A new species of the Asian leaf litter toad genus *Leptobrachella* (Amphibia, Anura, Megophryidae) from southwest China

Shi-Ze Li1,2*, Jing Liu1*, Gang Wei3, Bin Wang1,2

1 Department of Food Science and Engineering, Moutai Institute, Renhuai 564500, China 2 CAS Key Laboratory of Mountain Ecological Restoration and Bioresource Utilization and Ecological Restoration Biodiversity Conservation Key Laboratory of Sichuan Province, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, China 3 Biodiversity Conservation Key Laboratory, Guiyang College, Guiyang, 550002, China

Corresponding author: Bin Wang (wangbin@cib.ac.cn)

Academic editor: A. Crottini  |  Received 28 February 2020  |  Accepted 6 May 2020  |  Published 22 June 2020

Citation: Li S-Z, Liu J, Wei G, Wang B (2020) A new species of the Asian leaf litter toad genus *Leptobrachella* (Amphibia, Anura, Megophryidae) from southwest China. ZooKeys 943: 91–118. https://doi.org/10.3897/zookeys.943.51572

Abstract

A new species of the Asian leaf litter toad genus *Leptobrachella* from Guizhou Province, China is described based on molecular phylogenetic analyses, morphological comparisons, and bioacoustics data. Phylogenetic analyses based on the mitochondrial 16S rRNA gene sequences supported the new species as an independent clade nested into the *Leptobrachella* clade and sister to *L. bijie*. The new species could be distinguished from its congeners by a combination of the following characters: small body size (SVL 30.8–33.4 mm in seven adult males, and 34.2 mm in one adult female); dorsal skin shagreened, some of the granules forming longitudinal short skin ridges; tympanum distinctly discernible, slightly concave; internasal distance longer than interorbital distance; supra-axillary, femoral, pectoral and ventrolateral glands distinctly visible; absence of webbing and lateral fringes on fingers; toes with rudimentary webbing and shallow lateral fringes, relative finger lengths II < IV < I < III; heels overlapped when thighs are positioned at right angles to the body; and tibia-tarsal articulation reaches the tympanum.

Keywords

Guizhou, molecular phylogenetic analyses, morphology, new species, taxonomy

* Contributed equally as the first authors.
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 had been ever classified into *Leptolalax* Dubois, 1983 (e.g., Fei et al. 2009, 2012), and Chen et al. (2018) placed *Leptolalax* as a junior synonym of *Leptobrachella* based on large-scale molecular analyses. Currently, the genus *Leptobrachella* contains 76 species, of which 44 species have been described in the past ten years (Frost 2020). Currently, 21 species of the genus *Leptobrachella* are known from China: *Leptobrachella alpina* (Fei, Ye & Li, 1990) and *L. bourreti* (Dubois, 1983) from Yunnan and Guangxi; *L. eos* (Ohler, Wollenberg, Grosjean, Hendrix, Vences, Ziegler & Dubois, 2011) and *L. nyx* (Ohler, Wollenberg, Grosjean, Hendrix, Vences, Ziegler & Dubois, 2011) from Yunnan; *L. laui* (Sung, Yang & Wang, 2014) and *L. yunkaiensis* Wang, Li, Lyu & Wang, 2018 from southern Guangdong, including 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. purpureaventra* Wang, Li, Li, Chen & Wang, 2019, *L. bijie* Wang, Li, Li, Chen & Wang, 2019, and *L. suiyangensis* Luo, Xiao, Gao & Zhou, 2020 from Guizhou; *L. purpurus* (Yang, Zeng & Wang, 2018), *L. pelodytoides* (Boulenger, 1893), *L. tengchongensis* (Yang, Wang, Chen & Rao, 2016), and *L. yingjiangensis* (Yang, Zeng & Wang, 2018) from Yunnan; *L. ventripunctata* (Fei, Ye & Li, 1990) from Guizhou and Yunnan; *L. mangshanensis* (Hou, Zhang, Hu, Li, Shi, Chen, Mo & Wang, 2018) from southern Hunan; and *L. sungi* (Lathrop, Murphy, Orlov & Ho, 1998), *L. maerenshanensis* (Yuan, Sun, Chen, Rowley & Che, 2017), *L. shangsiensis* Chen, Liao, Zhou & Mo, 2019, and *L. wuhuangmontis* Wang, Yang & Wang, 2018 from Guangxi (Sung et al. 2014; Li et al. 2016; Yang et al. 2016, 2018; Yuan et al. 2017; Chen et al. 2018, 2019; Hou et al. 2018; Wang et al. 2018, 2019; Wang et al. 2019; Luo et al. 2020). Even more, a series of cryptic species in the genus were still proposed in Chen et al. (2018).

In recent years, we carried out a series of biodiversity surveys in Chishui City, Guizhou Province, China, and collected some specimens of the genus *Leptobrachella*. Molecular phylogenetic analyses, morphological comparisons, and bioacoustics comparisons consistently indicated these specimens as an undescribed species of *Leptobrachella*. Hence, we describe it herein as a new species.

Materials and methods

**Specimens.** Seven adult males and one adult female of the undescribed species were collected from the mountain streams in Chishui National Nature Reserve, Chishui City, Guizhou Province, China (for voucher information see Table 1; Fig. 1). After taking photographs, they were euthanized using isoflurane, and then the specimens were
fixed in 10% buffered formalin. Before fixing, muscle tissue was taken and preserved separately in 95% ethanol. Specimens were deposited in Chengdu Institute of Biology, Chinese Academy of Sciences (CIB, CAS).

**Molecular phylogenetic analyses.** All eight specimens of the new taxon were included in the molecular 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) mainly based on previous studies (Chen et al. 2018; Wang et al. 2019; Luo et al. 2020). Corresponding sequences of *Leptobrachium tengchongensis*, one *Leptobrachium huashen*, and one *Megophrys major* were also downloaded from GenBank, and used as outgroups according to previous phylogenetic works (Chen et al. 2018; Wang et al. 2019; Luo et al. 2020).

