

# How little is known about “the little brown frogs”: description of three new species of the genus *Leptobrachella* (Anura: Megophryidae) from Yunnan Province, China

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## ABSTRACT

Asian leaf-litter toads of the genus *Leptobrachella* represent a great anuran diversification in Asia. Previous studies have suggested that the diversity of this genus is still underestimated. During herpetological surveys from 2013 to 2018, a series of *Leptobrachella* specimens were collected from the international border areas in the southern and western parts of Yunnan Province, China. Subsequent analyses based on morphological and molecular data revealed three distinct and previously unknown lineages, which we formally describe as

three new species herein. Among them, we describe a new species that occurs at the highest known elevation for *Leptobrachella* in China. Four species of *Leptobrachella*, including two new species, are found in the same reserve. Furthermore, our results

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suggest that the population from Longchuan County, Yunnan, may represent an additional new species of *Leptobrachella*, although we tentatively assigned it to *Leptobrachella* cf. *yingjiangensis* due to the small sample size examined. Lastly, we provide the first description of females of *L. yingjiangensis*. Our results further highlight that both micro-endemism and sympatric distributions of species are common patterns in *Leptobrachella*, that contribute to taxonomic and conservation challenges in these frogs. We provide an identification key for *Leptobrachella* known to occur in Yunnan. Given the lack of knowledge on species diversity of *Leptobrachella* along international border areas, we recommend that future studies include trans-boundary collaborative surveys.

**Keywords:** Biodiversity hotspot; Conservation; Cryptic diversity; Micro-endemism; National border; Speciation; Taxonomy

## INTRODUCTION

Cryptic diversity is a hot topic in biodiversity and evolutionary research. In amphibians, cryptic diversity is generally defined as the presence of evolutionarily distinct lineages with conservative morphology (Bickford et al., 2007). Lineages that match such a definition have been observed in different groups of anurans and a part of them are often referred to colloquially as “little brown frogs”, indicating groups of inconspicuous frogs harboring high cryptic diversity (Ohler et al., 2011; Schick et al., 2010). The existence of “little brown frogs” poses major challenges not only to taxonomic studies, but also to conservation and legislation that depend on accurate taxonomic identifications and geographic ranges for implementing appropriate biodiversity management. The representative example of the “little brown frogs” in Asia is the leaf-litter frogs of the genus *Leptobrachella* Smith, 1925, which represent one of the most species-rich, yet taxonomically most troubling, groups of Asian anurans.

Until recently, most species of *Leptobrachella* were regarded as members of the genus *Leptolalax* Dubois, 1980 (Chen et al., 2018). These species are generally small-sized, forest-floor specialists that inhabit montane evergreen forest throughout tropical and subtropical Asia, ranging from the eastern Himalayas to the islands of Southeast Asia (Frost, 2019). With a total of 75 species recognized to date, species diversity within the genus has increased rapidly in recent years. More than 60% of recognized species were described in the last two decades (e.g., Chen et al., 2018; Eto et al., 2018; Frost, 2019; Hou et al., 2018; Nguyen et al., 2018; Poyarkov et al., 2015; Rowley et al., 2015a, 2015b, 2016, 2017; Van Duong et al., 2018; Yuan et al., 2017), which is attributed to intensified survey efforts and use of more integrative taxonomic approaches. In China alone, 11 out of

17 species of *Leptobrachella* have been described in the past five years, with most recorded from southern and southwestern regions (Frost, 2019; Wang et al., 2019).

A recent large-scale phylogenetic study has suggested that the diversity of *Leptobrachella* in China is still underestimated, and many of the previously believed widespread taxa need further examination (Chen et al., 2018). During herpetological surveys in Yunnan Province, China, from 2013 to 2018, a number of *Leptobrachella* specimens were collected, especially from the international border areas in the southern and western parts of the province (Figure 1). Subsequent genetic analyses and morphological comparisons revealed that three of the sampled populations each represent a distinct evolutionary lineage that warrants formal recognition as a unique species.

