 2010) | Rowley et al. 2010a |
| 2   | *L. alpina* (Fei, Ye & Li, 1990) | Fei et al. 1990 |
| 3   | *L. applebyi* (Rowley & Cao, 2009) | Rowley and Cao 2009 |
| 4   | *L. anquai* (Matsui, 1997) | Matsui 1997 |
| 5   | *L. ardru* (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016) | Rowley et al. 2016 |
| 6   | *L. baluensis* (Smith, 1931) | Dring 1983; Eto et al. 2016, 2018 |
| 7   | *L. brefci* (Rowley, Tran & Hoang, 2011) | Rowley et al. 2011 |
| 8   | *L. bijie* (Wang, Li, Li, Chen & Wang, 2019) | Wang et al. 2019 |
| 9   | *L. boudangensis* (Eto, Matsui, Hamidy, Munir & Iskandar, 2018) | Eto et al. 2018 |
| 10  | *L. botafordi* (Dubois, 1983) | Rowley et al. 2013 |
| 11  | *L. bourreti* (Dring, 1983) | Ohler et al. 2011 |
| 12  | *L. brevicrus* (Dring, 1983) | Dring 1983; Eto et al. 2015 |
| 13  | *L. chishuiensis* Li, Liu, Wei & Wang, 2020 | Li et al. 2020a |
| 14  | *L. crocea* (Rowley, Hoang, Le, Dau & Cao, 2010) | Rowley et al. 2010b |
| 15  | *L. dringi* (Dubois, 1987) | Inger et al. 1995; Matsui and Dehling 2013 |
| 16  | *L. eos* (Ohler, Wollenberg, Grosjean, Hendrix, Vences, Ziegler & Dubois, 2011) | Ohler et al. 2011 |
| 17  | *L. euthi* (Rowley, Hoang, Dau, Le & Cao, 2012) | Chen et al. 2020 |
| 18  | *L. flaviglandulosa* (Chen, Yuan & Che, 2020) | Chen et al. 2020 |
| 19  | *L. fritinniens* (Dehling & Matsui, 2013) | Dehling and Matsui 2013 |
| 20  | *L. fuliginosa* (Matsui, 2006) | Matsui 2006 |
| 21  | *L. fusca* (Eto, Matsui, Hamidy, Munir & Iskandar, 2018) | Eto et al. 2018 |
| 22  | *L. gracilis* (Günther, 1872) | Günther 1872; Dehling 2012a |
| 23  | *L. hamidi* (Matsui, 1997) | Matsui 1997 |
| 24  | *L. heteropus* (Boulenger, 1900) | Boulenger 1900 |
| 25  | *L. lateralis* (Anderson, 1871) | Anderson 1871; Hunttoe et al. 2008 |
| 26  | *L. laui* (Sung, Yang & Wang, 2014) | Sung et al. 2014 |
| 27  | *L. liui* (Fei & Ye, 1990) | Fei et al. 2009; Sung et al. 2014 |
| 28  | *L. macrops* (Duong, Do, Ngo, Nguyen & Poyarkov, 2018) | Duong et al. 2018 |
| 29  | *L. maculosa* (Rowley, Stuart, Neang, Hoang, Dau, Nguyen & Emmett, 2015) | Rowley et al. 2015 |
| 30  | *L. marmorata* (Matsui, Zainudin & Nishikawa, 2014) | Matsui et al. 2014a |
| 31  | *L. marina* (Inger, Lakim, Biun & Yambun, 1977) | Inger et al. 1997 |
| 32  | *L. melica* (Rowley, Stuart, Neang & Emmett, 2010) | Rowley et al. 2010c |
| 33  | *L. melina* (Taylor, 1962) | Taylor 1962; Ohler et al. 2011 |
| 34  | *L. melanoleuca* (Matsui, 2006) | Matsui 2006 |
| 35  | *L. minima* (Taylor, 1962) | Taylor 1962; Ohler et al. 2011 |
| 36  | *L. melanoleuca* (Matsui, 2006) | Matsui 2006 |
| 37  | *L. nyx* (Ohler, Wollenberg, Grosjean, Hendrix, Vences, Ziegler & Dubois, 2011) | Ohler et al. 2011 |
| 38  | *L. oshaensis* (Liu, 1950) | Liu 1950, 1961; This paper |
| 39  | *L. paludis* (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016) | Rowley et al. 2016 |
| 40  | *L. para* (Dring, 1983) | Dring 1983 |
| 41  | *L. petrops* (Rowley, Dau, Hoang, Le, Cutajar & Nguyen, 2017) | Rowley et al. 2017a |
| 42  | *L. picta* (Malkmus, 1992) | Malkmus 1992 |
| 43  | *L. platyccephala* (Dehling, 2012) | Dehling 2012b |
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 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).
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 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). *Leptobrachella jinshaensis* sp. nov. has longer call interval (132.7 ± 8.6, *N* = 109) than *L. bijie* (101.9 ± 6.4, *N* = 33), and has lower dominant frequency (4525 ± 0.065 Hz) than *L. bijie* (4780.4 ± 76.5 Hz) and *L. chishuiensis* (6064–6284 Hz). 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 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.
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*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-
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,
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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
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 ± 0.33, n = 109). Call duration was 117–156 ms (mean 132.7 ± 8.6, n = 109). Call interval was 62–106 ms (mean 84.3 ± 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
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The duration of first type of note with 35–71 ms (mean 48.77 ± 7.90, n = 109), the duration of the second type of note with 39–78 ms (mean 52.93 ± 8.85, n = 122), the duration between notes 18–40 ms (mean 23 ± 5.68, n = 122). The dominant frequency of calls is 4500–4688 Hz (mean 4525 ± 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 (22.2–27.76), 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. fritinniens, L. gracilis, L. hamidi, L. heteropus, L. kajangensis, L. kecil, L. marmorata, L. melanoleuca, L. maura,
By tympanum distinctly visible, Leptobrachella jinshaensis sp. nov. differs from Leptobrachella 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. maoershanensis, L. marmorata, L. melica, L. minima, L. namdongensis, L. namdongensis, L. niveimontis, L. nokrekensis, L. nyx, L. pluvialis, L. pluvialis, L. puhaoensis, L. purpuris, L. purpuraventra, L. pyrrhops, L. sabahmontauts, L. shangsiensis, L. suisiangensis, 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. namdongensis, 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. maoershanensis (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 (1.9–22 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. puboatensis* 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 (308–400), having narrow lateral fringes on toes (vs. wide in the latter), toe webbing rudimentary in the latter; differs from *L. suiyangensis* 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).