Total DNA was extracted using a standard phenol-chloroform extraction protocol (Sambrook et al. 1989). The mitochondrial 16S rRNA gene (16S) sequences were amplified, and the primers P7 (5’-CGCCTGTTTACCAAAAACAT-3’) and P8 (5’-CCGGTCTGAACTCAGATCACGT-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 s, annealing at 51 °C for 30 s and extending at 72 °C for 70 s. Sequencing was conducted using an ABI3730 automated DNA sequencer in Shanghai DNA BioTechnologies Co., Ltd. (Shanghai, China). New sequences were deposited in GenBank (for 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. Phylogenetic analyses were conducted using Maximum Likelihood (ML) and Bayesian Inference (BI) methods, implemented in
| ID | Species                     | Voucher             | Locality                                                                 | GenBank accession number |
|----|-----------------------------|---------------------|--------------------------------------------------------------------------|--------------------------|
| 1  | *Leptobrachella chishuiensis* sp. nov. | CIBCS20190518047 | Chishui National Nature Reserve, Chishui City, Guizhou Province, China | MT117053                 |
| 2  | *Leptobrachella chishuiensis* sp. nov. | CIBCS20190518042 | Chishui National Nature Reserve, Chishui City, Guizhou Province, China | MT117054                 |
| 3  | *Leptobrachella chishuiensis* sp. nov. | CIBCS20190518043 | Chishui National Nature Reserve, Chishui City, Guizhou Province, China | MT117055                 |
| 4  | *Leptobrachella chishuiensis* sp. nov. | CIBCS20190518049 | Chishui National Nature Reserve, Chishui City, Guizhou Province, China | MT117056                 |
| 5  | *Leptobrachella chishuiensis* sp. nov. | CIBCS20190518046 | Chishui National Nature Reserve, Chishui City, Guizhou Province, China | MT117057                 |
| 6  | *Leptobrachella chishuiensis* sp. nov. | CIBCS20190518045 | Chishui National Nature Reserve, Chishui City, Guizhou Province, China | MT117058                 |
| 7  | *Leptobrachella chishuiensis* sp. nov. | CIBCS20190518044 | Chishui National Nature Reserve, Chishui City, Guizhou Province, China | MT330118                 |
| 8  | *Leptobrachella chishuiensis* sp. nov. | CIBCS20190518048 | Chishui National Nature Reserve, Chishui City, Guizhou Province, China | MT330119                 |
| 9  | *Leptobrachella bijie* | SYS a007313/ CIB110002 | Mt. Zhaosi Nature Reserve, Bijie City, Guizhou Province, China | MK414532                 |
| 10 | *Leptobrachella bijie* | SYS a007314 | Mt. Zhaosi Nature Reserve, Bijie City, Guizhou Province, China | MK414533                 |
| 11 | *Leptobrachella bijie* | SYS a007315 | Mt. Zhaosi Nature Reserve, Bijie City, Guizhou Province, China | MK414534                 |
| 12 | *Leptobrachella purpuraventra* | SYS a007081 | Wujing Nature Reserve, Bijie City, Guizhou Province, China | MK414517                 |
| 13 | *Leptobrachella purpuraventra* | SYS a007277/ CIB110003 | Wujing Nature Reserve, Bijie City, Guizhou Province, China | MK414518                 |
| 14 | *Leptobrachella purpuraventra* | SYS a007278 | Wujing Nature Reserve, Bijie City, Guizhou Province, China | MK414519                 |
| 15 | *Leptobrachella suiyangensis* | GZNU20180606002 | Huoqiuba Nature Reserve, Suiyang County, Guizhou, China | MK829648                 |
| 16 | *Leptobrachella suiyangensis* | GZNU20180606006 | Huoqiuba Nature Reserve, Suiyang County, Guizhou, China | MK829649                 |
| 17 | *Leptobrachella suiyangensis* | GZNU20180606005 | Huoqiuba Nature Reserve, Suiyang County, Guizhou, China | MK829650                 |
| 18 | *Leptobrachella purpurus* | SYS a006530 | Yingjiang County, Yunnan Province, China | MG520354                 |
| 19 | *Leptobrachella alpina* | KIZ046816 | Huangcaoling, Yunnan Province, China | MH055866                 |
| 20 | *Leptobrachella bourreti* | AMS R 177673 | Lao Cai Province, Vietnam | KR018124                 |
| 21 | *Leptobrachella oshanensis* | KIZ025776 | Emei Shan, Emei Shan City, Sichuan Province, China | MH055895                 |
| 22 | *Leptobrachella eos* | MNHN:2004.0278 | Phongsaly Province, Laos | JN848450                 |
| 23 | *Leptobrachella tengchongensis* | SYS a004958 | Tengchong County, Yunnan Province, China | KU589209                 |
| 24 | *Leptobrachella mangshanensis* | MSZTC201701 | Mt. Mang, Yizhang County, Hunan Province, China | MG132196                 |
| 25 | *Leptobrachella linii* | SYS a001597 | Mt. Wuji, Wuyishan City, Fujian Province, China | KM014547                 |
| 26 | *Leptobrachella laui* | SYS a001507 | Mt. Wuutong, Shenzhen City, Guangdong Province, China | KM014544                 |
| 27 | *Leptobrachella yuankaiensis* | SYS a004664 / CIB107272 | Dawuling Forest Station, Maoming City, Guangdong Province, China | MH055885                 |
| 28 | *Leptobrachella maoshanensis* | KIZ019385 | Mt. Maor Nature Reserve, Ziyuan County, Guangxi Province, China | KY986930                 |
| 29 | *Leptobrachella khasiorum* | SDBDU 2009.329 | East Khasi Hills, Meghalaya, India | KY022303                 |
| 30 | *Leptobrachella yingjiangensis* | SYS a006532 | Yingjiang County, Yunnan Province, China | MG520351                 |
| 31 | *Leptobrachella petrops* | AMS:R184826 | Vietnam | KJ459997                 |
| 32 | *Leptobrachella pahuensis* | AMS:R184852 | Pu Hoat Nature Reserve, Nghe An Province, Vietnam | KJ849588                 |
| 33 | *Leptobrachella namdangensis* | VNUF A.2017.37 | Thanh Hoa Provincen, Vietnam | MK965389                 |
| ID | Species                        | Voucher          | Locality                                           | GenBank accession number |
|----|--------------------------------|------------------|----------------------------------------------------|--------------------------|
| 34 | Leptobrachella tios           | VNMN A 2015.4/AMS R 176480 | Gia Lai Province, Vietnam                          | KT824769                |
| 35 | Leptobrachella firihi         | AMS R 176524     | Kon Tum Province, Vietnam                          | JQ739206                |
| 36 | Leptobrachella minimus        | KUHE:19201       | Thailand                                           | LC201981                |
| 37 | Leptobrachella vestrapunctata | SY a004536       | Zhushibe, Yunnan Province, China                   | MH055831                |
| 38 | Leptobrachella aerea          | ZFMK 86362       | Quang Binh Province, Vietnam                       | JN848409                |
| 39 | Leptobrachella wuhausagromontis| SY a003500 /CIB107274 | Mt. Wuhuanc, Pubei County, Guangxi Zhuang minority Autonomous Region, China | MH605581                |
| 40 | Leptobrachella plusialis      | MNHN:1999.5675   | Mu. Fan Si Pan, Lao Cai Province, Vietnam          | JN848391                |
| 41 | Leptobrachella shangsiensis   | NHMG1704003      | Shangsi County, Guangxi Zhuang minority Autonomous Region, China | MK095463                |
| 42 | Leptobrachella nahanogensis   | ROM 7035         | Na Hang Nature Reserve, Tuyen Quang, Vietnam       | MH055853                |
| 43 | Leptobrachella nyx            | AMNH A163810     | Ha Giang Province, Vietnam                         | DQ283381                |
| 44 | Leptobrachella zhongyupingi   | KIZ07258         | Pang Num Poo, Chiang Mai Province, Thailand        | MH055864                |
| 45 | Leptobrachella sungi          | ROM 20236        | Tam Dao, Vinh Phuc, Vietnam                        | MH055858                |
| 46 | Leptobrachella tuberosa       | ZMMU-NAP-02275   | Kon Ka Kinh National Park, Gia Lai, Vietnam       | MH055859                |
| 47 | Leptobrachella butserdii      | VNMN 03682       | Fansipan, Lao Cai, Vietnam                        | MH055953                |
| 48 | Leptobrachella pallida        | UNS00510         | Lam Dong Province, Vietnam                         | KR018112                |
| 49 | Leptobrachella kalonensis     | IEBR A.2015.15   | Binh Thuan Province, Vietnam                      | KR018114                |
| 50 | Leptobrachella kiedopensi     | NAP-01453        | Lam Dong Province, Vietnam                         | KP017573                |
| 51 | Leptobrachella tadosensis     | UNS00515         | Dak Nong Province, Vietnam                         | KR018121                |
| 52 | Leptobrachella maculona       | AMS R 177660     | Ninh Thuan Province, Vietnam                       | KR018119                |
| 53 | Leptobrachella pyphiphi       | ZMMU ABV-00148   | Loc Bao, Lam Dong Province, Vietnam                | KP017575                |
| 54 | Leptobrachella macropo        | IEBR A.2017.9    | Hon Den Mt., Phu Yen Province, Vietnam             | MG787990                |
| 55 | Leptobrachella melicina       | MVZ 258197       | Virachey National Park, Katanakiri Province, Cambodia | HM135399                |
| 56 | Leptobrachella applebyi       | AMS R171704      | Song Thanh, Quang Nam, Vietnam                     | HM135398                |
| 57 | Leptobrachella rowleyae       | ITBCZ 2783       | Son Tra, Da Nang City, Vietnam                     | MG682552                |
| 58 | Leptobrachella ardens         | AMS R 176463     | Gia Lai Province, Vietnam                          | KR018110                |
| 59 | Leptobrachella crocea         | AMS R 173740     | Kon Tum, Vietnam                                   | MH055954                |
| 60 | Leptobrachella melanoleuca    | KUHE 23840       | Sarat Thani, Thailand                              | LC201997                |
| 61 | Leptobrachella fuliginosa     | KUHE:20172       | Thailand                                           | LC201985                |
| 62 | Leptobrachella istiokai       | KUHE:55897       | Mulu NP, Sarawak, Borneo, Malaysia                 | LC137805                |
| 63 | Leptobrachella breviceus      | ZMH A09365       | Sarawak: Gunung Mulu National Park; Small stream of the Sungai Tapin, Malaysia | KJ831302                |
| 64 | Leptobrachella parva          | KUHE 55308       | Mulu NP, Sarawak, Borneo, Malaysia                 | LC056791                |
| 65 | Leptobrachella balisensis     | SP 21604         | Tambunan, Sabah, Borneo, Malaysia                  | LC056792                |
| 66 | Leptobrachella mjoberti       | KUHE 17064       | Gading NP, Sarawak, Borneo, Malaysia               | LC056785                |
| 67 | Leptobrachella julandringi    | SRC 00230/KUHE 49815 | Mulu NP, Sarawak, Borneo, Malaysia             | LC056779                |
| 68 | Leptobrachella arayai         | BORNEEISIS 22931 | Liwagu, Kinabalu, Borneo, Malaysia                 | AB847558                |
| 69 | Leptobrachella kemadi         | KUHE 17545       | Borneo, Malaysia                                   | AB969286                |
| 70 | Leptobrachella marmorata      | KUHE 53227       | Annah Rais, Padawan, Kuching Division, Sarawak, Malaysia | AB969289                |
| 71 | Leptobrachella maura          | SP 21450         | Kinabalu, Sabah, Malaysia                          | AB847559                |
| 72 | Leptobrachella gracilis       | KUHE 55624       | Camp 1, Gunung Mulu, Borneo, Malaysia              | AB847560                |
| 73 | Leptobrachella sabalmontana   | BORNEEISIS 12632 | Borneo, Malaysia                                   | AB847551                |
| 74 | Leptobrachella dringi         | KUHE 55610       | Camp 4 of Gunung Mulu, Malaysia                    | AB847553                |
| 75 | Leptobrachella picta          | UNIMAS 8705      | Borneo, Malaysia                                   | KJ831295                |
| 76 | Leptobrachella fritinniens    | KUHE 55371       | Headquarters, Gunung Mulu, Malaysia                | AB847557                |
| 77 | Leptobrachella sola           | KUHE 23261       | Hala Bala, Thailand                               | LC202007                |
| 78 | Leptobrachella heteropus      | KUHE 15487       | Larut, Peninsular, Malaysia                        | AB530453                |
| 79 | Leptobrachella kelil          | KUHE 52440       | Malaysia                                          | LC202004                |
| 80 | Leptobrachella kajangensis    | LSUHC 4439       | Tioman, Malaysia                                   | LC202002                |
| 81 | Leptobrachium tengchongense   | SYSa004604d      | Yunnan Province, China                            | KX066880                |
| 82 | Leptobrachium huahsen         | KIZ049025        | Yunnan Province, China                            | KX811931                |
| 83 | Megophrys major               | AMS R 173870     | Kon Tum, Vietnam                                  | KY476333                |
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 used in ML and BI analyses. For the ML analysis, branch supports were drawn from 10,000 nonparametric bootstrap replicates. In BI analysis, 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 60 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 eight adult specimens (Table 2) of the new taxon were measured. 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. purpuraventra* were retrieved from Wang et al. (2019). Twenty-three morphometric characters of adult specimens were measured:

- **ED** eye diameter (distance from the anterior corner to the posterior corner of the eye);
- **FIL** first finger length (distance from base to tip of finger I);
- **FIIL** second finger length (distance from base to tip of finger II);
- **FIIL** third finger length (distance from base to tip of finger III);
- **FIVL** fourth finger length (distance from base to tip of finger IV);
- **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);
- **TYD** maximal tympanum diameter;
- **TEY** tympanum-eye distance (distance from anterior edge of tympanum to posterior corner of eye);
A new species of *Leptobrachella*

**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;

**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 was log-transformed for the following morphometric analyses. Mann-Whitney *U* tests were conducted to test the significance of differences on morphometric characters between the undescribed species, *L. bijie* and *L. purpuraventra*. 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* and *L. purpuraventra* being available from Wang et al. (2019), the morphometric analyses were conducted only based on these 14 morphometric characters for male group.

The new taxon was also compared with all other congeners of *Leptobrachella* based on morphological characters. Comparative morphological data were obtained from literatures (Table 3).

**Bioacoustics analyses.** The advertisement calls of the new taxon were recorded from the holotype specimen CIBCS20190518047 in the field on 18 May 2019 in Chishui National Nature Reserve, Chishui City, Guizhou Province, China. The advertisement call of the new species was recorded in the stream at ambient air temperature of 20 °C and air humidity of 87%. 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 visualized and edited with SoundRuler v. 0.9.6.0 (Gridi-Papp 2003–2007) and Raven Pro v. 1.5 software (Cornell Laboratory of Ornithology, Ithaca, NY, USA). Ambient temperature of the type locality was taken by a digital hygrothermograph.

**Results**

Aligned sequence matrix of 16S contained 537 bps. ML and BI analyses based on the 16S matrix resulted in essentially identical topologies (Fig. 2). All six samples of the new taxon were clustered into one monophyletic group (node supports in ML and BI: 94 and 0.95) nested into *Leptobrachella*, and was a sister taxon to *L. bijie* (node supports in ML and BI: 92 and 1.00). The genetic distance between the new taxon and its closest relatives *L. bijie* was 2.1%, at the same level with that between *L. alpina* and *L. purpurus* (2.1%; Suppl. material 1: Table S1).
Table 2. Measurements of *Leptobrachella chishuiensis* sp. nov. Units in mm. See abbreviations for characters in the Materials and methods section.

| Species                  | Voucher number | Sex | SVL | HDL | HDW | SL  | IND | IOD | UEW | ED  | TYD | LAI | LW  | THL | TW  | TL  | TFL | FL  | FIL | FIIL | FIIIL | FIVL | FIVL |
|--------------------------|----------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| *Leptobrachella chishuiensis* sp. nov. | CIBCS20190518047 | male | 32.4 | 12.3 | 11.8 | 5.1 | 3.8 | 3.1 | 3.3 | 4.6 | 2.6 | 17.0 | 3.2 | 16.0 | 4.3 | 16.2 | 22.3 | 15.6 | 3.4 | 3.1 | 5.0 | 3.2 |
| *Leptobrachella chishuiensis* sp. nov. | CIBCS20190518042 | male | 32.7 | 12.2 | 11.9 | 5.8 | 3.5 | 3.1 | 3.1 | 5.0 | 2.2 | 15.4 | 3.1 | 15.3 | 3.6 | 15.5 | 22.3 | 14.7 | 3.6 | 3.4 | 5.5 | 3.5 |
| *Leptobrachella chishuiensis* sp. nov. | CIBCS20190518043 | male | 33.0 | 11.9 | 11.7 | 5.1 | 3.5 | 2.8 | 3.0 | 4.0 | 2.2 | 15.3 | 3.1 | 15.2 | 4.2 | 15.5 | 22.2 | 15.3 | 3.3 | 3.0 | 4.9 | 3.2 |
| *Leptobrachella chishuiensis* sp. nov. | CIBCS20190518049 | male | 30.9 | 11.9 | 10.8 | 5.0 | 3.5 | 3.0 | 3.1 | 4.1 | 2.2 | 14.9 | 2.6 | 13.9 | 3.4 | 15.3 | 21.1 | 14.4 | 3.0 | 2.8 | 5.1 | 2.9 |
| *Leptobrachella chishuiensis* sp. nov. | CIBCS20190518044 | male | 33.4 | 11.1 | 11.6 | 5.4 | 3.8 | 3.1 | 3.3 | 4.4 | 2.2 | 16.3 | 2.8 | 17.1 | 3.8 | 16.8 | 22.1 | 15.9 | 3.8 | 3.0 | 5.0 | 3.5 |
| *Leptobrachella chishuiensis* sp. nov. | CIBCS20190518045 | male | 30.8 | 11.8 | 11.4 | 4.8 | 3.6 | 3.0 | 3.0 | 4.1 | 2.0 | 15.5 | 3.0 | 14.2 | 4.1 | 15.2 | 21.2 | 15.1 | 3.6 | 3.1 | 5.0 | 3.1 |
| *Leptobrachella chishuiensis* sp. nov. | CIBCS20190518048 | male | 31.6 | 11.5 | 10.6 | 5.0 | 3.7 | 2.7 | 3.3 | 4.2 | 2.6 | 14.7 | 2.9 | 13.7 | 3.3 | 14.9 | 20.9 | 15.0 | 3.2 | 2.8 | 5.0 | 2.9 |
| *Leptobrachella chishuiensis* sp. nov. | CIBCS20190518046 | female | 34.2 | 12.7 | 12.0 | 5.3 | 3.4 | 2.7 | 3.0 | 4.4 | 2.4 | 16.3 | 3.3 | 15.3 | 4.2 | 16.0 | 22.2 | 16.3 | 3.4 | 3.0 | 5.6 | 3.3 |
Table 3. References for morphological characters for congeners of the genus *Leptobrachella*.