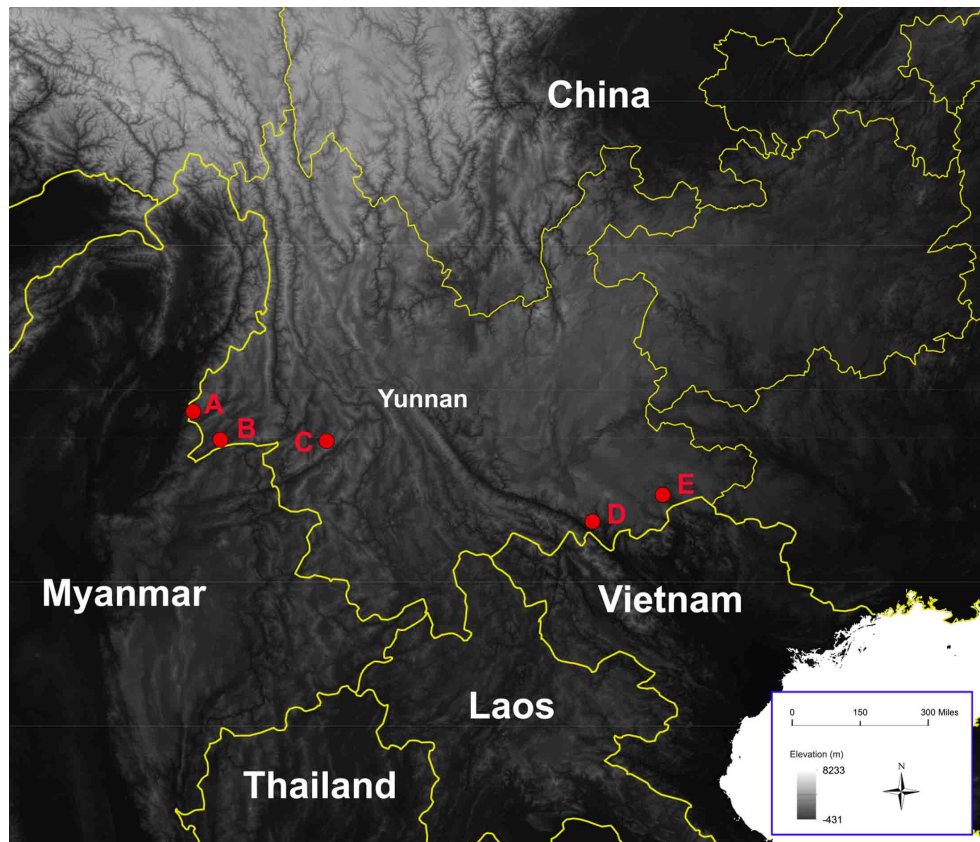
## MATERIALS AND METHODS

### Sampling

32 specimens were collected between 2013 and 2018 from 5 Nature Reserves along the international border areas in Yunnan Province, China (Figure 1). All specimens were euthanized and fixed in 10% formalin, and subsequently stored in 75% ethanol after 24 hours. Liver and muscle tissues were taken from specimens before fixation and stored in 95% ethanol at  $-80^{\circ}\text{C}$ . These newly collected specimens were deposited at the Museum of the Kunming Institute of Zoology (KIZ), Chinese Academy of Sciences (CAS), Kunming, China. For molecular analysis, a total of 95 samples, including 32 newly collected specimens, were used as ingroups in this study (Supplementary Table S1). The final dataset contained 63 recognized species of *Leptobrachella* available in GenBank. *Pelobates fuscus*, *Megophrys montana*, and *Leptobrachium huashen* were chosen as outgroups based on previous phylogenetic studies (Chen et al., 2018; Yang et al., 2018). The protocols (No. SYDW-20130814-71) of the Animal Care and Ethics Committee at the Kunming Institute of Zoology were followed for the proper treatments of animals.

### DNA extraction, polymerase chain reaction (PCR), and sequencing

DNA was extracted from tissue samples using a standard phenol-chloroform extraction protocol (Sambrook et al., 1989). Mitochondrial fragments of the 16S ribosomal RNA gene (16S rRNA) were amplified and sequenced using the primer pairs 16S rRNA-F (CGCCTGTTTAYCAAAAACAT) and 16S rRNA-R (CCGGTYTGAAGTCAATCAGT) (Bossuyt & Milinkovitch, 2000). PCR amplification was performed in a 25 $\mu\text{L}$  reaction volume with an initial denaturation step at  $95^{\circ}\text{C}$  for 5 min, 35 cycles of denaturation at  $95^{\circ}\text{C}$  for 1 min, annealing at  $55^{\circ}\text{C}$  for 1 min, extension at  $72^{\circ}\text{C}$  for 1 min, and final extension at  $72^{\circ}\text{C}$  for 10 min. The PCR products were purified with a Gel Extraction Mini Kit (Watson Biotechnologies, China), and then sequenced in both directions with a BigDye Terminator Cycle Sequencing Kit (v3.1, Applied Biosystems, USA) using an ABI PRISM 3730



**Figure 1** Sampling localities of *Leptobranchella* used in this study

A: Jinzhuzhai Village, Yingjiang, Yunnan, China; B: Gongdong, Longchuan, Yunnan, China; C: Daxueshan Nature Reserve, Lincang, Yunnan, China; D: Daweishan, Pingbian, Yunnan, China; E: Xiaoqiaogou Nature Reserve, Wenshan, Yunnan, China.

DNA Analyzer (Applied Biosystems, USA). New sequences were deposited in GenBank under accession Nos. MT302618–MT302642. (Supplementary Table S1).

### Phylogenetic analyses

The newly obtained nucleotide sequences were first assembled and edited using dnastar lasergene 7.1. New sequences incorporated with homologous data retrieved from GenBank were aligned using MUSCLE 3.8 (Edgar, 2004), and then inspected by eye for accuracy and trimmed to minimize missing characters in mega6 (Tamura et al., 2013).

Phylogenetic reconstruction was achieved using Bayesian inference (BI) and maximum likelihood (ML) methods based on the 16S rRNA gene. The best-fit model of evolution for 16S rRNA was determined using the Bayesian information criterion (BIC; Posada, 2008) by jModelTest 2.1.7 (Darriba et al., 2012). The BI analyses were conducted using GTR+I+G model with 50 million generations and sampled every 1 000 generations. Convergence was assessed in Tracer 1.5 (Rambaut & Drummond, 2009) based on having a average standard deviation of split frequencies less than 0.01 and effective sample size (ESS) values great than 200. We excluded the first 25% of trees as burn-in before the log-likelihood scores stabilized. Maximum likelihood analyses

were performed using RAxML-HPC BlackBox 8.2.10 (Stamatakis, 2014) on the CIPRES Science Gateway (Miller et al., 2010). The analyses used the proportion of invariable sites estimated from the data and 1 000 bootstrap pseudoreplicates under the GTR+G model. Nodes in the trees were considered well-supported when Bayesian posterior probabilities (PP) were  $\geq 0.95$  and ML bootstrap support (BS) was  $\geq 70\%$  (Douady et al., 2003; Huelsenbeck and Rannala, 2004).

### Morphology and morphometrics

Measurements were recorded to the nearest 0.1 mm with digital calipers by Kai Xu following Fei et al. (2009) and Rowley et al. (2013). Measurements included: SVL: Snout-vent length; HDL: Head length from tip of snout to jaw angle; HDW: Head width at commissure of jaws; SNT: Snout length from tip of snout to anterior corner of eye; EYE: Diameter of exposed portion of eyeball; IOD: Interorbital distance; IND: Internarial distance; UEW: Upper eyelid width measured as greatest width of upper eyelid; NEL: Nostril-eyelid length; NSL: Nostril-snout length; TMP: Horizontal diameter of tympanum; TEY: Distance from anterior edge of tympanum to posterior corner of eye; TIB: Tibia length with hindlimb flexed; ML: Manus length from tip of third digit to proximal edge of inner palmar tubercle; HLL: Hindlimb length from tip of fourth toe to

vent; FOT: Foot length from proximal edge of inner metatarsal tubercle to tip of fourth toe; FAL: Forearm length; FEM: Maximum diameter of femoral gland; FIIL: Third finger length; IMT: Inner metatarsal tubercle length; PEC: Maximum diameter of pectoral gland; THL: Distance from vent to knee.

