A new species of *Leptobrachella* (Anura, Megophryidae) from Guizhou Province, China

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Abstract

This study describes a new species of the genus *Leptobrachella*, *Leptobrachella suiyangensis* sp. nov., from the Huoqiuba Nature Reserve, Suiyang County, Guizhou Province, China, based on morphological data and phylogenetic analyses (16S rRNA mtDNA). The new species can be distinguished from other congeners by the molecular divergence and by a combination of morphological characters, including body size, dorsal and ventral patterns, dorsal skin texture, size of the pectoral and femoral glands, degree of webbing and fringing on the toes and fingers, dorsum coloration, and iris coloration in life. Currently, the genus *Leptobrachella* contains 75 species, 21 of which are found in China, including seven species reported from Guizhou Province. The uncorrected sequence divergence percentage between *Leptobrachella suiyangensis* sp. nov. and all homologous DNA sequences available for the 16S rRNA gene was found to be >4.7%. The new record of the species and its relationships with others in the same genus imply that species distribution, habitat variation, environmental adaptation, and diversity of the genus *Leptobrachella* in southwest China need to be further investigated.

* These authors contributed equally to this paper
Keywords
Leptobrachella suiyangensis sp. nov., mitochondrial DNA, morphology, Southwest China

Introduction

The genus *Leptolalax* Dubois, 1983 in the family Megophryidae Bonaparte, 1850 is regarded to be closely associated with the genus *Leptobrachella* Smith, 1925 and has been assigned as a synonym of the genus *Leptobrachella* based on a large-scale molecular analysis (Chen et al. 2018). The genus *Leptobrachella* is now considered to contain 74 species. The genus is widely distributed from southwestern China to northeastern India and Myanmar (Fei et al. 2012; Frost 2019), extending to mainland Indochina, peninsular Malaysia, and the islands of Borneo (Rowley et al. 2016, 2017a; Yang et al. 2016; Yuan et al. 2017; Wang et al. 2018; Nguyen et al. 2018). Currently, 20 species of this genus are known from China. They are: *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. purpuraventra* Wang, Li, Li, Chen & Wang, 2019 and *L. bijie* Wang, Li, Li, Chen & Wang, 2019 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. maoershanensis* (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; Yang et al. 2016, 2018; Yuan et al. 2017; Wang et al. 2018, 2019; Hou et al. 2018; Chen et al. 2018, 2019; Wang et al. 2019; AmphibiaChina 2019).

During a field survey in June 2018 in a montane evergreen forest, Suiyang County, Guizhou Province (Fig. 1), we collected three different species of the family Megophryidae co-occurring in this small-fragmented forest. The specimens could be morphologically separated from one another. Subsequent studies based on morphological and molecular data indicated that two of the three could be classified as *Megophrys minor* Stejneger and *M. spinata* Liu and Hu, while the third population, differing significantly from the other two, was further analyzed via morphological characters. Subsequent 16S rRNA sequences from these specimens revealed that the collection represented distinct evolving lineages and belong to the genus *Lepobrachella*. Combining morphological characters, acoustic data, and molecular divergence, we described the specimens as a new species.
Materials and methods

Sampling

Eight specimens collected from the aforementioned area (Fig. 1) were euthanized with chlorobutanol solution and fixed in 10% formalin for 24 h, and then stored in 75% ethanol. Liver and muscular tissues were taken before fixing and preserved in 95% alcohol at -20 °C. All of the specimens are kept at the College of Life Sciences, Guizhou Normal University (GZNU), Guiyang City, Guizhou Province, China.

DNA Extraction, PCR and sequencing

DNA samples were extracted from muscular tissues with a DNA extraction kit (Tiangen Biotech (Beijing) Co. Ltd). The mitochondrial gene and 16S ribosomal RNA gene (16S rRNA) were sequenced (951bp). The fragmented genes were amplified with primer pairs L3975 (5’-CGCCTGTTCACCAAAACAT-3’) and H4551 (5’-CCGGGTCTGAACTCAGATCACGT-3’) for 16S rRNA (Simon et al. 1994). PCR amplifications were performed in a 20 μl reaction volume with the following cycling conditions: an initial denaturing step at 95 °C for five min; 35 cycles of denaturing at 95 °C for 40 s, annealing at 53 °C for 40 s and extending at 72 °C for 1 min, followed by a final extending

Figure 1. Collection locality (red circle) of Leptobrachella suiyangensis sp. nov. from Suiyang County, Guizhou province, China used in this study.
step of 72 °C for 10 min. PCR products were purified with spin columns. The purified products were sequenced with both forward and reverse primers using a BigDye Terminator Cycle Sequencing Kit according to the guidelines of the manufacturer. The products were sequenced on an ABI Prism 3730 automated DNA sequencer at Shanghai Majorbio Bio-pharm Technology Co. Ltd. All sequences have been deposited in GenBank (Table 1). For molecular analyses, a total of 77 sequences (74 sequences downloaded from GenBank and three our new sequences) from 55 species of the genus Leptobrachella were used, including one undescribed species from China, that is, the populations from Huoqiuba Nature Reserve, Suiyang County, and Guizhou Province. Three species which sequences downloaded from GenBank are used as outgroups (Leptobrachium huashen Fei & Ye, 2005, Leptobrachium cf. chapaense (Bourret, 1937) and Megophrys major Boulenger, 1908 (Chen et al. 2018; Wang et al. 2019; Table 1).

Table 1. Localities and voucher data for all specimens used in this study.