| ID | *Leptobrachella* species | Literature obtained |
|----|--------------------------|---------------------|
| 1  | *L. aerea* (Rowley, Stuart, Richards, Phimmachak & Sivongxay, 2010) | Rowley et al. 2010c |
| 2  | *L. alpina* (Fei, Ye & Li, 1990) | Fei et al. 2009 |
| 3  | *L. applebyi* (Rowley & Cao, 2009) | Rowley and Cao 2009 |
| 4  | *L. arayaui* (Matsui, 1997) | Matsui 1997 |
| 5  | *L. arwen* (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016) | Rowley et al. 2016 |
| 6  | *L. baliensis* Smith, 1931 | Dring 1983; Eto et al. 2016 |
| 7  | *L. bidopenensis* (Rowley, Le, Tran & Hoang, 2011) | Rowley et al. 2011 |
| 8  | *L. bijiez* Wang, Li, Li, Chen & Wang, 2019 | Wang et al. 2019 |
| 9  | *L. bondangensis* Eto, Matsui, Hamidy, Munir & Iskandar, 2018 | Eto et al. 2018 |
| 10 | *L. botsfordi* (Rowley, Dau & Nguyen, 2013) | Rowley et al. 2013 |
| 11 | *L. bourreti* (Dubois, 1983) | Ohler et al. 2011 |
| 12 | *L. brevicornis* Dring, 1983 | Dring 1983; Eto et al. 2015 |
| 13 | *L. crocea* (Rowley, Hoang, Le, Dau & Cao, 2010) | Rowley et al. 2010a |
| 14 | *L. dringi* (Dubois, 1987) | Inger et al. 1995; Matsui and Dehling 2012 |
| 15 | *L. es* (Ohler, Wollenberg, Grosjean, Hendrix, Vences, Ziegler & Dubois, 2011) | Ohler et al. 2011 |
| 16 | *L. fchri* (Rowley, Hoang, Dau, Le & Cao, 2012) | Rowley et al. 2012 |
| 17 | *L. fritinniens* (Dehling & Matsui, 2013) | Dehling and Matsui 2013 |
| 18 | *L. fuliginosa* (Matsui, 2006) | Matsui 2006 |
| 19 | *L. fusi* Eto, Matsui, Hamidy, Munir & Iskandar, 2018 | Eto et al. 2018 |
| 20 | *L. gracilis* (Günther, 1872) | Günther 1872; Dehling 2012b |
| 21 | *L. hamidi* (Matsui, 1997) | Matsui 1997 |
| 22 | *L. heteropus* (Boulenger, 1900) | Boulenger 1900 |
| 23 | *L. isos* (Rowley, Stuart, Neang, Hoang, Dau, Nguyen & Emmett, 2015) | Rowley et al. 2015a |
| 24 | *L. jarlandrangi* Eto, Matsui & Nishikawa, 2015 | Eto et al. 2015 |
| 25 | *L. kajangensis* (Grismer, Grismer & Youmans, 2004) | Grismer et al. 2004 |
| 26 | *L. kalonensis* (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016) | Rowley et al. 2016 |
| 27 | *L. kecil* (Matsui, Belabut, Ahmad & Yong, 2009) | Matsui et al. 2009 |
| 28 | *L. khai* (Das, Tron, Rangan & Hooroo, 2010) | Das et al. 2010 |
| 29 | *L. lateralis* (Anderson, 1871) | Anderson 1871; Humtsoe et al. 2008 |
| 30 | *L. laui* (Sung, Yang & Wang, 2014) | Sung et al. 2014 |
| 31 | *L. liti* (Fei & Ye, 1990) | Fei et al. 2009; Sung et al. 2014 |
| 32 | *L. macrops* (Duong, Do, Ngo, Nguyen & Poyarkov, 2018) | Duong et al. 2018 |
| 33 | *L. maculosa* (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016) | Rowley et al. 2016 |
| 34 | *L. mangshanensis* (Hou, Zhang, Hu, Li, Shi, Chen, Mo, & Wang, 2018) | Hou et al. 2018 |
| 35 | *L. novobalavasa* (Yuan, Sun, Chen, Rowley & Che, 2017) | Yuan et al. 2017 |
| 36 | *L. nivias* (Matsui, Zainudin & Nishikawa, 2014) | Matsui et al. 2014b |
| 37 | *L. nlassen* (Inger, Lakim, Bin & Yambun, 1997) | Inger et al. 1997 |
| 38 | *L. melanoleuca* (Matsui, 2006) | Matsui 2006 |
| 39 | *L. nix* (Ohler, Wollenberg, Grosjean, Hendrix, Vences, Ziegler & Dubois, 2011) | Ohler et al. 2011 |
| 40 | *L. oblongata* (Lathrop, Murphy, Orlov & Ho, 1998) | Lathrop et al. 1998 |
| 41 | *L. oblongata* (Lin, 1950) | Günther 1895 |
| 42 | *L. okorek* (Mathew & Sen, 2010) | Mathew and Sen 2010 |
| 43 | *L. nyx* (Ohler, Wollenberg, Grosjean, Hendrix, Vences, Ziegler & Dubois, 2011) | Ohler et al. 2011 |
| 44 | *L. oshane†* (Liu, 1950) | Fei et al. 2009 |
| 45 | *L. palis* (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016) | Rowley et al. 2016 |
| 46 | *L. palmaris* Inger & Stuebing, 1992 | Inger and Stuebing 1992 |
| 47 | *L. parvus* Dring, 1983 | Dring 1983 |
| 48 | *L. peleytoides* (Boulenger, 1893) | Boulenger 1893; Ohler et al. 2011 |
| 49 | *L. petra* (Rowley, Dau, Hoang, Le, Cutajar & Nguyen, 2017) | Rowley et al. 2017a |
| 50 | *L. picta* (Malkmus, 1992) | Malkmus 1992 |
In PCA for male group, the total variation of the first two principal components was 64.6%. In males on the two-dimensional plots of PC1 vs. PC2, the undescribed species could be distinctly separated from \textit{L. bijie} and \textit{L. purpuraventra} (Fig. 3). The results of Mann-Whitney \textit{U} tests indicated that in males, the new taxon was significantly different from \textit{L. bijie} and \textit{L. purpuraventra} on many morphometric characters (all \( p \)-values < 0.05; Table 4).

There were many differences in sonograms and waveforms of calls between the new species \textit{L. bijie} and \textit{L. purpuraventra}. Firstly, a call contains 1–4 notes in the new species and only contains two notes of each call in \textit{L. bijie} and \textit{L. purpuraventra}. Secondly, the dominant frequency of the new species is higher than \textit{L. bijie} and \textit{L. purpuraventra}.

Based on the molecular, morphological, and bioacoustics differences, the specimens from Chishui City, Guizhou Province, China represent a new species which is described as follows.

**Taxonomic account**

\textit{Leptobrachella chishuiensis} sp. nov.

http://zoobank.org/DE8BA5C5-CB7B-4872-B489-61E7EFCF9B8C

Figs 4–6; Tables 1–5

**Type material.** \textbf{Holotype.} CIBCS20190518047, adult male (Figs 4, 5), collected by Shi-Ze Li in Chishui National Nature Reserve (28.436708N, 105.997794E, ca. 465 m a. s. l.), Chishui City, Guizhou Province, China on 18 May 2019.
A new species of Leptobrachella

Paratypes. Six adult males and one adult female from Chishui City, Guizhou Province, China, collected by Shize LI and Jing LIU. One female CIBCS20190518046 and two adult males CIBCS 20190518048 and CIBCS20190518049 collected by Jing LIU on 18 May 2019, four adult males CIBCS 20190518042, CIBCS 20190518043, CIBCS20190518044 and CIBCS20190518045 collected by Shize LI on 18 May 2019.
Figure 3. Plots of the first principal component (PC1) versus the second (PC2) for *Leptobrachella chishuiensis* sp. nov., *L. bijie*, and *L. purpuraventra* in males from a principal component analysis.

Figure 4. The holotype specimen CIBCS20190518047 of *Leptobrachella chishuiensis* sp. nov. in preservative **A** dorsal view **B** ventral view **C** frontal view of tongue **D** ventral view of hand **E** ventral view of foot.
A new species of *Leptobrachella*

**Diagnosis.** *Leptobrachella chishuiensis* sp. nov. is assigned to the genus *Leptobrachella* based on molecular phylogenetic analyses and the following morphological characters: small body size; having an elevated inner metacarpal tubercle; having macro-glands on body (including supra-axillary, femoral and ventrolateral glands); lacking vomerine teeth; having small tubercles on eyelids; anterior tip of snout with whitish vertical bar (Dubois 1983; Matsui 1997, 2006; Lathrop et al. 1998; Delorme et al. 2006; Das et al. 2010; Luo et al. 2020).

*Leptobrachella chishuiensis* sp. nov. could be distinguished from its congeners by a combination of the following characters: (1) small body size (SVL 30.8–33.4 mm in seven adult males, and 34.2 mm in one adult female); (2) dorsal skin shagreened, some of the granules forming longitudinal short skin ridges; (3) tympanum distinctly discernible, slightly concave; (4) internasal distance longer than interorbital distance; (5) supra-axillary, femoral, pectoral and ventrolateral glands distinctly visible; (6) absence of webbing and lateral fringes on fingers; (7) toes with rudimentary webbing and shallow lateral fringes; (8) relative finger lengths II < IV < I < III; (9) heels overlapped when thighs are positioned at right angles to the body; and (10) tibia-tarsal articulation reaches the tympanum.