Sex was determined by direct observation of calls in life, the presence of eggs in the abdomen as seen via external inspection, or the presence of internal vocal sac openings. Due to the high likelihood of unrecognized diversity within the genus (Chen et al., 2018; Rowley et al., 2016), we relied on examination of topotypic material and/or the original species descriptions where available.

## RESULTS

### Morphology

All collected specimens were assigned to the genus *Leptobranchella* based on the following morphological characters: (1) comparatively small size, SVL less than 60.0 mm, (2) the presence of an elevated inner palmar tubercle not continuous to the thumb, (3) presence of macroglands on body including supra-axillary, pectoral, femoral and ventrolateral glands, (4) vomerine teeth absent, (5) small tubercles on eyelids present, and (6) anterior tip of snout with whitish vertical bar (Das et al., 2010; Delorme et al., 2006; Dubois, 1983; Lathrop et al., 1998; Matsui, 1997, 2006).

Furthermore, the three focal populations from Daxueshan Nature Reserve, Yongde County, and Xiaoqiaogou Nature Reserve, Xichou County, could be reliably differentiated from each other and all known congeners of *Leptobranchella* on the basis of body size, degree of webbing and fringing on the fingers and toes, ventral coloration and pattern, size and coloration of body macroglands, dorsal skin texture, iris coloration in life, pattern of markings on flanks, tympanum pattern, pattern of longitudinal ridges under toes and supratympanic line coloration, which supports the recognition of the three new species of the genus *Leptobranchella* based on morphological evidence. Their descriptions are provided below.

The morphology of one female individual (KIZ027521) from Yingjiang, Yunnan, mostly matches the description of *L. yingjiangensis*, sharing the following diagnostic features: dorsal skin shagreened and scattered, fine, round brown tubercles; tympanum distinct, upper half black; finger webbing absent; toes with rudimentary webbing and wide lateral fringes; pectoral gland smaller than femoral gland; ventrolateral glands distinct; tiny white flecks present on edges of dark brown markings/blotches on dorsum; flanks with distinct irregular black spots; ventral surface of body creamy white; and iris bicolored, upper half orange yellow, lower half sliver white. However, this female individual (KIZ027521) had distinctly larger body size than known males of *L. yingjiangensis* (SVL 33.56 mm in Supplementary Table S2 vs. 25.7–27.6 mm in Yang et al., 2018), and the belly and throat of our individual had larger and more distinct scattered, dark brown flecks than *L. yingjiangensis* from Yang et al. (2018).

### Phylogenetics and genetic distances

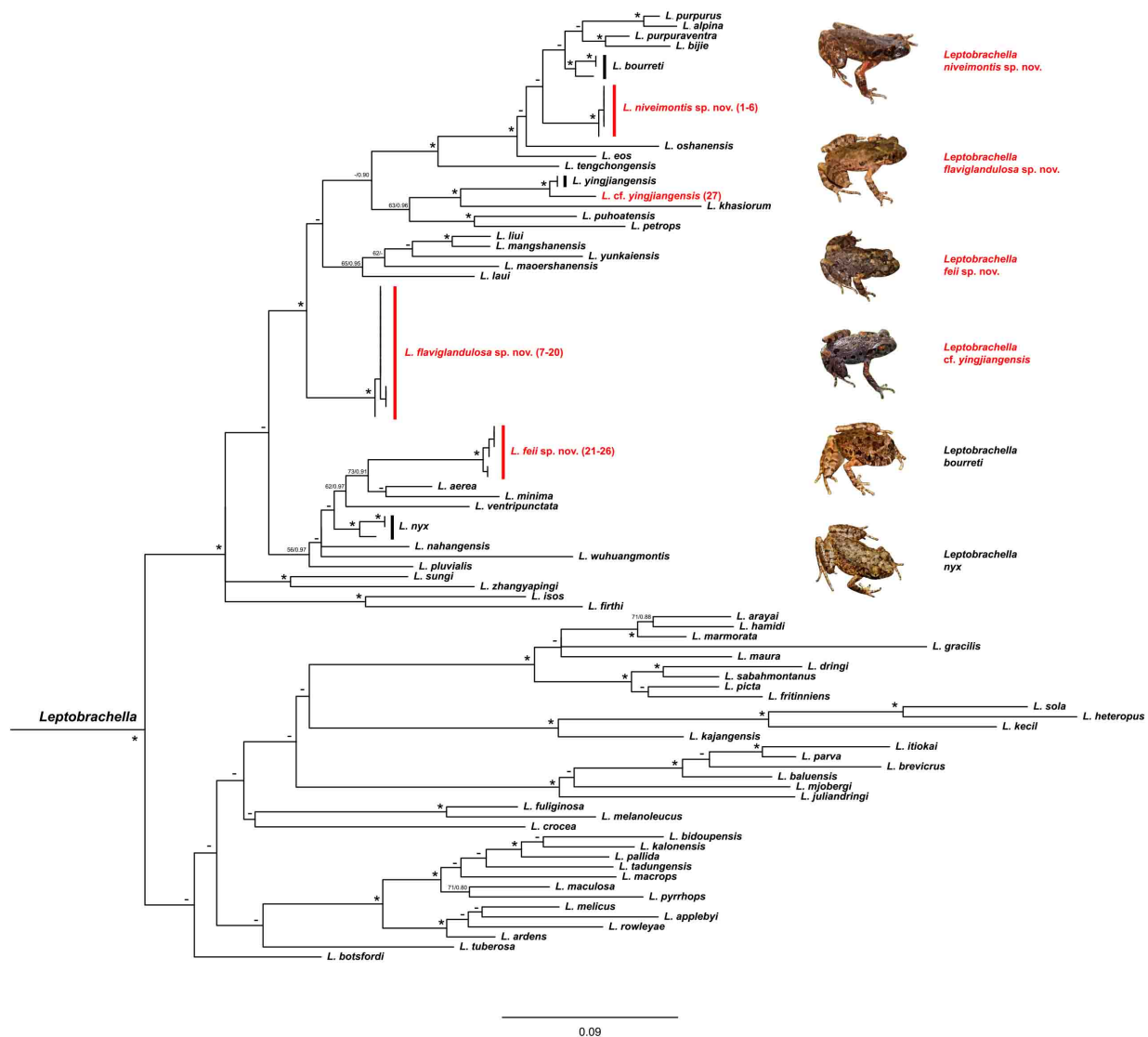
Bayesian inference (BI) and maximum likelihood (ML) analyses resulted in essentially identical topologies (Figure 2). The genealogy of *Leptobranchella* based on 16S rRNA gene in general agreed with phylogenetic relationships of the genus as reported by Chen et al. (2018). Although the basal relationship within *Leptobranchella* was poorly resolved, all the newly collected samples clustered into one major clade (ML=83, BI=1.00; Figure 2), which corresponded to Clade A of Chen et al. (2018). The species in this clade is widely distributed in southern China and Indochina and is the most species-rich clade within *Leptobranchella*.