| ID | Species                        | Locality                                      | Voucher no.                 | GenBank no.       |
|----|--------------------------------|-----------------------------------------------|-----------------------------|-------------------|
| 1  | Leptobrachella suiyangensis sp.nov. | Suiyang County, Guizhou, China                | GZNU20180606002             | MK829648          |
| 2  | Leptobrachella suiyangensis sp.nov. | Suiyang County, Guizhou, China                | GZNU20180606005             | MK829649          |
| 3  | Leptobrachella suiyangensis sp.nov. | Suiyang County, Guizhou, China                | GZNU20180606006             | MK829650          |
| 4  | Leptobrachella aerea             | Võlály, Savannakhet, Laos                     | NCSM 76038                  | MH055809          |
| 5  | Leptobrachella aerea             | Phong Nha-Ke Bang, Quang Binh, Vietnam        | RH60165                     | JN848437          |
| 6  | Leptobrachella alpina            | Huangcaoling, Yunnan, China                   | KIZ046816                   | MH055866          |
| 7  | Leptobrachella applebyi          | Song Thanh Nature Reserve, Quang Nam, Vietnam |                         |                  |
| 8  | Leptobrachella balsensis         | Tambunan, Sabah, Borneo, Malaysia             | SP 21604                    | LC056792          |
| 9  | Leptobrachella bidoupensis       | Bidoup, Lam Dong, Vietnam                     | NCSM 77321                  | HQ902883          |
| 10 | Leptobrachella bijie             | Zhaozishan Nature Reserve, Bijie City, Guizhou, China | SYS a007313/CIB110002       | MK41532           |
| 11 | Leptobrachella bijie             | Zhaozishan Nature Reserve, Bijie City, Guizhou, China | SYS a007314                | MK41533           |
| 12 | Leptobrachella botsfordi         | Fansipan, Lao Cai, Vietnam                    | AMS R 176540                | MH055952          |
| 13 | Leptobrachella bourreti          | Sapa, Lao Cai, Vietnam                        | 1999.566                    | KR827860          |
| 14 | Leptobrachella brevicrus         | Gunung Mulu National Park, Sarawak, Malaysia  | UNIMAS 8957                | KJ831303          |
| 15 | Leptobrachella dringi            | Gunung Mulu, Malaysia                         | KUHE:55610                  | AB847553          |
| 16 | Leptobrachella eos               | Boung Tay, Phongsaly, Laos                    | NCSM 80551                  | MH055887          |
| 17 | Leptobrachella eos               | Zhushihve, Yunnan, China                      | SYa003959                   | MH055888          |
| 18 | Leptobrachella firthi            | Ngoc Linh Nature Reserve, Kon Tum, Vietnam    | AMS: R 176506               | JQ732907          |
| 19 | Leptobrachella frietinensis      | Gunung Mulu, Malaysia                         | KUHE55371                   | AB847557          |
| 20 | Leptobrachella gracilis          | Gunung Mulu, Malaysia                         | KUHE55624                   | AB847560          |
| 21 | Leptobrachella hamidi            | Bukit Lanjan, Selangor, Malaysia              | KUHE17545                  | AB809086          |
| 22 | Leptobrachella heteropus         | Larut, Perak, Malaysia                        | KUHE15487                   | AB850453          |
| 23 | Leptobrachella his                | Gia Lai, Vietnam                              | AMS R 176469                | K82024767         |
| 24 | Leptobrachella itokai            | Mulu NP, Sarawak, Borneo, Malaysia            | KUHE 55845                  | LC137802          |
| 25 | Leptobrachella juliandringi      | Mulu NP, Sarawak, Borneo, Malaysia            | KUHE 55333                  | LC056780          |
| 26 | Leptobrachella kajangemensis     | Tiroman, Malaysia                             | LUHC 4431                   | LC200201          |
| 27 | Leptobrachella kecil             | Cameron, Malaysia                             | KUHE 52440                  | LC200204          |
| 28 | Leptobrachella khasiorum         | Khasi Hills, Meghalaya, India                 | SDBDU 2009.329              | KY022303          |
| 29 | Leptobrachella liui              | Wuyi Shan, Fujian, China                      | SYa001597                   | KM014547          |
| 30 | Leptobrachella liui              | Wuyi Shan, Fujian, China                      | ZYCA907                     | MH055908          |
| 31 | Leptobrachella laui              | Shenzhen, Guangdong, China                    | SYa002450                   | MH055904          |
| 32 | Leptobrachella laui              | Shenzhen, Guangdong, China                    | SYa001515                   | KM014545          |
| ID  | Species                           | Locality                              | Voucher no.          | GenBank no.          |
|-----|-----------------------------------|---------------------------------------|----------------------|----------------------|
| 33  | *Leptobrachella macrops*          | Phu Yen, Vietnam                      | ZMMU-A5823           | MG787993             |
| 34  | *Leptobrachella mangshanensis*    | Mangshan, Hunan, China                | MSZT201701           | MG132196             |
| 35  | *Leptobrachella mangshanensis*    | Mangshan, Hunan, China                | MSZT201702           | MG132197             |
| 36  | *Leptobrachella maoershanensis*   | Mao'er Shan, Guangxi, China           | KIZ07614             | MH055927             |
| 37  | *Leptobrachella maoershanensis*   | Mao'er Shan, Guangxi, China           | KIZ027236            | MH055928             |
| 38  | *Leptobrachella marmorata*        | Borneo, Malaysia                      | KUYE53227            | AB909289             |
| 39  | *Leptobrachella maurus*           | Borneo, Malaysia                      | SP21450              | AB847559             |
| 40  | *Leptobrachella melanoleucus*     | Surat Thani, Thailand                 | KUHE:23845           | LC201999             |
| 41  | *Leptobrachella melica*           | Cambodia, Ratanakiri                  | MVZ258198            | HM133600             |
| 42  | *Leptobrachella minimina*         | Doi Chiang Dao, Chiangmai, Thailand   | THNHM04718           | JN848402             |
| 43  | *Leptobrachella minimina*         | Doi Suthep, Thailand                  | KUHE:19201           | LC201981             |
| 44  | *Leptobrachella mjobergi*         | Gading NP, Sarawak, Borneo, Malaysia  | KUHE:47872           | LC056787             |
| 45  | *Leptobrachella nahangensis*      | Na Hang Nature Reserve, Tuyen Quang,  | ROM 7035             | MH055853             |
|     |                                   | Vietnam                               |                      |                      |
| 46  | *Leptobrachella nahangensis*      | Na Hang, Tuyen Quang, Vietnam         | ZMMU-NAP-02259       | MH055854             |
| 47  | *Leptobrachella nyx*              | Ha Giang, Vietnam                     | ROM 36692            | MH055816             |
| 48  | *Leptobrachella oshanensis*       | Emei Shan, Sichuan, China            | KIZ025776            | MH055895             |
| 49  | *Leptobrachella oshanensis*       | Emei Shan, Sichuan, China            | Tissue ID: YPX37492  | MH055896             |
| 50  | *Leptobrachella pallida*          | Vietnam: Lam Dong                     | UNS05511             | KJ350190             |
| 51  | *Leptobrachella parva*            | Mulu National Park, Sarawak, Malaysia | KUHE:55308           | LC056791             |
| 52  | *Leptobrachella petrops*          | Cham Chu Nature Reserve, Tuyen Quang, | VNMN:2016 A.06       | KY459998             |
|     |                                   | Vietnam                               |                      |                      |
| 53  | *Leptobrachella picta*            | Borneo, Malaysia                      | UNIMAS 8705          | KJ831295             |
| 54  | *Leptobrachella phyladis*         | Fansipan, Lao Cai, Vietnam            | ROM 30685            | MH055843             |
| 55  | *Leptobrachella phyladis*         | Sapa, Lao Cai, Vietnam                | ZMMU-A-5222-02262    | MH055844             |
| 56  | *Leptobrachella puhoatensis*      | Pu Hu, Thanh Hoa, Vietnam             | VNMN2016 A.23        | KY849887             |
| 57  | *Leptobrachella purpurea*         | Yingjiang, Yunnan Province, China     | SY006530             | MG520354             |
| 58  | *Leptobrachella purpurea*         | Yingjiang, Yunnan Province, China     | SY006531             | MG520355             |
| 59  | *Leptobrachella purpuravenata*    | Wujing Nature Reserve, Bieje City, Guizhou, China | SY007081 | MK414517             |
|     |                                   |                                       |                      |                      |
| 60  | *Leptobrachella purpuravenata*    | Wujing Nature Reserve, Bieje City, Guizhou, | SY007277/CIB110003  | MK414518             |
|     |                                   | Vietnam                              |                      |                      |
| 61  | *Leptobrachella pyrrohs*          | Lam Dong, Vietnam                     | ZMMU A-5208          | KP017575             |
| 62  | *Leptobrachella sabihomontana*    | Borneo, Malaysia                      | BORNEENSIS 12632     | AB847551             |
| 63  | *Leptobrachella shangleri*        | Guangxi, China                       | NHMG410132           | MK095460             |
| 64  | *Leptobrachella shangleri*        | Guangxi, China                       | NHMG410133           | MK095461             |
| 65  | *Leptobrachella solis*            | Hala-Bala, Thailand                  | KUHE:23261           | LC200207             |
| 66  | *Leptobrachella solis*            | Tam Dao, Vinh Phuc, Vietnam           | ROM 20236            | MH055858             |
| 67  | *Leptobrachella tengchongensis*   | Gaoligong Shan, Yunnan, China        | SY004598             | KU589209             |
| 68  | *Leptobrachella tengchongensis*   | Gaoligong Shan, Yunnan, China        | SY003766             | MH055897             |
| 69  | *Leptobrachella ventripunctatus*  | Zhushih, Yunnan, China               | SY004536             | MH055831             |
| 70  | *Leptobrachella wuhanensis*       | Mr. Wuhiang, Pubei County, Guangxi, China | SY003485 | MH055577             |
|     |                                   |                                       |                      |                      |
| 71  | *Leptobrachella wuhanensis*       | Mr. Wuhiang, Pubei County, Guangxi, China | SY003486 | MH055578             |
| 72  | *Leptobrachella yingjiangensis*   | Yingjiang, Yunnan, China             | SY006533             | MG520350             |
| 73  | *Leptobrachella yingjiangensis*   | Yingjiang, Yunnan, China             | SY006532             | MG520351             |
| 74  | *Leptobrachella yunkaiensis*      | Dawulang Forest Station, Maoming City, Guangdong, China | SY004664 | MH055854             |
| 75  | *Leptobrachella yunkaiensis*      | Dawulang Forest Station, Maoming City, Guangdong, China | SY004664 / | MH055854             |
|     |                                   | Guangdong, China                     | CIB107272            | MH055854             |
| 76  | *Leptobrachella zhangyanpi*       | Chiang Mai, Thailand                 | KIZ07258             | MH055864             |
| 77  | *Leptobrachella zhangyanpi*       | Pang Num Poo, Chiang Mai, Province, Thailand | JK-2013 | JX069799             |
| 78  | *Leptobrachialis huashen*         | Yunnan, China                        | KIZ049025            | KX811931             |
| 79  | *Leptobrachialis cf. chinae*      | Sapa, Lao Cai, Vietnam               | AMS R 171623         | KR018126             |
| 80  | *Megophrys major*                 | Kon Tum, Vietnam                     | AMS R 173820         | KY476313             |
Phylogenetic analyses

All sequences were aligned by MUSCLE v. 3.6 with the default settings (Edgar 2004). Trimming with the gaps partially deleted was performed in MEGA 7.0 (Kumar et al. 2016), while within high variable regions, all gaps were removed.