**Description of holotype.** Measurements in mm. Adult male (CIBCS20190518047). SVL 32.4. Head length slightly longer than head width (HDL/HDW ratio 1.04); 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, internarial distance longer than interorbital distance (IND/IOD ratio 1.23); pineal ocellus absent; vertical pupil; snout length larger than eye diameter; tympanum distinct, rounded, and slightly concave, diameter smaller than that of the eye (TMP/ED ratio 0.57); upper margin of tympanum in contact with supratympanic ridge; distinct black supratympanic line present; vomerine teeth absent; tongue notched behind; supratympanic ridge distinct, extending from posterior corner of eye to supra-axillary gland.

Tips of fingers rounded, slightly swollen; relative finger lengths II < IV < I < III (FIL/FIIL ratio 1.1, FIVL/FIIL ratio 1.03); absence of webbing; nuptial pad and subarticular tubercles absent; inner palmar tubercle large, rounded separated from small, round outer palmar tubercle.

Hindlimbs slender, tibia 49% of snout-vent length; heels overlapped when thighs are positioned at right angles to the body, tibiotarsal articulation reaching tympanum when leg stretched forward; tibia length slightly longer than thigh length; relative toe lengths I < II < V < III < IV; tips of toes rounded, slightly dilated; subarticular tubercle small, distinct at the base of each toes; toes without webbing; narrow lateral fringes present 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 on the flank of dorsal; ventral skin smooth; dense tiny granules present on surface of chest and ventral surface of thigh and tibia; pectoral gland and
femoral gland 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 scattered with irregular light orange pigmentation. A dark brown inverted triangular pattern between anterior corner of eyes. Tympanum brown, a dark brown bar above tympanum, and a dark brown bar under the eye; 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 coloration; fingers and toes with distinct dark bars. Ventral surface of throat grey purple, chest and belly white, presence of distinct nebulous greyish speckling on flanks; ventral surface of limbs grey purple. Supra-axillary gland, femoral, pectoral and ventrolateral glands white (Fig. 5).

**Preserved holotype colouration.** Dorsum of body and limbs fade to dark brown; transverse bars on limbs become more distinct ventral surface of body and limbs fade to greyish white. Supra-axillary, femoral, pectoral and ventrolateral glands fade to greyish white (Fig. 4).

**Variations.** Morphological measurements were showed in Table 2. All specimens were similar in morphology but some individuals different from the holotype in color pattern. In some adult males, a dark brown inverted triangular pattern between anterior corner of eyes, in connected to the dark brown W-shaped marking on interorbital region (Fig. 6A); in adult female, the color of dorsum is blacker (Fig. 6B) and some patchiness on the chest and the flank of belly (Fig. 6C); in some adult males, the throat and bell creamy and white patchiness sparse on the ventral surface of limbs (Fig. 6D); in some specimens, the tibiotarsal articulation reaching tympanum to eye when leg stretched forward.

**Advertisement call.** A total of 32 advertisement calls of *Leptobrachella chishuiensis* sp. nov. were recorded in Chishui City, Guizhou Province, China on 18 May 2019 between 21:00–22:00. The call description is based on recordings of the holotype CIBCS20190518047 (Fig. 7) from a branch of bush nearby a stream. Each call contains 1–4 notes (mean 2.34 ± 0.827, N = 32). Call duration was 75–353 ms (mean 200 ± 67, N = 32). Call interval was 8–98 ms (mean 60 ± 21, N = 31) with a peak frequency was 6140.15 ± 69.35 (6064–6284 Hz, N = 32). Each note had a duration of 52–950 ms (mean 104 ± 107, N = 69), and the intervals between notes had a duration of 0.1–25 ms (mean 5.3 ± 8.5, N = 37). Amplitude modulation within note was apparent, beginning with high energy pulses then decreasing towards the end of each note.

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

**Comparisons.** The new species was compared with 52 congeners on morphology (Table 4). By having small body size (SVL 30.8–33.4 mm in seven adult males, and 34.2 mm in one adult female), *Leptobrachella chishuiensis* sp. nov. differs from the larger *L. bourreti* (42.0–45.0 mm in females), *L. eos* (33.1–34.7 mm in males and 40.7 in female), *L. lateralis* (36.6 mm in females), *L. nahangensis* (40.8 mm in male), *L. nyx* (37.0–41.0 mm in females), *L. platycephalus* (35.1 mm in male), *L. sungi* (48.3–
A new species of Leptobrachella

Figure 5. Photos of the holotype CIBCS20190518047 of *Leptobrachella chishuiensis* 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.

52.7 mm in males and 56.7–58.9 mm in females), and *L. zhangyapingi* (45.8–52.5 mm in males), and differs from the smaller *L. aerea* (25.1–28.9 mm in males), *L. alpina* (24.0–26.4 mm in males), *L. applebyi* (19.6–22.3 mm in males), *L. ardens* (21.3–24.7 mm in males), *L. baluensis* (14.9–15.9 mm in males), *L. bidoupensis* (18.5–25.4 mm in males), *L. bijie* (29.0–30.4 mm in males), *L. bondangensis* (17.8 mm in male), *L. brevicrus* (17.1–17.8 mm in males), *L. crocea* (22.2–27.3 mm in males), *L. frthi* (26.4–29.2 mm in males), *L. fuliginosa* (28.2–30.0 mm in males), *L. fisca* (16.3 mm in male), *L. isos* (23.7–27.9 mm in males), *L. itiokai* (15.2–16.7 mm in males), *L. juliandringi* (17.0–17.2 mm in males and 18.9–19.1 mm in females), *L. khasiorum* (24.5–27.3 mm in males), *L. lateralis* (26.9–28.3 mm in males), *L. laui* (24.8–26.7 mm in males), *L. liui* (23.0–28.7 mm in males), *L. macrops* (28.0–29.3 mm in males), *L. maculosa* (24.2–26.6 mm in males), *L. mangshanensis* (22.22–27.76 mm in males), *L. melica* (19.5–22.8 mm in males), *mjobergi* (15.7–19.0 mm in males), *L. natunae* (17.6 mm in male), *L. pallida* (24.5–27.7 mm in males), *L. palmate* (14.4–16.8 mm in males), *L. parva* (15.0–16.9 mm in males), *L. petrops* (23.6–27.6 mm in males), *L. pluvialis* (21.3–22.3 mm in males), *L. purpuraventra* (27.3–29.8 mm in males), *L. puboatensis* (24.2–28.1 mm in males), *L. purpura* (25.0–27.5 mm in males), *L. rowleyae* (23.4–25.4 mm in males), *L. shangsiensis* (24.9–29.4 mm in males), *L. suiyangensis* (28.7–29.7 mm in males), *L. tadungensis* (23.3–28.2 mm in males), *L. tengchongensis*
Figure 6. Colour variation in *Leptobrachella chishuiensis* sp. nov. A dorsal view of the male specimen CIBCS20190518042 B dorsal view of the female specimen CIBCS20190518046 C ventral view of the female specimen CIBCS20190518046 D ventral view of the female specimen CIBCS20190518049.

(23.9–26.0 mm in males), *L. tuberosa* (24.4–29.5 mm in males), *L. ventripunctata* (25.5–28.0 mm in males), *L. wuhuangmontis* (25.6–30.0 mm in males), *L. yingjiangensis* (25.7–27.6 mm in males), and *L. yunkaiensis* (25.9–29.3 mm in males).

By supra-axillary and ventrolateral glands present, *Leptobrachella chishuiensis* sp. nov. differs from *L. arayai*, *L. dringi*, *L. fritinniens*, *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. absent in the latter).

By having black spots on flanks, *Leptobrachella chishuiensis* sp. nov. differs from *L. aerea*, *L. botsfordi*, *L. frthi*, and *L. tuberosa* (vs. lacking in the latter).

By toes with rudimentary webbing, *Leptobrachella chishuiensis* sp. nov. differs from *L. kalonensis* and *L. oshanensis* (vs. lacking webbing on toes in the latter), and differs from *L. pelodytoides* (vs. toes with wide webbing in the latter).

By having shallow lateral fringes on toes, *Leptobrachella chishuiensis* sp. nov. differs from *L. aerea*, *L. frthi*, *L. liui*, and *L. yunkaiensis* (vs. having prominently wide
A new species of *Leptobrachella* 107

lateral fringes on toes in the latter), and differs from *L. kalonensis*, *L. macrops*, *L. minima*, *L. nyx*, *L. oshanensis*, *L. pyrrhops*, and *L. tuberosa* (vs. lacking lateral fringes on toes in the latter).