The newly collected female specimen (KIZ027521) from Yingjiang was nested within *L. yingjiangensis* with strong support and genetically identical to the holotype of *L. yingjiangensis*, which confirmed the morphological diagnosis. In addition, one specimen (KIZ048513) collected from Longchuan County was recovered as sister to *L. yingjiangensis*, with sequence divergence of uncorrected *p*-distance of 2.9% in 16S rRNA. The geographical distance between the Longchuan sample and *L. yingjiangensis* was ~60 km.

For the putative new species, the individuals from Yongde and Xichou counties formed five monophyletic groups. Among them, samples from Yongde County (samples 1–6) formed a distinct monophyletic group (within-group genetic differentiation up to *p*-distance=0.2%). Phylogenetically, the samples from Yongde County formed a group with *L. bourreti*, *L. purpurus*, *L. purpuraventra*, *L. bijie*, *L. alpina*, *L. oshanensis*, and *L. eos* from Southwest China and Indochina with strong support. On the other hand, the sympatric samples from Xichou County were divided into four highly divergent clades with strong nodal supports (Figure 2): two were nested within a clade containing recognized species (i.e. *L. bourreti* and *L. nyx*), while the other two formed their own previously unknown clades. For the two new distinct clades, samples 7–20 formed a highly divergent clade with shallow within-group genetic differentiation (*p*-distance=0.0–0.4%); this clade was recovered as basal to many Chinese and Northern Indochinese species, although the relationship was not well resolved (Figure 2). Samples 21–26 (within-group differentiation up to *p*-distance=0.6%) formed a group with *L. aerea* and *L. minima* from Indochina (Figure 2).

The three putative new species showed obvious genetic divergence from other congeners. When compared with closely related recognized congeners, the minimum uncorrected genetic distance was 4.2% between the clade from Yongde County and *L. bourreti*, 5.8% between the clade containing the samples (7–20) from Xichou County and *L. liui*; and 4.7% between the clade containing the samples (21–26) from Xichou County and *L. aerea* (Supplementary Table S3). Moreover, the minimum uncorrected genetic distance among these three putative new species comprised 7.6% (Supplementary Table S3). These levels of pairwise divergence of the 16S rRNA gene are greater than the accepted threshold of species-level genetic divergence in





**Figure 2** Bayesian phylogram of *Leptobranchella* inferred from 16S mtDNA gene fragments

“\*” denotes high support by Bayesian posterior probabilities (BPP>0.95) and bootstrap support values (BS>70%); “-” represents low support values. Scale bar represents 0.1 nucleotide substitutions per site. Outgroup taxa not shown. Photos showing species of *Leptobranchella* newly collected from Yunnan. Numbers in brackets after species names correspond to Samples IDs in Supplementary Table S1.

anurans (Vences et al., 2005) and exceed many known interspecific distances for *Leptobranchella* (Supplementary Table S3).

Thus, based on the congruence in genetic differentiation and diagnostic morphological features, we herein describe the three distinct lineages of *Leptobranchella* as three new species.

### Taxonomic accounts

***Leptobranchella niveimontis* sp. nov.** Chen, Poyarkov, Yuan & Che

**Chresonymy:** *Leptolalax* sp. 1 — Chen et al., 2018: 165.

**Holotype:** KIZ028277, adult male, collected from Wumulong Ganhe Protection Station, Daxueshan Nature Reserve,

Yongde County, Lincang City, Yunnan Province, China (N99.6455°, E24.1773°, 2 601 m a.s.l.; Figure 1), on 18 April 2017 by Zhi-Yong Yuan.

**Paratypes:** KIZ028275–76, two adult males, from same locality as holotype, collected on 18 April 2017 by Zhiyong Yuan; KIZ015743–44, two adult males; KIZ015734, adult female; KIZ015763, adult female from Sancha River, Daxueshan Nature Reserve, Yongde County, Lincang City, Yunnan Province, China (N99.6422°, E24.1704°, 2 569 m a.s.l.; Figure 1) collected by Da-Hu Zou and Kai Xu in April 2016.