Phylogenetic trees were constructed with both Maximum Likelihood (ML) and Bayesian Inference (BI). The ML was conducted in IQ-TREE (Nguyen et al. 2015) with 2000 ultrafast bootstrapping (Hoang et al. 2018) and was performed until a correlation coefficient of at least 0.99 was reached. The BI was performed in MrBayes v. 3.2.1 (Ronquist et al. 2012), and the best-fit model was obtained by the Akaike Information Criterion (AIC) computed with PartitionFinder 2 (Lanfear et al. 2016), resulting in the best-fitting nucleotide substitution models of GTR + I + G with for BI and ML analysis. Two independent processes were conducted for 10 million generations, sampling every 1000, with four independent chains and a burn-in of 25%. Convergence was assessed referring to the criteria of all parameters having reached stationarity and having obtained satisfactory effective sample sizes (>200) using Tracer v. 1.6. (Rambaut et al. 2014). Nodes in the trees were considered well supported when Bayesian posterior probabilities (BPP) were $\geq 0.95$ and ML ultrafast bootstrap values (UFB) was $\geq 95\%$ (Chen et al. 2018; Hoang et al. 2018). Uncorrected $p$-distances based on 16S rRNA were calculated in MEGA v. 7.0 (Kumar et al. 2016).

Morphological and morphometric analyses

Morphometric data were taken from eight of most well-preserved adult specimens. Measurements were recorded to the nearest 0.1 mm (Watters et al. 2016) with digital calipers following the methods of Fei et al. (2009) and Rowley et al. (2013). These measurements were as follows:

- **SVL**: snout-vent length (from tip of snout to vent)
- **HDL**: head length (from tip of snout to rear of jaws)
- **HDW**: head width (head width at commissure of jaws)
- **SNT**: snout length (from tip of snout to the anterior corner of the eye)
- **EYE**: eye diameter (diameter of the exposed portion of the eyeballs)
- **IOD**: interorbital distance (minimum distance between upper eyelids)
- **IND**: internasal distance (distance between nares)
- **UEW**: upper eyelid width (measured as the greatest width of the upper eyelid)
- **NEL**: nostril-eyelid length (distance from nostril to eyelid)
- **NSL**: nostril-snout length (distance from nostril to snout)
- **TMP**: tympanum diameter (horizontal diameter of tympanum)
- **TEY**: tympanum-eye distance (distance from anterior edge of tympanum to posterior corner of eye)
- **TIB**: tibia length (distance from knee to heel)
- **ML**: manus length (distance from tip of third digit to proximal edge of inner palmar tubercle)
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LAHL length of the lower arm and hand (distance from tip of the third finger to elbow)
HLL hindlimb length (distance from tip of fourth toe to vent)
FOT foot length (from proximal edge of the inner metatarsal tubercle to the tip of the fourth toe)

Sex was determined by direct observation of calls in life, the presence of internal vocal sac openings, and the presence of eggs in the abdomen through external inspection. Comparative morphological data of *Leptobrachella* species were obtained from the references listed in Table 2. Due to the high likelihood of undiagnosed diversity within the genus (Rowley et al. 2016; Yang et al. 2016), where available, we relied on examination of topotypic material and/or original species descriptions.

| 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. arayai* (Matsui, 1997) | Matsui 1997 |
| 5 | *L. ardens* (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016) | Rowley et al. 2016 |
| 6 | *L. bahenensis* Smith, 1931 | Dring 1983; Eto et al. 2016 |
| 7 | *L. bidonensis* (Rowley, Le, Tran & Hoang, 2011) | Rowley et al. 2011 |
| 8 | *L. bijire* 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. brevicrus* 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. euf* (Ohler, Wollenberg, Grosjean, Hendrix, Vences, Ziegler & Dubois, 2011) | Ohler et al. 2011 |
| 16 | *L. ethers* (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. fusov* 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. heteropys* (Boulenger, 1900) | Boulenger 1900 |
| 23 | *L. ios* (Rowley, Stuart, Neang, Hoang, Dau, Nguyen & Emmert, 2015) | Rowley et al. 2015a |
| 24 | *L. itiokai* Eto, Matsui & Nishikawa, 2016 | Eto et al. 2016 |
| 25 | *L. julandring* Eto, Matsui & Nishikawa, 2015 | Eto et al. 2015 |
| 26 | *L. kajangensis* (Grismer, Grismer & Youmans, 2004) | Grismer et al. 2004 |
| 27 | *L. kalonensis* (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016) | Rowley et al. 2016 |
| 28 | *L. kecil* (Matsui, Belabut, Ahmad & Yong, 2009) | Matsui et al. 2009 |
| 29 | *L. khasiorum* (Das, Tron, Rangad & Hooroo, 2010) | Das et al. 2010 |
| 30 | *L. laternalis* (Anderson, 1871) | Anderson 1871; Hummsoe et al. 2008 |
| 31 | *L. lani* (Sung, Yang & Wang, 2014) | Sung et al. 2014 |
| 32 | *L. liui* (Fei & Ye, 1990) | Fei et al. 2009; Sung et al. 2014 |
| 33 | *L. macrops* (Duong, Do, Ngo, Nguyen & Poyarkov, 2018) | Duong et al. 2018 |
| 34 | *L. maculata* (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016) | Rowley et al. 2016 |
| 35 | *L. manghanensis* (Hou, Zhang, Hu, Li, Shi, Chen, Mo & Wang, 2018) | Hou et al. 2018 |
| 36 | *L. maershanensis* (Yuan, Sun, Chen, Rowley & Che, 2017) | Yuan et al. 2017 |
| 37 | *L. marmorata* (Matsui, Zainudin & Nishikawa, 2014) | Matsui et al. 2014b |
### Results

Phylogenetic trees from Maximum likelihood (ML) and Bayesian inference (BI) were constructed based on DNA sequences of the mitochondrial 16S rRNA gene with a length of 500 bp. The trees present identical topologies (Fig. 2) with the clustered population of *Leptobrachella* from Huoqiuba Nature Reserve, in which *L. alpina* + *L. purpurus* and the population of *Leptobrachella* from Huoqiuba Nature Reserve show relatively high node supporting values (0.68 in BI and 71% in ML) and exhibit a separate evolving lineage. The smallest pairwise genetic divergence between the population from Suiyang County and all other species of the genus *Leptobrachella* is 4.71%. This indicates that there is substantial genetic divergence between the species in *Leptobrachella* and the specimens from Suiyang County, indicating that this new population can be regarded to be a separate lineage and is valid to be described as a new species as below.