By having dorsal surface shagreened and granular, lacking enlarge tubercles or warts, *Leptobrachella chishuiensis* sp. nov. differs from the following species: *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. maoresbanensis* (dorsum shagreened with tubercles), *L. minima* (dorsum smooth), *L. nyx* (dorsum with round tubercles), *L. pelodytoides* (dorsum with small, smooth warts), *L. tamdil* (dorsum weakly tuberculate, with low, oval tubercles), *L. tuberosa* (dorsum highly tuberculate), *L. yunkaiensis* (dorsum with raised warts), and *L. wuhuangmontis* (dorsum rough with conical tubercles).

By the finger II < I, *Leptobrachella chishuiensis* sp. nov. differs from *L. tamdil* (vs. II > I in the latter).

By head length slightly longer than wide, *Leptobrachella chishuiensis* sp. nov. differs from *L. namdongensis* (vs. head wider than long in the latter).

Six *Leptobrachella* species were reported to be distributed in Guizhou Province, China, they are: *L. liui*, *L. oshanensis*, *L. purpuraventra*, *L. bijie*, *L. ventripunctata*, and *L. suiyangensis* (Fei et al. 2012; Li et al. 2016; Wang et al. 2019; Luo et al. 2020). We make a comparative note between them and the new species as follows. *Leptobrachella chishuiensis* sp. nov. differs from *L. liui* by having shallow lateral fringes on toes (vs. wide lateral fringes on the toes in the latter), dorsal surface shagreened with small granules, lacking enlarge tubercles or warts (vs. dorsum with round tubercles in the latter); from *L. oshanensis* by having rudimentary webbing on the toes (vs. lack webbing on the toes in the latter), having shallow lateral fringes on toes (vs. lacking lateral fringes on the toes in the latter), from *L. suiyangensis* by heels overlapping when thighs are positioned at right angles to the body (vs. just meeting in the latter), tibia-tarsal articulation reaches tympanum or tympanum to eye (vs. reaches to the anterior corner of eye in the latter); from *L. ventripunctata* by bigger body size (SVL 30.8–33.4 mm in adult males vs. SVL 25.5–28.0 mm in males in the latter), chest and belly without large dark brown spots (vs. with large dark brown spots in the latter).

*Leptobrachella chishuiensis* sp. nov. is genetically closer to *L. bijie* and *L. purpuraventra*. The new species differs from *L. bijie* by the following characters: larger body size (SVL 30.8–33.4 mm in males vs. SVL 29.0–30.4 mm in males in the latter), internasal distance longer than interorbital distance (vs. equal to interorbital distance in the latter), heels overlapping (vs. just meeting in the latter), tibia-tarsal articulation reaches the tympanum or tympanum to eye (vs. reaching the region between middle of eye to anterior corner of eye in the latter), one call contains 1–4 notes (vs. 2 notes in each call in the latter), having shorter call interval (60 ± 21, N = 31 in the new species vs. 101.9 ± 6.4, N = 33 in the latter), having significantly higher value of SVL in males, and having significantly higher value of HDL, HDW, SL, IND, IOD, TEY, TL and FL to SVL in males (all P-values < 0.05; Table 5).
Table 4. Diagnosis characters on morphology of *Leptobrachella chishuiensis* sp. nov. from other congeners.

| ID | Species                  | Male SVL (mm) | Black spots on flanks | Toes webbing | Fringes on toes | Ventral coloration | Dorsal skin texture                  |
|----|--------------------------|---------------|-----------------------|--------------|-----------------|-------------------|---------------------------------------|
| 1  | *L. chishuiensis* sp. nov.| 30.8–33.4     | Yes                   | Rudimentary  | Narrow          | White with distinct nebulous greyish speckling on chest and ventrolateral flanks | Shagreened and granular              |
| 2  | *L. aerea*               | 25.1–28.9     | No                    | Rudimentary  | Wide            | Near immaculate creamy white, brown speckling on margins             | Finely tuberculate                  |
| 3  | *L. alpina*              | 24.0–26.4     | Yes                   | Rudimentary  | Wide in males   | Creamy-white with dark spots                                         | Relatively smooth, some with small warts|
| 4  | *L. applebyi*            | 19.6–22.3     | Yes                   | Rudimentary  | No               | Reddish brown with white speckling                                     | Smooth                               |
| 5  | *L. ardens*              | 21.3–24.7     | Yes                   | No           | No               | Reddish brown with white speckling                                     | Smooth                               |
| 6  | *L. bedouensis*          | 18.5–25.4     | Yes                   | Rudimentary  | Weak             | Reddish brown with white speckling                                     | Smooth                               |
| 7  | *L. bijie*               | 29.0–30.4     | Yes                   | Rudimentary  | Narrow          | White with distinct nebulous greyish speckling on chest and ventrolateral flanks | Shagreened and granular              |
| 8  | *L. botsfordi*           | 29.1–32.6     | No                    | Rudimentary  | Narrow          | Reddish brown with white speckling                                     | Shagreened                           |
| 9  | *L. bourreti*            | 28.0–36.2     | Yes                   | Rudimentary  | Weak             | Creamy white                                                         | Relatively smooth, some with small warts|
| 10 | *L. crocea*              | 22.3–27.3     | No                    | Rudimentary  | No               | Bright orange                                                        | Highly tuberculate                   |
| 11 | *L. on*                  | 33.1–34.7     | No                    | Rudimentary  | Wide             | Creamy white                                                         | Shagreened                           |
| 12 | *L. forbi*               | 26.4–29.2     | No                    | Rudimentary  | Wide in males   | Creamy white                                                         | Isolated, scattered tubercles        |
| 13 | *L. fuliginosa*          | 28.2–30.0     | Yes                   | Rudimentary  | Weak             | White with brown dusting                                              | Mostly smooth, females more tuber-culate|
| 14 | *L. issi*                | 23.7–27.9     | No                    | Rudimentary  | Wide in males   | Creamy white with white dusting on margins                           | Mostly smooth, females more tuber-culate|
| 15 | *L. kalonensis*          | 25.8–30.6     | Yes                   | No           | No               | Pale, speckled brown                                                 | Smooth                               |
| 16 | *L. khasiorum*           | 24.5–27.3     | Yes                   | Rudimentary  | Wide             | Creamy white                                                         | Smooth                               |
| 17 | *L. lateralis*           | 26.9–28.3     | Yes                   | Rudimentary  | No               | Creamy white                                                         | Roughly tuberculate                   |
| 18 | *L. leui*                | 24.8–26.7     | Yes                   | Rudimentary  | Wide             | Creamy white with dark brown dusting on margins                      | Round granular tubercles             |
| 19 | *L. liui*                | 23.0–28.7     | Yes                   | Rudimentary  | Wide             | Creamy white with dark brown spots on chest and margins              | Round granular tubercles with glandular folds |
| 20 | *L. macrops*             | 28.0–29.3     | Yes                   | Rudimentary  | No               | Greyish-violet with white speckling                                  | Roughly granular with larger tubercles|
| 21 | *L. maculos*             | 24.2–26.6     | Yes                   | No           | No               | Brown, less white speckling                                           | Mostly smooth                         |
| 22 | *L. marungshenensis*     | 22.2–27.76    | Yes                   | Rudimentary  | Weak             | White speckles on throat and belly                                   | Nearly smooth                         |
| 23 | *L. maoshanensis*        | 25.2–30.4     | Yes                   | Rudimentary  | Narrow           | Creamy white chest and belly with irregular black spots              | Longitudinal folds                   |
| 24 | *L. marinima*            | 32.3–38.0     | Yes                   | Rudimentary  | No               | Chest and belly immaculate white                                     | Nearly smooth, scattered with small tubercles of varying sizes |
| 25 | *L. melica*              | 19.5–22.8     | Yes                   | Rudimentary  | No               | Reddish brown with white speckling                                   | Smooth                               |
| 26 | *L. minimum*             | 25.7–31.4     | Yes                   | Rudimentary  | No               | Creamy white                                                         | Smooth                               |
| 27 | *L. nabangensis*         | 40.8          | Yes                   | Rudimentary  | No               | Creamy white with light specking on throat and chest                 | Smooth                               |
| 28 | *L. namdongensis*        | 30.9          | Yes                   | Rudimentary  | No               | Creamy white with brown dusting on margins                          | Finely tuberculate                   |
| ID | Species            | Male SVL (mm) | Black spots on flanks | Toes webbing | Fringes on toes | Ventral coloration                                                                 | Dorsal skin texture                                                                 |
|----|--------------------|---------------|-----------------------|--------------|----------------|-----------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|
| 29 | *L. nokrekensis*   | 26.0–33.0     | Yes                   | Rudimentary  | unknown        | White with distinct nebulous greyish speckling on chest and ventrolateral flanks   | Tubercles and longitudinal folds                                                   |
| 30 | *L. nyx*           | 26.7–32.6     | Yes                   | Rudimentary  | No             | Creamy white with white with brown margins                                         | Rounded tubercles                                                                  |
| 31 | *L. oshamensis*    | 26.6–30.7     | Yes                   | No           | No             | Whitish with no markings or only small, light grey spots                           | Smooth with few glandular ridges                                                   |
| 32 | *L. pallida*       | 24.5–27.7     | No                    | No           | No             | Reddish brown with white speckling                                                | Tuberculate                                                                         |
| 33 | *L. pelodytoida*   | 27.5–32.3     | Yes                   | Wide         | Narrow         | Ventral coloration                                                                  |                                                                                      |
| 34 | *L. petrope*       | 23.6–27.6     | No                    | No           | Narrow         | Immaculate creamy white                                                            |                                                                                      |
| 35 | *L. pluvisalis*    | 21.3–22.3     | Yes                   | Rudimentary  | No             | Dirty white with dark brown marbling                                               | Smooth, flattened tubercles on flanks                                              |
| 36 | *L. puboatemii*    | 24.2–28.1     | Yes                   | Rudimentary  | No             | Reddish brown with white dusting                                                  | Longitudinal skin ridges                                                           |
| 37 | *L. purpurus*      | 25.0–27.5     | Yes                   | Rudimentary  | Wide           | Dull white with indistinct grey dusting                                            | Shagreen with small tubercles                                                      |
| 38 | *L. purpuraventra* | 27.3–29.8     | Yes                   | Rudimentary  | Narrow         | Grey purple with distinct nebulous greyish speckling on chest and ventrolateral flanks | Shagreened and granular                                                            |
| 39 | *L. pyrhops*       | 30.8–34.3     | Yes                   | Rudimentary  | No             | Reddish brown with white speckling                                                | Slightly shagreened                                                                |
| 40 | *L. russлогae*     | 23.4–25.4     | Yes                   | No           | No             | Pinkish milk-white to light brown chest and belly with numerous white speckles     | Smooth with numerous tiny tubercles                                               |
| 41 | *L. sabahmentaus*  | 25–28         | Yes                   | Rudimentary  | Narrow         | Cream-coloured with dark brown speckling                                            | with tiny tubercles, weakly wrinkled                                               |
| 42 | *L. shangienis*    | 24.9–29.4     | Rudimentary           | Narrow       | Ventrall surface yellowish creamy-white with marble texture                        | Smooth                                                                              |
| 43 | *L. sheng*         | 48.3–52.7     | No or small           | Wide         | Weak           | White                                                                              | Granular                                                                           |
| 44 | *L. suiyangensis*  | 28.7–29.7     | Yes                   | Rudimentary  | Narrow         | Yellowish creamy-white with marble texture and belly or with irregular light brown speckling | Shagreen with small granules                                                   |
| 45 | *L. tadungensis*   | 23.3–28.2     | Yes                   | No           | No             | Reddish brown with white speckling                                                | Smooth                                                                              |
| 46 | *L. tamdid*        | 32.3          | Yes                   | Wide         | Wide           | White                                                                              | Weakly tuberculate                                                                 |
| 47 | *L. tengdongensis* | 23.9–26.0     | Yes                   | Rudimentary  | Narrow         | White with dark brown blotches                                                    | Shagreened with small tubercles                                                   |
| 48 | *L. tubornai*      | 24.4–29.5     | No                    | Rudimentary  | No             | White with small grey spots/streaks                                                | Highly tuberculate                                                                 |
| 49 | *L. ventripunctata*| 25.5–28.0     | Yes                   | Rudimentary  | No             | Chest and belly with dark brown spots                                              | Longitudinal skin ridges                                                           |
| 50 | *L. wubahuangmontis*| 25.6–30.0     | Yes                   | Rudimentary  | Narrow         | Greyish white mixed by tiny white and black dots                                   | Rough, scattered with dense conical tubercles                                      |
| 51 | *L. yingjiangensis*| 25.7–27.6     | Yes                   | Rudimentary  | Wide           | Creamy white with dark brown flecks on chest and margins                           | Shagreened with small tubercles                                                   |
| 52 | *L. yunkaiensis*   | 25.9–29.3     | Yes                   | Rudimentary  | Wide           | Belly pink with distinct or indistinct speckling                                  | Shagreened with short skin ridges and raised warts                                 |
| 53 | *L. zhangyingi*    | 45.8–52.5     | No                    | Rudimentary  | Wide           | Creamy-white with white with brown                                                 | Mostly smooth with distinct tubercules                                            |
Shi-Ze Li et al. / ZooKeys 943: 91–118 (2020)