**Diagnosis:** *Leptobranchella niveimontis* sp. nov. can be distinguished from its congeners by the following combination

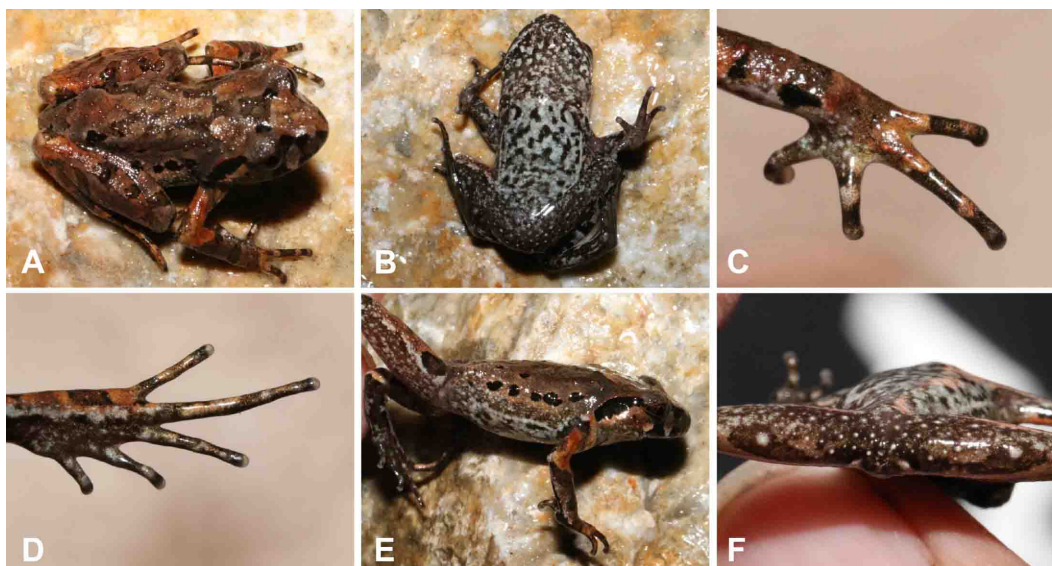
of morphological characters: (1) body size small (SVL 22.5–23.6 mm in four adult males, 28.5–28.7 mm in two females); (2) tympanum distinct, upper half black; (3) skin on dorsum scattered with fine reddish tubercles; (4) ventral sides marbled with distinct irregular black speckling on bluish-white background; (5) black blotches distinct, scattered on dorsum and flanks; (6) ventrolateral glands distinct, forming continuous white line; (7) finger webbing and fringes absent; (8) toe webbing rudimentary, toe lateral fringes narrow; (9) longitudinal ridges distinct under toes; (10) supratympanic ridges distinct, with reddish-brown pigmentation; (11) numerous rounded whitish speckles present on cloacal region; (12) pectoral gland white, larger than femoral gland; (13) iris distinctly bicolored, typically bright orange red in upper half, fading to silver white in lower half.

**Description of holotype:** Adult male, SVL 23.56 mm; head slightly longer than wide (HDL/HDW=1.09), triangular in dorsal view; snout rounded in both ventral and lateral views, protruding slightly beyond lower jaw (Figure 3); nostril oval-shaped, located closer to tip of snout than to anterior margin of eye; loreal region oblique; canthus rostralis indistinct; eyes large (EYE/HDL=0.38), diameter of eye comprising 86% of snout length, eyes notably protuberant in both dorsal and lateral views, pupil vertical; tympanum distinct, rounded, tympanum diameter smaller than eye (TMP/EYE=0.60); tympanic annulus notably elevated; vomerine teeth absent; tongue long, notched at posterior tip; vocal sac openings slit-like, paired, located posteriolaterally on mouth floor; supratympanic ridge distinct with reddish-brown pigmentation, running from eye towards axilla.

Fore-limbs relatively long (FAL/SVL=0.24), fingers long and slender (ML/SVL=0.26), without webbing and lateral fringes (Figure 3); relative length of fingers: I<II<IV<III; tips of fingers

rounded, slightly swollen; subarticular tubercles absent on fingers, inner metacarpal tubercle large and rounded, separated from much smaller outer metacarpal tubercle. Hind limbs relatively long, tibiotarsal articulation of adpressed limb reaching beyond eye, but not reaching snout; heels meeting when tibiae positioned at right angles to body axis; tibia about half of snout–vent length (TIB/SVL=0.51); relative toe lengths: I<II<V<III<IV; toe tips rounded and slightly swollen; rudimentary webbing present between all five toes; lateral fringes narrow, present on all toes; toe subarticular tubercles indistinct; distinct dermal ridges present under toes, uninterrupted at the articulations; moderate in size, oval inner metatarsal tubercle distinct, outer metatarsal tubercle absent. Dorsal skin relatively smooth, with small reddish tubercles and longitudinal folds; tiny white warts scattered on flanks (Figure 3); ventral skin smooth; supra-axillary gland raised, located in axillary region dorsally from insertion of fore-limb, oval; pectoral gland large and oval; round femoral glands small and distinct on rear of thighs, located closer to knee than to vent; ventrolateral glands forming continuous white line on flanks (Figure 3).

**Coloration of holotype:** In life: dorsal surface of head and trunk reddish-brown with some dark-brown, irregularly shaped markings; distinct reverse-triangle black marking between eyes; roughly W-shaped marking on scapular region; transverse black bars on dorsal surfaces of fingers and toes, lower arms, tarsus, thighs and tibia (Figure 3); upper lip barred with black strokes; supratympanic ridge reddish; a large black marking under supratympanic ridge covering upper half of tympanum; dorsal surfaces of elbow to upper arm and tibiotarsal articulations with distinctive reddish-brown coloration; distinct, irregularly-shaped black blotches scattered on flanks from groin to axilla; fine, distinct reddish tubercles scattered on



**Figure 3** Holotype of *Leptobrachella niveimontis* sp. nov. (KIZ028277) in life

A: Dorsolateral view; B: Ventral view; C: Left hand in dorsal view; D: Left foot in dorsal view; E: Lateral view; F: Cloacal area view.

upper eyelids, snout, dorsal surfaces of head, body and limbs. Black and bluish-white marbling all over ventral surfaces of throat, chest and belly; denser on throat than on chest and belly; ventral surfaces of limbs black, covered with small white speckles. Supra-axillary gland whitish; ventrolateral glands, pectoral glands and femoral glands white (Figure 3). Iris distinctly bicolored, typically bright orange-red in upper half and silvery-white in lower half. In preservative, the coloration of dorsum and flanks fades to dark brown with greyish-pink limbs (Figure 4A). After three years in alcohol, black markings on dorsum, flanks and transverse cross-bars on limbs still visible. Ventral surfaces of throat, chest and belly dull white with well-discernable marbling; ventral surfaces of limbs turn brown. Macrogrands still distinct and turn whitish. Iris coloration fades to completely black. In general, coloration pattern over body remains distinct and unchanged (Figure 4A).