| ID | Leptobrachella species                                                                 | Literature obtained                      |
|----|-----------------------------------------------------------------------------------------|------------------------------------------|
| 38 | *L. maura* (Inger, Lakim, Biun & Yambun, 1997)                                          | Inger et al. 1997                         |
| 39 | *L. melanoleuca* (Matsui, 2006)                                                         | Matsui 2006                               |
| 40 | *L. metica* (Rowley, Stuar, Neang & Emmett, 2010)                                       | Rowley et al. 2010b                       |
| 41 | *L. minima* (Taylor, 1962)                                                             | Taylor 1962; Ohler et al. 2011           |
| 42 | *L. mijolbergi* Smith, 1925                                                            | Eto et al. 2015                           |
| 43 | *L. nahanagensis* (Lathrop, Murphy, Orlov & Ho, 1998)                                   | Lathrop et al. 1998                       |
| 44 | *L. natunae* (Günther, 1895)                                                            | Günther 1895                              |
| 45 | *L. nokrekensis* (Mathew & Sen, 2010)                                                   | Mathew and Sen 2010                       |
| 46 | *L. nyx* (Ohler, Wollenberg, Grosjean, Hendrix, Vences, Ziegler & Dubois, 2011)        | Ohler et al. 2011                         |
| 47 | *L. oshanensis* (Liu, 1950)                                                            | Fei et al. 2009                           |
| 48 | *L. pallida* (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016)    | Rowley et al. 2016                       |
| 49 | *L. palmaris* Inger & Stuebing, 1992                                                    | Inger and Stuebing 1992                  |
| 50 | *L. parva* Dring, 1983                                                                 | Dring 1983                                |
| 51 | *L. peladystoides* (Boulenger, 1893)                                                    | Boulenger 1893; Ohler et al. 2011         |
| 52 | *L. petropis* (Rowley, Dau, Hoang, Le, Cutajar & Nguyen, 2017)                          | Rowley et al. 2017a                       |
| 53 | *L. picta* (Malkmus, 1992)                                                             | Malkmus 1992                              |
| 54 | *L. platycephala* (Dehling, 2012)                                                       | Dehling 2012a                             |
| 55 | *L. plusialis* (Ohler, Marquis, Swan & Grosjean, 2000)                                  | Ohler et al. 2000, 2011                  |
| 56 | *L. pahoetheni* (Rowley, Dau & Cao, 2017)                                               | Rowley et al. 2017b                      |
| 57 | *L. perparaventra* Wang, Li, Li, Chen & Wang, 2019                                       | Wang et al. 2019                         |
| 58 | *L. perpurus* (Yang, Zeng & Wang, 2018)                                                  | Yang et al. 2018                         |
| 59 | *L. pyrrhos* (Poyarkov, Rowley, Gogoleva, Vassilieva, Galoyan & Orlov, 2015)            | Poyarkov et al. 2015                     |
| 60 | *L. rouleae* (Nguyen, Poyarkov, Le, Vo, Ninh, Duong, Murphy & Sang, 2018)              | Nguyen et al. 2018                       |
| 61 | *L. salaboniana* (Matsui, Nishikawa & Yambun, 2014)                                     | Matsui et al. 2014a                      |
| 62 | *L. sausaucie* Dring, 1983                                                              | Dring 1983                               |
| 63 | *L. shangsiensis* Chen, Liao, Zhou & Mo, 2019                                           | Chen et al. 2019                          |
| 64 | *L. sola* (Matsui, 2006)                                                                | Matsui 2006                              |
| 65 | *L. songi* (Lathrop, Murphy, Orlov & Ho, 1998)                                          | Lathrop et al. 1998                      |
| 66 | *L. tadangensis* (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016) | Rowley et al. 2016                      |
| 67 | *L. tanndil* (Sengupta, Saito, Laiemngsa, Das & Das, 2010)                              | Sengupta et al. 2010                     |
| 68 | *L. tengchongensis* (Yang, Wang, Chen & Rao, 2016)                                      | Yang et al. 2016                         |
| 69 | *L. tuberosa* (Inger, Orlov & Darevsky, 1999)                                           | Inger et al. 1999                        |
| 70 | *L. ventripunctata* (Fei, Ye & Li, 1990)                                                 | Fei et al. 2009                          |
| 71 | *L. wubuangenonis* Wang, Yang & Wang, 2018                                              | Wang et al. 2018                         |
| 72 | *L. yingjiangensis* (Yang, Zeng & Wang, 2018)                                           | Yang et al. 2018                         |
| 73 | *L. yunkauenensis* Wang, Li, Lyu & Wang, 2018                                            | Wang et al. 2018                         |
| 74 | *L. zhangyapingi* (Jiang, Yan, Suwannapoom, Chomdej & Che, 2013)                        | Jiang et al. 2013                        |
Figure 2. Bayesian inference tree derived from partial DNA sequences of the mitochondrial 16S rRNA gene. Numbers before slashes indicate Bayesian posterior probabilities (displayed >0.60 values), and numbers after slashes are ultrafast bootstrap support for maximum likelihood (2000 replicates) analyses (>60 retained). The symbol “–” represents value below 0.60/60. The scale bar represents 0.05 nucleotide substitutions per site.
**Taxonomic account**

*Leptobrachella suiyangensis* sp. nov.

http://zoobank.org/75EDCF88-0293-40E9-83FE-47785145864C

Table 3, Figs 3, 4

**Type material.** **Holotype.** GZNU20180606007, adult male, collected by Tao Luo (TL hereafter) on 7 June 2018 from the Huoqiuba Nature Reserve (28.4805°N, 107.0764°E, 1501 m. a.s.l.; Fig. 1), Suiyang County, Guizhou Province, China.

**Paratypes.** Five adult males (GZNU20180606002, GZNU20180606005, GZNU20180606006, GZNU20180606008), and three adult females (GZNU20180606001, GZNU20180606003, GZNU20180606004). They were collected from the holotype locality on 6 June 2018.

**Etymology.** The specific epithet “suiyangensis” refers to the name of the holotype locality, Taibai Town in Suiyang County of Guizhou Province, China. We suggest as its English name “Suiyang Leaf-litter Toad,” and its Chinese name as “Sui Yang Zhang Tu Chan (绥阳掌突蟾).”

**Diagnosis.** The specimens were assigned to the genus *Leptobrachella* on the basis of the following characters: (1) small body size; (2) having an elevated inner metacarpal tubercle; (3) having macro-glands on body (including supra-axillary, femoral and ventrolateral glands); (4) lacking vomerine teeth; (5) having small tubercles on eyelids; (6) 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). *Leptobrachella suiyangensis* sp. nov. can be distinguished from its congeners by referring to the following characters: (1) small body size (SVL 28.7–29.7 mm in males, 30.5–33.5 mm in females); (2) dorsal skin shagreened, with some of the granules forming longitudinal short skin ridges; (3) tympanum distinctly discernible, slightly concave, with a deep, black, supratympanic line; (4) ventrolateral glands are distinct, forming a dotted line; (5) dorsal surface shagreened and granular, lacking enlarged tubercles or warts, with some of the granules forming short longitudinal folds; (6) flanks with several distinct and large dark blotches; (7) ventral surface of throat grey-white, and surface of chest and belly yellowish creamy-white with marbled texture or with irregular light brown speckling; (8) supra-axillary, femoral, pectoral and ventrolateral glands are distinctly visible; (9) absence of webbing and lateral fringes on fingers, and toes feature rudimentary webbing and a weak lateral fringes; (10) relatively short hindlimbs (TIB/SVL ratio in males 0.46–0.47); (11) longitudinal ridges under the toes are interrupted at the articulations; (12) relative finger lengths I < II < IV < III, relative toe lengths I < II < V < III < IV; (13) dorsum greyish-brown, with small light-orange granules and distinct darker brown markings scattered with irregular light-orange pigmentation, and bicolored iris, coppery orange on the upper half and silver grey on the lower half.

**Description of the holotype.** GZNU20180606007 (adult male), small body size (SVL 28.7 mm); the head length is slightly larger than the head width (HDL/HDW ratio 1.06); the snout is slightly protruding, projecting beyond the margin of the lower
Leptobrachella suiyangensis sp. nov.