| Character | *Leptobrachella jinshaensis* sp. nov. | *L. chishuiensis* | *L. bijie* | P-value |
|-----------|--------------------------------------|-------------------|------------|---------|
|           | Male (n = 5)                          | Male (n = 7)      | Male (n = 8)|         |
| Ranging   | Mean ± SD                            | Ranging           | Ranging    | Mean ± SD | Lj vs. LC | Lj vs LB |
| SVL       | 29.7–31.2                            | 30.8 ± 0.6        | 30.8–33.4  | 32.1 ± 1.0 | 29.0–30.4 | 29.7 ± 0.6 | 0.088 | 0.019 |
| HDL       | 10.0–11.4                            | 10.7 ± 0.6        | 11.1–12.3  | 11.8 ± 0.4 | 10.0–10.6 | 10.2 ± 0.2 | 0.123 | 0.661 |
| HDW       | 10.0–10.4                            | 10.2 ± 0.2        | 10.6–11.9  | 11.4 ± 0.5 | 9.5–10.2  | 9.8 ± 0.3  | 0.012 | 0.463 |
| SL        | 4.5–4.9                              | 4.6 ± 0.1         | 4.8–5.8    | 5.2 ± 0.3  | 4.0–4.7   | 4.2 ± 0.2  | 0.019 | 0.057 |
| IND       | 2.8–3.5                              | 3.2 ± 0.3         | 3.5–3.8    | 3.7 ± 0.1  | 2.8–3.4   | 3.1 ± 0.2  | 0.062 | 0.046 |
| IOD       | 3.1–4.0                              | 3.5 ± 0.4         | 2.7–3.1    | 3.0 ± 0.2  | 2.8–3.4   | 3.1 ± 0.2  | 0.004 | 0.242 |
| UEW       | 2.7–3.2                              | 2.9 ± 0.2         | 3.0–3.3    | 3.2 ± 0.1  | /         | /         | 0.223 | /     |
| ED        | 3.7–4.3                              | 4.0 ± 0.2         | 4.0–5.0    | 4.4 ± 0.4  | 3.6–4.1   | 3.8 ± 0.2  | 0.064 | 0.558 |
| TYD       | 2.5–3.2                              | 2.7 ± 0.3         | 2.0–2.6    | 2.3 ± 0.2  | 1.9–2.2   | 2.0 ± 0.1  | 0.019 | 0.003 |
| TET       | 0.9–1.4                              | 1.0 ± 0.2         | 1.2–1.6    | 1.4 ± 0.2  | 0.9–1.1   | 1.0 ± 0.1  | 0.042 | 0.464 |
| LAL       | 13.7–15.4                            | 14.6 ± 0.7        | 14.7–17.0  | 15.6 ± 0.8 | 14.0–14.8 | 14.3 ± 0.3 | 0.570 | 0.661 |
| LW        | 2.1–2.6                              | 2.3 ± 0.2         | 2.6–3.2    | 3.0 ± 0.2  | /         | /         | 0.004 | /     |
| ML        | 7.2–8.4                              | 7.9 ± 0.5         | 7.9–8.8    | 8.2 ± 0.39 | 7.4–8.3   | 7.8 ± 0.3  | 0.935 | 0.770 |
| HLL       | 41.3–46.4                            | 44.4 ± 2.0        | 43.3–49.7  | 49.7 ± 2.7 | 43.0–45.5 | 43.7 ± 0.8 | 0.291 | 0.464 |
| THL       | 14.0–15.2                            | 14.6 ± 0.5        | 13.7–17.1  | 15.1 ± 1.2 | /         | /         | 0.465 | /     |
| TW        | 3.2–4.9                              | 3.8 ± 0.7         | 3.3–4.3    | 3.8 ± 0.4  | /         | /         | 0.935 | /     |
| TL        | 14.5–15.6                            | 15.1 ± 0.4        | 14.9–16.8  | 15.6 ± 0.6 | 13.5–14.4 | 13. ± 0.3  | 0.685 | 0.008 |
| TFL       | 19.3–21.4                            | 20.6 ± 1.0        | 20.9–22.3  | 21.7 ± 0.6 | /         | /         | 0.962 | /     |
| FL        | 13.0–14.4                            | 13.7 ± 0.7        | 14.4–15.9  | 15.1 ± 0.5 | 13.0–13.8 | 13.3 ± 0.2 | 0.019 | 0.558 |
having lower dominant frequency of 4525 ± 0.065 Hz vs. 4780.4 ± 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), tibiotoral 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 ± 0.065, 20 °C) vs. 6064–6284 Hz (6140.15 ± 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)”.

![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).](image-url)
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.

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**Supplementary material 1**

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

Authors: Bin Wang

Data type: molecular data

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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

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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

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