**Morphological variation:** All individuals of the type series are similar in morphometric ratios (Table 1). Representative photographs of paratypes in life are shown in Figure 5. Females are distinctly larger than males (SVL 22.5–23.3 mm in three adult males and 28.5–28.7 mm in two females). There is a slight variation in number and size of dark blotches on flanks: the holotype (KIZ028277) and two paratypes (KIZ028275 and KIZ028276) have larger blotches than the other paratypes: Dorsal color in life varies from brown to

reddish-brown. Marbling on ventral surfaces of throat, chest and belly is distinct in holotype, but is less distinct in paratype KIZ015743, or very distinct, expanding to ventral surface of hind limbs in paratype KIZ015734. The elbows and upper arms are pink in the holotype but are pale yellow in paratypes KIZ015763 and KIZ015743 (Figures 3, 5). Lastly, in general, the skin texture appears more tuberculate in life (Figure 3) than in preservative (Figure 4A).

**Etymology:** The specific epithet “*niveimontis*” is a toponymic adjective derived from the Latin words “*niveus*” for “snowy”, and “*montanus*” meaning “related to mountains”, given in reference to the type locality of the new species, Mt. Daxue (literally “Great Snowy Mountain”) of Yunnan Province, China, and also in reference to the ecology of the new species, inhabiting high elevations in low temperature climate zones. For the common name, we recommend “Mt. Daxue Leaf Litter Toad” (English) and “Xue Shan Zhang Tu Chan” (雪山掌突蟾) (Chinese).

**Distribution and habits:** *Leptobranchella niveimontis* sp. nov. is presently known only from its type locality, near Wumulong Ganhe Protection Station of Daxueshan Nature Reserve in Yongde County, Lincang, Yunnan Province, China. The new species was found along small creeks or rocky streams in well-preserved montane evergreen broadleaf forest at elevations between 2 569 m and 2 601 m a.s.l. (Figure 6). *Leptobranchella niveimontis* sp. nov. is the fifth known species

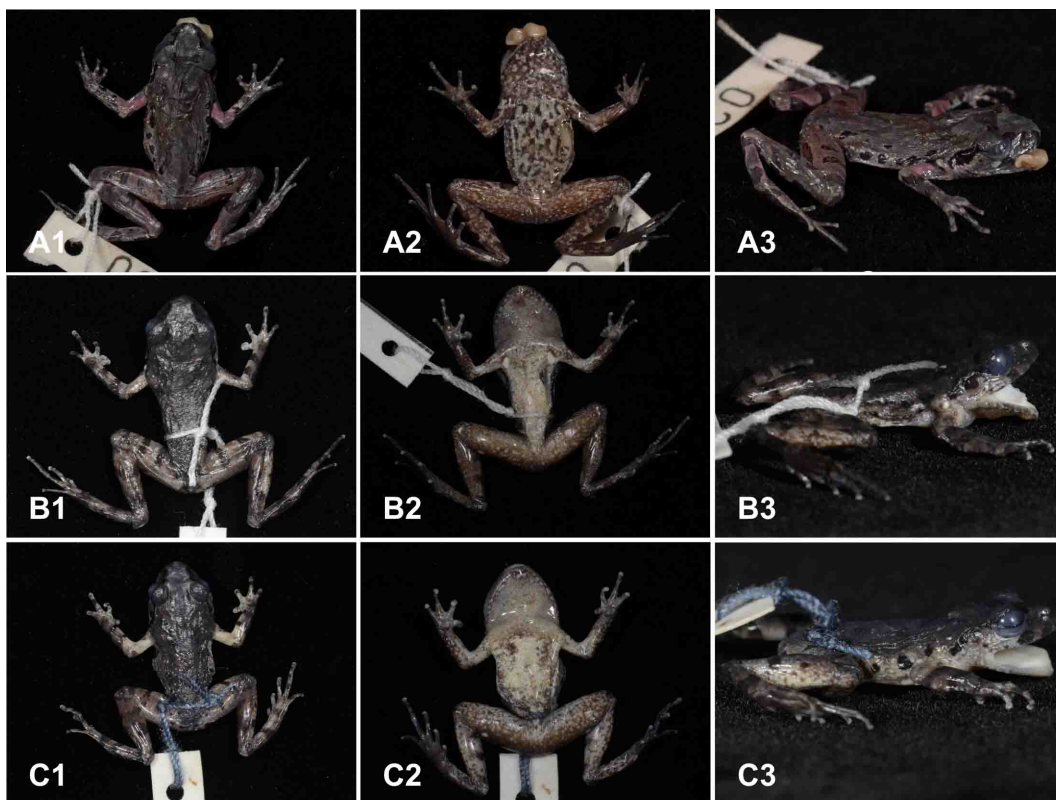


Figure 4 Holotypes of three new species of *Leptobranchella* in preservation in (1) dorsal, (2) ventral, and (3) dorsolateral views A: *Leptobranchella niveimontis* sp. nov.; B: *Leptobranchella flaviglandulosa* sp. nov.; C: *Leptobranchella feii* sp. nov.