Figure 3. Holotype of *Leptobrachella suiyangensis* sp. nov. (GZNU20180606007) in life. A Dorsal view B Dorsolateral view C Ventral view D Right eye shown iris coloration E Volar view of the left hand F Plantar view of the left foot.

jaw; the nostril is between the snout and the eye (NSL/NEL ratio 0.39); the canthus rostralis is gently rounded; the loreal region is slightly concave; the interorbital space is flat; larger (IOD 2.9 mm) than the upper eyelid (1.6 mm in width), and the internarial distance is 2.8 mm; with vertical pupil; snout length is slightly larger than eye diameter (SNT/EYE ratio 1.71); tympanum is distinct and rounded, its diameter (TMP 2.1 mm) is smaller than that of the eye diameter (EYE 2.4 mm) and longer than the tympanum-eye distance (TMP/TEY ratio 1.91); deep black supratympanic line is present; weakly black supratympanic line exists (Fig. 3C); tympanic rim is distinctly elevated relative to the skin of the temporal region; supratympanic ridge is distinct, extending from the eye to the supra-axillary gland; a few indistinct tubercles present on supratympanic ridge; absent vomerine teeth; vocal sac openings is slit-like, located posterior-laterally on the floor of the mouth close to the margins of the mandible; long and wide tongue, with a small shallow notch at the posterior tip.

The tips of the fingers are rounded, slightly swollen; relative finger lengths are presented as: I < II < IV < III; nuptial pad is absent; absent subarticular tubercles (Fig. 3F); a large, round inner palmar tubercle is distinctly separated from a small, round outer palmar tubercle; finger webbing and dermal fringes absent. Toe tips are similar to those
of the fingers; the relative toe length is presented as: I < II < V < III < IV; absent subarticular tubercles; distinct dermal ridges present under the 3rd to 5th toes; pronounced larger, oval inner metatarsal tubercle, outer metatarsal tubercle is absent; rudimentary toe webbing; weak lateral fringes present on all toes. Tibia is slightly shorter than half of the snout-vent length (TIB/SVL ratio 0.46); tibiotarsal articulation reaches to the anterior eye; heels meet each other when thighs are appressed at right angles referring to the body.

Dorsal skin is shagreened and scattered with fine and rounded granules, some of the granules forming short longitudinal folds; ventral skin smooth; large pectoral gland, elongated oval, 1.5 mm in length; small femoral gland, rounded, 0.7 mm in diameter, situated on the posterovernal surface of the thigh, closer to tibiotarsal articulation than to the vent; risen supra-axillary gland, 1.3 mm in diameter; ventrolateral gland is distinct as small white dots forming an incomplete line (Fig. 3D).

**Measurements of holotype (in mm).** Holotype: SVL 28.7, HDL 9.9, HDW 9.3, SNT 4.1, EYE 2.4, IOD 2.9, INT 2.8, UEW 1.6, NEL 2.8, NSL 1.1, TMP 2.1, TEY 1.1, TIB 13.1, HND 7.2, LAHL 13.4, HLL 43.3, FOT 12.5.

**Coloration of holotype in life.** Dorsal skin purple-brown; brown-purplish with dark-brown marks between the eyes and the scapular region, which are scattered with some deep yellow-orange granules more concentrated on the upper eyelid (Fig. 3C). A dark brown Y-pattern exists between eyes, linked with dark brown W-shaped marks between axillae. Tympanum is light brown-grey; black-brown tubercles present on dorsum of the body and the limb; those on dorsal side are much more distinct and dense; anterior upper lip features distinct blackish-brown patches; transverse dark-brown bars exist on dorsal surface of the limbs two or three (elbow and upper arms are an exception); indistinct black or brown blotches present on the flanks from groin to axilla; elbow and upper arms have no dark bars but with distinct dark-orange coloration; fingers and toes show indistinct brown blotches; a black spot is present on the loreal region; lower edge of the upper drum ridge is prominently black; ventral surface of the throat is grey-white, and surface of chest and belly is yellowish creamy-white, ventral part with distinct or indistinct light brown speckling mixed with marble texture; ventral surface of the thighs is dark grey and scattered with small light white spots. Supra-axillary gland milky yellow; iris is bicolored, coppery orange on the upper half and silver grey on the lower half.

**Coloration of holotype in preservative.** In preservation, there are dark brown marks on the dorsum and flanks; dorsum of the body and hindlimbs are dark brown, while dorsum of the forelimbs is yellowish brown; transverse bars on the limbs become more distinct, and dark-brown patterns, marks and spots on the back are indistinct; ventral surface of the body is yellowish brown with brown marbling on the sides and chest; orange supra-axillary, femoral, pectoral and ventrolateral glands fade to greyish white.

**Variations.** Measurements of the type series are shown in Table 4. Females (mean of SVL (32.0 ± 1.5 mm, n = 3) have larger body size than males (mean of SVL 29.2 ± 0.4 mm, n = 5) (Table 4). In life (Fig. 5), all paratypes match overall characters of the holotype, except the surface of the belly that is scattered with brown speckling
Leptobrachella suiyangensis sp. nov.

Figure 4. Holotype of *Leptobrachella suiyangensis* sp. nov. (GZNU20180606007) in preservative. A Dorsal view B Ventral views C Lateral views.

Table 3. Measurements (in mm) of the type series of *Leptobrachella suiyangensis* sp. nov. (H = holotype, P = paratype, M = male, F = female, other abbreviations defined in text).

| Specimen           | Type status | Sex | SVL | HDL | HDW | SNT | EYE | IOD | IND | UEW | NEL | NSL | TMP | TEY | TIB | HND | LAHL | HLL | FOT |
|--------------------|-------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| GZNU20180606007    | H           | M   | 28.7| 9.9 | 9.3 | 4.1 | 2.4 | 2.9 | 2.8 | 1.6 | 2.8 | 1.1 | 2.1 | 1.1 | 13.1| 7.2 | 13.4 | 43.3| 12.5|
| GZNU20180606008    | P           | M   | 29.2| 10.5| 9.8 | 4.6 | 2.8 | 2.8 | 2.9 | 2.1 | 2.3 | 1.7 | 1.2 | 1.9 | 13.4| 7.0 | 13.2 | 43.4| 12.9|
| GZNU20180606002    | P           | M   | 29.7| 12.1| 10.1| 5.0 | 3.9 | 3.2 | 3.7 | 3.1 | 2.3 | 1.3 | 2.3 | 1.4 | 13.8| 7.1 | 13.3 | 44.4| 12.3|
| GZNU20180606005    | P           | M   | 29.0| 11.8| 10.3| 4.5 | 3.3 | 3.4 | 3.1 | 2.0 | 2.7 | 1.1 | 1.9 | 1.6 | 13.5| 6.5 | 13.4 | 41.8| 12.9|
| GZNU20180606006    | P           | M   | 29.2| 11.4| 10.4| 4.0 | 3.8 | 3.2 | 3.2 | 2.6 | 2.6 | 2.2 | 1.8 | 1.3 | 13.6| 7.4 | 13.3 | 42.8| 12.6|
| GZNU20180606001    | P           | F   | 32.0| 12.6| 10.7| 4.7 | 3.7 | 3.5 | 3.5 | 3.0 | 2.4 | 1.3 | 2.6 | 1.7 | 15.2| 7.1 | 13.4 | 44.7| 13.9|
| GZNU20180606003    | P           | F   | 30.5| 10.3| 10.9| 4.7 | 3.7 | 3.1 | 3.1 | 2.2 | 2.3 | 1.5 | 3.5 | 1.4 | 15.2| 7.4 | 13.8 | 45.3| 16.6|
| GZNU20180606004    | P           | F   | 33.5| 13.1| 12.1| 4.9 | 3.6 | 3.1 | 3.6 | 2.8 | 3.1 | 1.8 | 3.8 | 1.7 | 17.4| 8.1 | 16.7 | 53.8| 14.4|
Table 4. Measurements (in mm), and body proportions of *Leptobrachella suiyangensis* sp. nov. from Suiyang County, Guizhou Province, China.