Leptobrachella chishuiensis sp. nov. differs from L. purpuraventra by larger body size (SVL 30.8–33.4 mm in seven adult males vs. SVL 27.3–29.8 mm in eleven adult males in the latter), tibia-tarsal articulation reaches the tympanum or tympanum to eye (vs. reaching the middle of eye in the latter), the call contains 1–4 notes (vs. 2 notes in each call in the latter), having longer call duration (200 ± 67, N = 32 vs. 192.2 ± 13.0 as the longest call duration in L. purpuraventra), shorter call interval (60 ± 21, N = 31 vs. 90.8 ± 5.6, N = 20 as the shortest call interval in L. bijie), having significantly higher value of SVL in males, and having significantly higher value of SVL, HDL, HDW, SL, IOD, ED, TYD, LAL, TL and FL to SVL in males (all P-values < 0.05; Table 5).

Ecology. Leptobrachella chishuiensis sp. nov. is known from the type locality, Chishui National Nature Reserve (28.383333–28.45 N, 105.05–109.75 E), Chishui City, Guizhou Province, China at elevations between 270–604 m a.s.l. This new species is found in bamboo forest nearby the streams (Fig. 8), and four sympatric amphibian species, i.e. Megophrys omeimontis, Odorrana margaratae (Liu, 1950), Zhangixalus omeimontis (Stejneger, 1924), and Rana omeimontis Ye & Fei, 1993 were found nearby.

Etymology. This specific name chishuiensis refers to the distribution of this species, Chishui City, Guizhou Province, China. We propose the common English name “Chishui leaf litter toads” (English) and its Chinese as “Chi Shui Zhang Tu Chan (赤水掌突蟾)”.