**Table 1** Measurements of type series of *Leptobranchella niveimontis* sp. nov. (All measurements are in mm)

Species	<i>Leptobranchella niveimontis</i> sp. nov.						
	Holotype	Paratype	Paratype	Paratype	Paratype	Paratype	Paratype
Voucher Nos.	KIZ 028277	KIZ 015743	KIZ 015744	KIZ 028275	KIZ 028276	KIZ 015734	KIZ 015763
Sex	M	M	M	M	M	F	F
EYE	3.00	3.02	3.15	3.24	2.88	3.51	3.46
FAL	5.57	4.92	5.31	5.69	4.82	6.43	6.09
FEM	1.00	0.97	0.91	1.00	1.10	1.04	1.06
FIIL	3.99	3.64	3.33	4.01	3.84	4.45	3.82
FOT	11.64	10.85	10.93	10.87	10.90	12.85	12.66
HDL	7.95	8.15	8.00	8.60	7.49	9.64	9.52
HDW	7.31	7.93	7.68	7.92	6.99	8.82	9.66
HLL	39.81	35.19	36.39	37.91	35.31	43.76	43.19
IMT	1.19	1.47	1.21	0.87	1.10	1.49	43.19
IND	2.79	2.49	2.75	2.62	2.01	2.88	2.86
IOD	2.84	2.81	2.69	3.03	2.48	3.23	3.00
ML	6.13	6.20	5.69	6.21	5.76	7.17	6.84
NEL	1.88	1.76	1.47	1.57	1.40	2.24	2.29
NSL	1.53	1.43	1.95	1.45	1.50	1.68	1.73
PEC	1.75	1.01	1.19	1.24	1.21	1.86	1.50
SNT	3.50	3.26	3.19	3.56	3.23	3.80	3.80
SVL	23.56	22.83	22.94	23.31	22.46	28.49	28.65
TEY	0.90	0.76	0.81	0.93	1.01	1.20	0.96
THL	11.52	10.90	10.58	11.39	10.75	13.03	11.86
TIB	12.01	10.91	11.30	11.74	10.69	14.51	13.57
TMP	1.81	1.59	1.93	1.87	1.41	2.13	1.78
UEW	2.16	2.19	1.98	2.25	2.01	2.83	2.69

For abbreviations see Material and Methods. M: Male; F: Female.



**Figure 5** Paratypes of *Leptobranchella niveimontis* sp. nov. in life

A: KIZ015734; B: KIZ015743.

of the genus inhabiting elevations above 2 000 m, and to the best of our knowledge it also occurs at the highest elevation as compared to other Chinese species of *Leptobranchella*. During our field surveys, calling males were recorded mostly

inside the rocky crevices, and some were found hiding under leaf litter. Ubiquitous orthopteran-like calls could be heard along the stream from April to May, and very few calls were detected during July. The gravid female collected in April





**Figure 6** Breeding habitat of *Leptobranchella niveimontis* sp. nov. near Wumulong Ganhe Protection Station, Daxueshan Nature Reserve, Yunnan Province, China

carried pure white eggs.

**Comparison:** Compared with the 26 known congeners occurring south of the Isthmus of Kra, *Leptobranchella niveimontis* sp. nov. can be easily distinguished from *L. arayai*, *L. dringi*, *L. fritinniens*, *L. gracilis*, *L. hamidi*, *L. heteropus*, *L. kajangensis*, *L. kecil*, *L. marmorata*, *L. melanoleuca*, *L. maura*, *L. picta*, *L. platycephala*, *L. sabahmontana*, and *L. sola* by the presence of supra-axillary and ventrolateral glands (vs. absence); and can be further distinguished from the following species by having rounded digit tips (vs. pointed digit tips in all species listed below) and an obviously larger adult body size (SVL 22.5–23.6 mm in males): *L. baluensis* (14.9–15.9 mm in males), *L. brevicrus*

(17.1–17.8 mm in males), *L. bondangensis* (17.8 mm in males), *L. fusca* (16.3 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. natunae* (17.6 mm in males), *L. parva* (15.0–16.9 mm in males), *L. palmata* (14.4–16.8 mm in males) and *L. serasanae* (16.9 mm in female).

*Leptobranchella niveimontis* sp. nov. differs from all other species of *Leptobranchella* occurring north of the Isthmus of Kra by a combination of body size, degree of webbing and fringing on the toes, externally distinct tympanum, dorsal skin texture, ventral coloration, distinct black blotches scattered on dorsum and flanks, presence of a distinct reddish supratympanic line, presence of rounded whitish speckles on

**Table 2** Selected diagnostic characters for species described herein and species in genus *Leptobranchella* occurring north of Isthmus of Kra (modified from Rowley et al., 2017; Wang et al., 2019; Yang et al., 2018; Yuan et al., 2017 )

Species	Male SVL (mm)	Black spots on flanks	Toes webbing	Fringes on toes	Ventral coloration	Dorsal skin texture
<i>niveimontis</i> sp. nov.	22.5–23.6	Yes	Rudimentary	Narrow	Marbling with black speckling	Relatively smooth with small tubercles
<i>flaviglandulosa</i> sp. nov.	23.0–27.0	Yes	Poorly developed	Narrow	Whitish, black speckling on margins	Shagreened with yellowish-brown tubercles
<i>feii</i> sp. nov.	21.5–22.8	Yes	Rudimentary	Narrow	Creamy white with black blotches	Shagreened with small tubercles and ridge
<i>aerea</i>	25.1–28.9	No	Rudimentary	Wide	Near immaculate creamy white, brown specking on margins	Finely tuberculate
<i>alpina</i>	24.0–26.4	Yes	Rudimentary	Wide in males	Creamy-white with dark spots	Relatively smooth, some with small warts
<i>applebyi</i>	19.6–22.3	Yes	Rudimentary	No	Reddish brown with white speckling	Smooth
<i>ardens</i>	21.3–24.7	Yes	No	No	Reddish brown with white speckling	Smooth-finely shagreened
<i>bidouzensis</i>	18.5–25.4	Yes	Rudimentary	Weak	Reddish brown with white speckling	Smooth
<i>bijie</i>	29.0–30.4	Yes	Rudimentary	Narrow	White with distinct nebulous greyish speckling on chest and ventrolateral flanks	Shagreened and granular
<i>botsfordi</i>	29.1–32.6	No	Rudimentary	Narrow	Reddish brown with white speckling	Shagreened
<i>bouretti</i>	28.0–36.2	Yes	Rudimentary	Weak	Creamy white	Relatively smooth, some with small warts
<i>crocea</i>	22.2–27.3	No	Rudimentary	No	Bright orange	Highly tuberculate
<i>eos</i>	33.1–34.7	No	Rudimentary	Wide	Creamy white	Shagreened