| Measurements | Males Range (mean ± SD), n = 5 | Females Range (mean ± SD), n = 3 |
|--------------|---------------------------------|----------------------------------|
| SVL          | 28.7–29.7 (29.2 ± 0.4)          | 30.5–33.5 (32.0 ± 1.5)           |
| HDL          | 9.9–12.1 (11.1 ± 0.9)           | 10.3–13.1 (12.0 ± 1.5)           |
| HDW          | 9.3–10.4 (10.0 ± 0.4)           | 10.7–12.1 (11.2 ± 0.8)           |
| SNT          | 4.0–5.0 (4.4 ± 0.4)             | 4.7–4.9 (4.8 ± 0.1)              |
| EYE          | 2.4–3.9 (3.2 ± 0.6)             | 3.6–3.7 (3.7 ± 0.1)              |
| IOD          | 2.8–3.4 (3.1 ± 0.2)             | 3.1–3.5 (3.2 ± 0.2)              |
| INT          | 2.8–3.7 (3.1 ± 0.4)             | 3.1–3.6 (3.4 ± 0.3)              |
| UEW          | 1.6–3.1 (2.3 ± 0.6)             | 2.2–3.0 (2.7 ± 0.4)              |
| NEL          | 2.3–2.8 (2.5 ± 0.2)             | 2.3–3.1 (2.6 ± 0.4)              |
| NSL          | 1.1–2.2 (1.5 ± 0.5)             | 1.3–1.8 (1.5 ± 0.3)              |
| TMP          | 1.2–2.3 (1.9 ± 0.4)             | 2.6–3.8 (3.3 ± 0.6)              |
| TEY          | 1.1–1.9 (1.5 ± 0.3)             | 1.4–1.7 (1.6 ± 0.2)              |
| TIB          | 13.1–13.8 (13.5 ± 0.3)          | 15.2–17.4 (15.9 ± 1.3)           |
| HND          | 6.5–7.4 (7.0 ± 0.3)             | 7.1–8.1 (7.5 ± 0.5)              |
| LAHL         | 13.2–13.4 (13.3 ± 0.1)          | 13.4–16.7 (14.6 ± 1.8)           |
| HLL          | 41.8–44.4 (43.1 ± 0.9)          | 44.7–53.8 (47.9 ± 5.1)           |
| FOT          | 12.3–12.9 (12.6 ± 0.3)          | 13.9–16.6 (15.0 ± 1.4)           |
| HDL/HDW      | 1.06–1.20 (1.12 ± 0.06)         | 0.90–1.20 (1.07 ± 0.15)          |
| HDL/TLV      | 0.34–0.41 (0.38 ± 0.03)         | 0.30–0.40 (0.37 ± 0.06)          |
| SNT/HDL      | 0.35–0.44 (0.40 ± 0.03)         | 0.40–0.50 (0.43 ± 0.06)          |
| SNT/EYE      | 1.05–1.71 (1.41 ± 0.27)         | 1.30–1.40 (1.33 ± 0.06)          |
| EYE/TMP      | 1.14–2.33 (1.80 ± 0.45)         | 1.60–1.40 (1.17 ± 0.21)          |
| EYE/SNT      | 0.59–0.95 (0.73 ± 0.15)         | 0.70–0.80 (0.77 ± 0.06)          |
| TMP/EYE      | 0.43–0.88 (0.59 ± 0.18)         | 0.70–1.10 (0.93 ± 0.21)          |
| TIB/SVL      | 0.46–0.47 (0.46 ± 0.01)         | 0.50                             |
| LAHL/SVL     | 0.45–0.47 (0.46 ± 0.01)         | 0.40–0.50 (0.47 ± 0.06)          |
| HLL/SVL      | 1.44–1.51 (1.48 ± 0.03)         | 1.40–1.60 (1.50 ± 0.10)          |
| TIB/HLL      | 0.30–0.32 (0.31 ± 0.01)         | 0.30                             |

in the holotype (that for females is more distinct; GZNU20180606001). Under the condition of preservation, however, some specimens become slightly darker brown compared to the holotype.
**Distribution and habitats.** Currently, *Leptobrachella suiyangensis* sp. nov. is known only from its holotype locality, Huoqiuba Nature Reserve, Suiyang County, Guizhou Province, China (Fig. 1). The specimens were collected in a stream (ca 1.5 m in width and ca 10 cm in depth) and from nearby well-preserved bamboo forests (1501 m a.s.l.). During June, males were calling from under bamboo leaves; others perch on or under rocks by the side of the stream.

**Comparisons**

*Leptobrachella suiyangensis* sp. nov. differs from all other species of *Leptobrachella* based on morphological and molecular evidence. Phylogenetically, *L. suiyangensis* sp. nov., *L. alpina* and *L. purpurus* form a clade. Genetically, among this clade, the smallest genetic distance, at 5.49%, is between *L. suiyangensis* sp. nov. and *L. alpina*, and the largest genetic distance is 6.27% (*L. suiyangensis* sp. nov. and *L. purpurus*). Morphologically, the new species can be distinguished from *L. alpina* by having a larger body size of males (28.7–29.7 mm vs 24.0–26.4 mm); having narrower lateral fringes on the toes of the male (vs wide in males); dorsum purple-brown to dark purple-brown or grey-purple ground colour; ventral yellowish creamy-white with marbled texture on the chest and belly or with irregular light-brown speckling (vs almost uniformly gray-brown on dorsal part, ventral nearly immaculately creamy white, brown speckling on margins); ventrolateral glands are characterized by small white dots forming an incomplete line (vs small white dots forming a complete line longitudinally); shoulder-gland is orange-yellow (vs white, around gland); head length greater than head width, HDL/HDW ratio 1.12 (vs head length equal to head width, HDL/HDW ratio 1.00). The new species can be distinguished from *L. purpurus* by body size of males (28.7–29.7 mm vs 25.0–27.5 mm); having narrow lateral fringes on the toes of males (vs wide in males); dorsum purple-brown to dark purple-brown or grey-purple ground color, ventral yellowish creamy-white with marbled texture on the chest and belly or with irregular light-brown speckling (vs dorsum coloration purplish brown, ventral side dull white with an indistinct grey dusting); throat immaculate gray (vs throat immaculate pinkish; almost dark orange-yellow on the upper arm (vs upper arms with distinct coppery orange coloration); dark bars on dorsal surface of tibia and tarsus very narrow, especially those on dorsal skin of tarsus (vs relatively broader dark bars on dorsal surface of tibia and tarsus); tibiotarsal articulation reaches to the anterior eye (vs tibiotarsal articulation reaches to posterior corner of the eye); relative length of fingers I < II < IV < III (vs I = II = IV < III).

Compared with the 26 known congeners in the genus *Leptobrachella* found south of the Isthmus of Kra, referring to the presence or absence of supra-axillary and ventrolateral glands, *L. suiyangensis* 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. maura*, *L. melanoleuca*, *L. picta*, *L. platycephala*, *L. sabahmontana* and *L. sola*, all of which are lack of supra-axillary and ventrolateral glands (Dubois et al. 2010;
Dehling and Matsui 2013; Matsui et al. 2014). As for the comparison referring to the body size, the new species shows a significantly larger body size (SVL, 28.7–29.7 mm in males) than *L. baluensis* (14.9–15.9 mm in males), *L. brevicrus* (17.1–17.8 mm in males), *L. itiokai* (15.2–16.7 mm in males), *L. juliandringi* (17.0–17.2 mm in males), *L. mjobergi* (15.7–19.0 mm in males), *L. natuna* (17.6 mm in one adult male), *L. parva* (15.0–16.9 mm in males), *L. palmata* (14.4–16.8 mm in males), *L. serasan* (16.9 mm in one adult male), and *Leptobrachella* sp. 3 “baluensis” (15.0–16.0 mm in males).