| Leptobrachella chishuiensis sp. nov. | L. bijie | L. purpuraventra | P-value from Mann-Whitney U test |
|------------------------------------|----------|-----------------|---------------------------------|
| Range (Male (N = 7)) | Mean ± SD | Range (Male (N = 8)) | Mean ± SD | Range (Male (N = 11)) | Mean ± SD | L. chishuiensis vs. L. bijie | P-value |
| SVL | 30.8–33.4 | 32.1 ± 1.0 | 29.0–30.4 | 29.7 ± 0.6 | 27.3–29.8 | 28.9 ± 0.8 | 0.001 |
| HDL | 11.1–12.3 | 11.8 ± 0.4 | 12.7 | 10.9–10.6 | 10.2 ± 0.2 | 9.6–10.3 | 9.9 ± 0.3 | 0.021 |
| HDW | 10.6–11.9 | 11.4 ± 0.5 | 12.0 | 9.5–10.2 | 9.8 ± 0.3 | 9.3–9.8 | 9.6 ± 0.2 | 0.001 |
| SL | 4.8–5.8 | 5.2 ± 0.3 | 5.3 | 4.0–4.7 | 4.2 ± 0.2 | 3.5–4.1 | 3.8 ± 0.2 | 0.002 |
| IND | 3.5–3.8 | 3.7 ± 0.1 | 3.4 | 2.8–3.4 | 3.1 ± 0.2 | 2.7–3.5 | 3.1 ± 0.2 | 0.003 |
| IOD | 2.7–3.1 | 3.0 ± 0.2 | 2.7 | 2.8–3.4 | 3.1 ± 0.2 | 2.6–3.2 | 2.9 ± 0.2 | 0.008 |
| UEW | 3.0–3.3 | 3.2 ± 0.1 | 3.0 | / | / | / | / | / |
| ED | 4.0–5.0 | 4.4 ± 0.4 | 4.4 | 3.6–4.1 | 3.8 ± 0.2 | 3.1–3.6 | 3.4 ± 0.2 | 0.064 |
| TYD | 2.0–2.6 | 2.3 ± 0.2 | 2.4 | 1.9–2.2 | 2.0 ± 0.1 | 1.7–1.9 | 1.8 ± 0.1 | 0.247 |
| TEP | 1.2–1.6 | 1.4 ± 0.2 | 1.2 | 0.9–1.1 | 1.0 ± 0.1 | 1.1–1.3 | 1.2 ± 0.1 | 0.002 |
| LAL | 14.7–17.0 | 15.6 ± 0.8 | 16.3 | 14.0–14.8 | 14.3 ± 0.3 | 12.6–14.0 | 13.3 ± 0.4 | 0.643 |
| LW | 2.6–3.2 | 3.0 ± 0.2 | 3.3 | / | / | / | / | / |
| ML | 7.9–8.8 | 8.2 ± 0.3 | 8.7 | 7.4–8.3 | 7.8 ± 0.3 | 7.0–7.7 | 7.4 ± 0.2 | 0.247 |
| FIL | 3.0–3.8 | 3.4 ± 0.3 | 3.4 | / | / | / | / | / |
| FII | 2.8–3.4 | 3.0 ± 0.2 | 3.0 | / | / | / | / | / |
| FIII | 4.9–5.5 | 5.1 ± 0.2 | 5.6 | / | / | / | / | / |
| FIVL | 2.9–3.5 | 3.2 ± 0.2 | 3.3 | / | / | / | / | / |
| HLL | 43.3–49.7 | 49.7 ± 2.7 | 49.4 | 43.0–45.5 | 43.7 ± 0.8 | 39.0–44.6 | 41.4 ± 2.2 | 0.487 |
| THL | 13.7–17.1 | 15.1 ± 1.2 | 15.3 | / | / | / | / | / |
| TW | 3.3–4.3 | 3.8 ± 0.4 | 4.2 | / | / | / | / | / |
| TL | 14.9–16.8 | 15.6 ± 0.6 | 16.0 | 13.5–14.4 | 13.6 ± 0.3 | 12.5–14.0 | 13.1 ± 0.5 | 0.005 |
| TFL | 20.9–22.3 | 21.7 ± 0.6 | 22.2 | / | / | / | / | / |
| FL | 14.4–15.9 | 15.1 ± 0.5 | 16.3 | 13.0–13.8 | 13.3 ± 0.2 | 12.1–13.2 | 12.6 ± 0.4 | 0.004 |
A new species of *Leptobrachella*

Figure 7. Advertisement calls of the holotype CIBCS20190518047 of *Leptobrachella chishuiensis* 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.

Figure 8. Habitats of *Leptobrachella chishuiensis* sp. nov. in the type locality Chuishui National Nature Reserve, Chishui City, Guizhou Province, China A landscape of montane forests in the type locality B a mountain stream in the type locality (insert holotype CIBCS20190518047 in life in the field).

Discussion

The Asian leaf litter toads of *Leptobrachella* have low vagility and are in exclusive association with montane forests, and their populations are often highly structured. Underestimation of species diversity occurs in the genus, which suggests a high degree of localized diversification and micro-endemism (Fei et al. 2012; Chen et al. 2018). Many cryptic species were proposed by molecular analyses in areas where surveys are weak (Chen et al. 2018), but in Guizhou Province the investigation into the genus was poor although this area was likely to be an important transition zone for many clades or lineages (Chen et al. 2018). Additionally, in Guizhou Province, many new amphib-
ian species has been described in recent years (Zhang et al. 2017; Li et al. 2018a, b; Li et al. 2019a, b; Lyu et al. 2019; Wang et al. 2019; Wei et al. 2020), including two species of *Leptobrachella*, indicating the underestimated species diversity of amphibians in this region. To date, in Guizhou Province, seven *Leptobrachella* species were recorded, i.e., *Leptobrachella chishuiensis* sp. nov., *L. liui*, *L. oshanensis*, *L. purpuraventra*, *L. bijie*, *L. ventripunctata*, and *L. suiyangensis* (Fei et al. 2012; Li et al. 2016; Wang et al. 2019; Luo et al. 2020). It is expected that in this area, the species diversity of *Leptobrachella* may be underestimated, and more investigation should be conducted for detecting richness of the toad species.

The new species is found along clear water rocky streams from Chishui County, Guizhou Province, China, and little is known about the population status of the new species. Thus, further research on the true distribution, population size and trends, and conservation actions are required.

**Acknowledgements**

We would like to thank Jian Wang for supplying measurements of some species. This work was supported by Project supported by the Biodiversity investigation, Observation and Assessment Program (2019–2023) of Ministry of Ecology and Environment of China, National Natural Science Foundation of China (No. 31960099), Basic research project of science and technology department of Guizhou Province (No. [2020] 1Y083), Science and technology support project of science and technology department of Guizhou Provincial (No. [2020] 4Y029), Guizhou Provincial Department of Education Youth Science and Technology Talents Growth Project (Nos. KY [2018] 455 and KY [2018] 468). Forestry Science and Technology Research Project of Guizhou Forestry Department (No. [2020] 13).

**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
A new species of *Leptobrachella* 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 *Leptobrachella* (Anura: Megophryidae) from southern Guangxi, China. Zootaxa 4563: 67–82. https://doi.org/10.11646/zootaxa.4563.1.3

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nature methods 9: 1–772. 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.2339.1.3

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.

Delorme M, Dubois A, Grosjean S, Ohler A (2006) Une nouvelle ergotaxinomie des Megophryidae (Amphibia, Anura). Alytes 24(1–4): 6–21.

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 espec e novelle 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. [in Chinese]

Fei L, Ye CY (2005) Two new species of Megophryidae from China. In: Fei L, Ye C-Y, Jiang J-p, Xie F, Huang Y-Z (Eds) An Illustrated Key to Chinese Amphibians. Sichuan Publishing House of Science and Technology, Chongqing, 253–255. [in Chinese]

Fei L, Hu SQ, Ye CY, Huang YZ (2009) Fauna Sinica. Amphibia (Vol. 2). Anura. Science Press, Beijing, 957 pp. [in Chinese]
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. [in Chinese]

Frost DR (2020) Amphibian species of the world: an Online Reference. Version 6.0. Electronic Database. American Museum of Natural History, New York. http://research.amnh.org/vz/herpetology/amphibia/index.html [accessed 24 Feb 2020]

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): 07–321. https://doi.org/10.1093/sysbio/syq010

Günther A (1872) On the reptiles and amphibia of Borneo. Proceedings of the Scientific Meetings of the Zoological Society of London 1872: 586–600.

Günther A (1895) Te 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 Leptobrachella 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 2012: 24–34. https://doi.org/10.11646/zootaxa.2012.1.2

Inger RF , Stuebing RB (1992 “1991”) A new species of frog of the genus Leptobrachella Smith (Anura: Pelobatidae), with a key to the species from Borneo. Raffles Bulletin of Zoology. Singapore 39: 99–103.

Inger RF , Stuebing RB, Tan F (1995) New species and new records of anurans from Borneo. Raffles Bulletin of Zoology. Singapore 43: 115–132.

Inger RF, 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/10.11646/bhl.part.18855

Inger RF, Orlov N, Darevsky I (1999) Frogs of Vietnam: a report on new collections. Fieldiana Zoology 92: 1–46. https://doi.org/10.5962/bhl.title.3478

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
A new species of *Leptobrachella*

Li GR, Wei G, Zhang HB, Su HJ (2016) A new amphibian record in Guizhou Province—*Leptolalax ventripunctatus*. Chinese Journal of Wildlife 37(2): 178–180.

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. 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: Microhylidae) from Guizhou Province, China. Zootaxa 4624: 551–575. https://doi.org/10.11646/zootaxa.4624.4.7

Liu CC (1950) Amphibians of western China. Fieldiana Zoology Memoires 2: 1–397. [10 pl.] https://doi.org/10.5962/bhl.title.2977

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. liangshansensis* 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/Nordborneo. 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 J M (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

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 *Leptobrachella* (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 (2015a) 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
A new species of *Leptobrachella* 117

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, Saino 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

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

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, Filipski 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

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

Wang J, Yang JH, Li Y, Lyu ZT, Zeng ZC, Liu ZY, Ye YH, Wang YY (2018) Morphology and molecular genetics reveal two new *Leptobrachella* 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 *Leptobrachella* from northwestern Guizhou Province, China (Anura, Megophryidae). ZooKeys 848: 119–154. https://doi.org/10.3897/zookeys.848.29181

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, Megaskumbara 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

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
Supplementary material I

Table S1. Uncorrected $p$-distance between Leptobrachella species on the 16S rRNA gene
Authors: Shi-Ze Li, Jing Liu, Gang Wei, Bin Wang
Data type: genetic distance
Explanation note: Mean value of genetic distance is given in the lower half of the table.
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.943.51572.suppl1