From the remaining 48 known congeners in the genus *Leptobrachella* found north of the Isthmus of Kra (Table 5) with SVL 28.7–29.7 mm in males and SVL 30.5–33.5 mm in females, *L. suiyangensis* sp. nov. can be distinguished 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 one female), *L. lateralis* (36.6 mm in females), *L. nahangensis* (40.8 mm in one male), *L. nyx* (37.0–41.0 mm in females), *L. pyrrhops* (30.8–34.3 mm in males), *L. sungi* (48.3–52.7 mm in males and 56.7–58.9 mm in females), *L. tamdil* (32.3 mm in males) and *L. zhangyingi* (45.8–52.5 mm in males), and from the smaller *L. alpina* (24.0–26.4 mm in males and 29.2–29.4 mm in females), *L. khasiorum* (24.5–27.3 mm in males), *L. laui* (24.8–26.7 mm in males), *L. maculosa* (24.2–26.6 mm in males and 27.0 mm in one female), *L. melica* (19.5–22.7 mm in males), *L. maoershanensis* (29.1 mm in one female), *L. petrops* (23.6–27.6 mm in males), *L. pluvialis* (21.3–22.3 mm in males), *L. purpurus* (25.0–27.5 mm in males), *L. rowleyae* (23.1–28.1 mm in males and 27.0–27.8 mm in females), *L. tengchongensis* (23.9–26.0 mm in males and 28.8–28.9 mm in females) and *L. yingjiangensis* (25.7–27.6 mm in males).

In having irregular, light-brown speckling on the flanks, the new species differs from *L. aerea*, *L. botsfordi*, *L. crocea*, *L. firthi*, *L. isos*, *L. pallida*, *L. petrops* and *L. tuberosa*, all of which lack distinct irregular, light-brown speckling on the flanks. By having rudimentary webbing on the toes, the new species differs from *L. kalonensis*, *L. oshanensis*, *L. pallida*, *L. petrops*, and *L. tadungensis*, all of which lack webbing on the toes; and from *L. pelodytoides*, which has wide webbing on the toes. By having narrow lateral fringes on toes, the new species differs from *L. ardens*, *L. eos*, *L. firthi*, *L. isos*, *L. khasiorum*, *L. laui*, *L. liui*, *L. purpurus*, *L. tamdil*, *L. yingjiangensis* and *L. yunkaisiensis*, all of which have wide lateral fringes on the toes; from *L. bidoupensis*, *L. bourreti*, *L. fuliginosa* and *L. maoershanensis*, all of which have weak lateral fringes on the toes; and from *L. crocea*, *L. kalonensis*, *L. lateralis*, *L. macrops*, *L. minima*, *L. nyx*, *L. oshanensis*, *L. pallida*, *L. pyrrhops*, *L. tadungensis*, *L. tuberosa*, and *L. ventripunctata*, all of which lack lateral fringes on the toes. By having dorsal surface shagreened with small granules, and in lacking enlarge tubercles or warts, the new species differs from *L. applebyi*, *L. bidoupensis*, *L. kalonensis*, *L. melica*, *L. minima*, *L. nahangensis*, *L. shangsiensis* and *L. tadungensis*, all of which have the dorsum smooth, and *L. alpina* (dorsum smooth, some with small warts), *L. fuliginosa* (dorsum smooth with fine tubercles), *L. laui* (dorsum with round granular tubercle, lacking skin ridges), *L. liui* (dorsum with round tubercles), *L. macrops* (dorsum roughly granular with large tubercles), *L. maoershanensis*
Table 5. Selected diagnostic characters for species described herein and species in the genus *Leptobrachella* occurring north of the Isthmus of Kra (modified from Rowley et al. 2017; Yuan et al. 2017; Hou et al. 2018; Wang et al. 2018).

| ID | Species                | Males SVL (mm) | Black spots on flanks | Toes webbing | Fringes on toes | Ventral coloration                                                                 | Dorsal skin texture                          |
|----|------------------------|----------------|-----------------------|---------------|-----------------|-------------------------------------------------------------------------------------|----------------------------------------------|
| 1  | *L. suiyangensis* sp. nov. | 28.7–29.7      | Yes                    | Rudimentary   | Narrow          | Yellowish creamy-white with marble texture chest and belly or with irregular light brown speckling | Shagreen with small granules                  |
| 2  | *L. aerata*             | 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            | Narrow          | Reddish brown with white specking                                                   | Smooth- finely shagreened                    |
| 6  | *L. bidoupensis*        | 18.5–25.4      | Yes                    | Rudimentary   | Weak            | Reddish brown with white speckling                                                   | Smooth                                       |
| 7  | *L. bosfordi*           | 29.1–32.6      | No                     | Rudimentary   | Weak            | Reddish brown with white speckling                                                   | Shagreen                                    |
| 8  | *L. bourreti*           | 28.0–36.2      | Yes                    | Rudimentary   | Wide            | Creamy white                                                                         | Relatively smooth, some with small warts     |
| 9  | *L. crocea*             | 22.2–27.3      | No                     | Rudimentary   | No              | Bright orange                                                                       | Highly tuberculate                           |
| 10 | *L. eolus*              | 33.1–34.7      | No                     | Rudimentary   | Wide            | Creamy white                                                                         | Shagreen                                    |
| 11 | *L. fisthi*             | 26.4–29.2      | No                     | Rudimentary   | Wide in males   | Creamy white                                                                         | Shagreened with fine tubercles              |
| 12 | *L. fuliginosa*         | 28.2–30.0      | Yes                    | Rudimentary   | Weak            | White with brown dusting                                                              | Nearly smooth, few tubercles                 |
| 13 | *L. isos*               | 23.7–27.9      | No                     | Rudimentary   | Wide in males   | Creamy white with white dusting on margins                                           | Mostly smooth, females more tuberculate      |
| 14 | *L. kalomensis*         | 25.8–30.6      | Yes                    | No            | No              | Pale, speckled brown                                                                 | Smooth                                       |
| 15 | *L. khaoirum*           | 24.5–27.3      | Yes                    | Rudimentary   | Wide            | Creamy white                                                                         | Isolated, scattered tubercles               |
| 16 | *L. latensii*           | 26.9–28.3      | Yes                    | Rudimentary   | No              | Creamy white                                                                         | Roughly granular                            |
| 17 | *L. laui*               | 24.8–26.7      | Yes                    | Rudimentary   | Wide            | Creamy white with dark brown dusting on margins                                      | Round granular tubercles                    |
| 18 | *L. liui*               | 23.0–28.7      | Yes                    | Rudimentary   | Wide            | Creamy white with dark brown spots on chest and belly                               | Round granular tubercles with glandular folds |
| 19 | *L. macrops*            | 28.0–29.3      | Yes                    | Rudimentary   | No              | Greyish-violet with white speckling                                                  | Roughly granular with larger tubercles      |
| 20 | *L. maculosa*           | 24.2–26.6      | Yes                    | No            | No              | Brown, less white speckling                                                           | Dorsum mostly smooth with numerous tiny tubercles |
| 21 | *L. manghanensis*       | 22.2–27.8      | Yes                    | Rudimentary   | Weak            | White speckles on throat and belly                                                   | Nearly smooth                                |
| 22 | *L. maershanensis*      | 25.2–30.4      | Yes                    | Rudimentary   | Narrow          | Creamy white chest and belly with irregular black spots                              | Longitudinal folds                          |
| 23 | *L. melica*             | 19.5–22.7      | Yes                    | Rudimentary   | No              | Reddish brown with white speckling                                                   | Smooth                                       |
| 24 | *L. minima*             | 25.7–31.4      | Yes                    | Rudimentary   | No              | Creamy white                                                                         | Smooth                                       |
| 25 | *L. nabangensis*        | 40.8           | Yes                    | Rudimentary   | No              | Creamy white with light specking on throat and chest                                | Smooth                                       |
| No. | Species/Chinese Name | Length | Sex | Rudimentary | No Markings or Only Small Light Grey Spots | Color | Description |
|-----|---------------------|--------|-----|-------------|-----------------------------------------|-------|-------------|
| 26  | L. nokrekensis       | 26.0–33.0 | Yes | Rudimentary | Unknown | Creamy white with white with brown margins | Tubercles and longitudinal folds |
| 27  | L. nyx               | 26.7–32.6 | Yes | Rudimentary | No | Creamy white with white with brown margins | Rounded tubercles |
| 28  | L. oshanensis        | 26.6–30.7 | Yes | No | No | Whitish with no markings or only small, light grey spots | Smooth with few glandular ridges |
| 29  | L. pallida           | 24.5–27.7 | No | No | No | Creamy white with white speckling | Tuberculate |
| 30  | L. peledytoides      | 27.5–32.3 | Yes | Wide | Narrow | Whitish | Small, smooth warts |
| 31  | L. petrops           | 23.6–27.6 | Yes | No | No | Immaculate creamy white | Highly tuberculate |
| 32  | L. pluvialis         | 21.3–22.3 | Yes | No | No | Dirtly white with dark brown marbling | Smooth, flattened tubercles on flanks |
| 33  | L. pubnataenensis    | 24.2–28.1 | Yes | Rudimentary | Narrow | Reddish brown with white dusting | Longitudinal skin ridges |
| 34  | L. purpurus          | 25.0–27.5 | Yes | Rudimentary | Wide | Dull white with indistinct grey dusting | Shagreen with small tubercles |
| 35  | L. pyrrophi         | 30.8–34.3 | Yes | Rudimentary | No | Reddish brown with white speckling | Slightly shagreened |
| 36  | L. rowleyae          | 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 |
| 37  | L. sungi             | 48.3–52.7 | No or small | Wide | Weak | White | Granular |
| 38  | L. shangsiensis      | 24.9–29.4 | Yes | Narrow | Narrow | Yellowish creamy-white with marble texture | Smooth |
| 39  | L. tudungensis       | 23.3–28.2 | Yes | No | No | Reddish brown with white speckling | Smooth |
| 40  | L. tamdil           | 32.3     | Yes | Wide | Wide | White | Weakly tuberculate |
| 41  | L. tenghongensis     | 23.9–26.0 | Yes | Rudimentary | Narrow | White with dark brown blotches | Shagreened with small tubercles |
| 42  | L. tuberosa          | 24.4–29.5 | No | Rudimentary | No | White with small grey spots/streaks | Highly tuberculate |
| 43  | L. ventripunctata    | 25.5–28.0 | Yes | Rudimentary | No | Chest and belly with dark brown spots | Longitudinal skin ridges |
| 44  | L. wauhuangmontis    | 25.6–30.0 | Yes | Rudimentary | Narrow | Greyish white mixed by tiny white and black dots | Rough, scattered with dense conical tubercles |
| 45  | L. yingjiangensis    | 25.7–27.6 | Yes | Rudimentary | Wide | Creamy white with dark brown flecks on chest and margins | Shagreened with small tubercles |
| 46  | L. yunkaiensis       | 25.9–29.3 | Yes | Rudimentary | Wide | Belly pink with distinct or indistinct speckling | Shagreened with short skin ridges and raised warts |
| 47  | L. zhangyapingi      | 45.8–52.5 | No | Rudimentary | Wide | Creamy-white with white with brown margins | Mostly smooth with distinct tubercules |
| 48  | L. bijie             | 29.0–30.4 | Yes | Rudimentary | Narrow | White with distinct nebulous greyish speckling on chest and ventrolateral flanks | Shagreened and granular |
| 49  | L. purpuraventra     | 27.3–29.8 | Yes | Rudimentary | Narrow | Grey purple with distinct nebulous greyish speckling on chest and ventrolateral flanks | Shagreened and granular |
(dorsum smooth with small warts), *L. nokrekensis* (dorsum tubercles and longitudinal folds), *L. pelodytoides* (dorsum with small, smooth warts), *L. puhuatensis* (dorsum longitudinal skin ridges), *L. tuberosa* (dorsum highly tuberculate), *L. yunkaiensis* (dorsum with raised warts), *L. wuhuangmontis* (dorsum rough with conical tubercles), and *L. bijie* and *L. purpuraventra* (dorsum shagreened and granular). By the yellowish creamy-white with marbled chest and belly or with irregular light-brown speckling, the new species differs from *L. alpinus*, *L. applebyi*, *L. ardens*, *L. bidoupensis*, *L. botsfordi* and *L. pyrrhops* (ventral reddish brown with white speckling), *L. aerea* (ventral nearly immaculate creamy-white with brown specking on margins), *L. bijie* (ventral white with distinct nebulous greyish speckling on chest and ventrolateral flanks), *L. crocea* (ventral bright orange), *L. khasiorum*, *L. nokrekensis* and *L. yingjiangensis* (ventral creamy white), *L. macrops* (ventral greyish-violet with white speckling), *L. puhuatensis* (ventral reddish-brown with white dusting), *L. purpurus* (ventral dull white with indistinct grey dusting), *L. purpuraventra* (ventral grey-purple with distinct nebulous greyish speckling on the chest and ventrolateral flanks), *L. tuberosa* (ventral white with small grey spots and streaks), *L. ventripunctata* (chest and belly with large dark brown spots), *L. wuhuangmontis* (ventral greyish white), and *L. yunkaiensis* (belly pink with speckling). A comparative morphological data (selection) of *Leptobrachella suiyangensis* sp. nov. and 48 recognized *Leptobrachella* species occurring north of the Isthmus of Kra are listed in Table 5.

**Discussion**

Phylogenetic analyses based on mitochondrial DNA and nuclear DNA all suggested that the new species belongs to *Leptobrachella* but is separate from its congeners. Genetic distance of the 16S rRNA gene between the new species and its closely related species (*L. bijie*, *L. purpuraventra*, *L. alpina* and *L. purpurus*) was 4.71–6.27%, within the expected range of interspecific divergences in amphibians (Fouquet et al. 2007), and this genetic distance is much higher than between many sister species, of which, most species have been completely recognized as valid species. For example, in *Leptobrachella*, the *p*-distance = 2.35% between *L. purpurus* and *L. alpina*. Finally, a series of morphological characters were found to be different between the new species and its congeners. All in all, multiple pieces of evidence support the validity of the new species.

The new species described in this study increases the number of *Leptobrachella* to 75, with 21 recorded from China (Fei et al. 2012; Sung et al. 2014; Yang et al. 2016, 2018; Yuan et al. 2017; Hou et al. 2018; Wang et al. 2018, 2019; Chen et al. 2018, 2019; Frost 2019). Before the description of the new species herein, only 12 species were recorded from southwest China. This highlights the underestimation of the species diversity of the genus *Leptobrachella*. Additional field surveys are required to understand the true diversity of amphibians in this genus, which will be useful for conservation strategies.
Studies on the taxonomy and phylogeny of the genus *Leptobrachella* were difficult to perform because of the morphological conservativeness of the species; in the field, many species appear to be very similar morphologically, and there exist sympatric species. This likely hinders our understanding of these cryptic species (Ohler et al. 2010; Sung et al. 2014; Yang et al. 2016, 2018; Yuan et al. 2017; Hou et al. 2018; Wang et al. 2018, 2019; Chen et al. 2019). The high species diversity and the degree of endemism indicated that the speciation pattern and sympatry mechanism of species in the genus *Leptobrachella* also need additional investigation.

Currently, to our knowledge, *L. suiyangensis* sp. nov. is restricted to rocky streams in bamboo forests. However, the type locality of *L. suiyangensis* sp. nov. has faced habitat loss and human disturbance, such as artificial grazing and herb collection, which could possibly threaten this species. *Leptobrachella suiyangensis* sp. nov. is range-restricted to Kuankuoshui National Nature Reserve, which borders the nearby Huoqiuba Nature Reserve and is in the eastern Ta-lou Mountains. These areas feature subtropical evergreen broad-leaved forest and evergreen deciduous broad-leaved mixed forest. Thus, it is likely that other populations of *L. suiyangensis* sp. nov. may be discovered in the Kuankuoshui Nature Reserve in the near future.

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