Continued

Species	Male SVL (mm)	Black spots on flanks	Toes webbing	Fringes on toes	Ventral coloration	Dorsal skin texture
<i>firthi</i>	26.4–29.2	No	Rudimentary	Wide in males	Creamy white	Shagreened with fine tubercles
<i>fuliginosa</i>	28.2–30.0	Yes	Rudimentary	Weak	White with brown dusting	Nearly smooth, few tubercles
<i>isos</i>	23.7–27.9	No	Rudimentary	Wide in males	Creamy white with white dusting on margins	Mostly smooth, females more tuberculate
<i>kalonensis</i>	25.8–30.6	Yes	No	No	Pale, speckled brown	Smooth
<i>khasiorum</i>	24.5–27.3	Yes	Rudimentary	Wide	Creamy white	Isolated, scattered tubercles
<i>lateralis</i>	26.9–28.3	Yes	Rudimentary	No	Creamy white	Roughly granular
<i>lalui</i>	24.8–26.7	Yes	Rudimentary	Wide	Creamy white with dark brown dusting on margins	Round granular tubercles
<i>liui</i>	23.0–28.7	Yes	Rudimentary	Wide	Creamy white with dark brown spots on chest and margins	Round granular tubercles with glandular folds
<i>macrops</i>	28.0–29.3	Yes	Rudimentary	No	Greyish-violet with white speckling	Roughly granular with larger tubercles
<i>maculosa</i>	24.2–26.6	Yes	No	No	Brown, less white speckling	Mostly smooth
<i>mangshanensis</i>	22.2–27.8	Yes	Rudimentary	Weak	Throat grey-white and belly creamy white, scattered with white speckles	Smooth with orange tubercles and dark brown stripes
<i>maoershanensis</i>	25.2–30.4	Yes	Rudimentary	Narrow	Creamy white chest and belly with irregular black spots	Longitudinal folds
<i>melica</i>	19.5–22.7	Yes	Rudimentary	No	Reddish brown with white speckling	Smooth
<i>minima</i>	25.7–31.4	Yes	Rudimentary	No	Creamy white	Smooth
<i>nahangensis</i>	40.8	Yes	Rudimentary	No	Creamy white with light speckling on throat and chest	Smooth
<i>nokrekensis</i>	26.0–33.0	Yes	Rudimentary	Unknown	Creamy white	Tubercles and longitudinal folds
<i>nyx</i>	26.7–32.6	Yes but indistinct	Rudimentary	No	Creamy white with white with brown margins	Rounded tubercles
<i>oshanensis</i>	26.6–30.7	Yes	No	No	Whitish with no markings or only small, light grey spots	Smooth with few glandular ridges
<i>pallida</i>	24.5–27.7	No	No	No	Reddish brown with white speckling	Tuberculate
<i>pelodytoides</i>	27.5–32.3	Yes	One third webbing	Narrow	Whitish	Mostly smooth with smooth warts
<i>petrops</i>	23.6–27.6	No	No	Narrow	Immaculate creamy white	Highly tuberculate
<i>pluvialis</i>	21.3–22.3	Yes	Rudimentary	No	Dirty white with dark brown marbling	Smooth, flattened tubercles on flanks
<i>puhoatensis</i>	24.2–28.1	Yes	Rudimentary	Narrow	Reddish brown with white dusting	Longitudinal skin ridges
<i>purpuraventra</i>	27.3–29.8	Yes	Rudimentary	Narrow	Grey purple with distinct nebulous greyish speckling on chest and ventrolateral flanks	Shagreened and granular
<i>purpurus</i>	25.0–27.5	Yes	Rudimentary	Wide	Dull white with indistinct grey dusting	Shagreen with small tubercles
<i>pyrrhops</i>	30.8–34.3	Yes	Rudimentary	No	Reddish brown with white speckling	Slightly shagreened
<i>rowleyae</i>	23.4–25.4	Yes	No	No	Pinkish milk-white to light brown with white speckles	Smooth with numerous tiny tubercles
<i>sungi</i>	48.3–52.7	No or small	Wide	Weak	White	Granular
<i>tadungensis</i>	23.3–28.2	Yes	No	No	Reddish brown with white speckling	Smooth
<i>tamdil</i>	32.3	Yes	Wide	Wide	White	Weakly tuberculate
<i>tengchongensis</i>	23.9–26.0	Yes	Rudimentary	Narrow	White with dark brown blotches	Shagreened with small tubercles
<i>tuberosa</i>	24.4–29.5	No	Rudimentary	No	White with small grey spots/streaks	Highly tuberculate
<i>ventripunctata</i>	25.5–28.0	Yes	Rudimentary	Absent or narrow	Chest and belly with dark brown spots	Longitudinal skin ridges
<i>wuhuangmontis</i>	25.6–30.0	Yes	Rudimentary	Narrow	Greyish white mixed by tiny white and black dots	Rough with dense conical tubercles
<i>yingjiangensis</i>	25.7–27.6	Yes	Rudimentary	Wide	Creamy white with dark brown flecks on chest and margins	Shagreened with small tubercles
<i>yunkaiensis</i>	25.9–29.3	Yes	Rudimentary	Wide	Belly pink with distinct or indistinct speckling	Shagreened with short skin ridges and warts
<i>zhangyapingi</i>	45.8–52.5	Yes	Rudimentary	Wide	Near immaculate white	Mostly smooth with distinct tubercles

surface of cloacal region, and a bicolored iris (Table 2).

Specifically, *Leptobranchella niveimontis* sp. nov. can be easily distinguished from its phylogenetically close congeners as follows. The new species differs from *L. bourreti* by having a smaller body size (SVL 22.5–23.6 mm in males, 28.5–28.7 mm in females vs. 28.0–36.2 mm in males, 42.0–45.0 mm in females), distinct dermal ridges under toes (vs. indistinct), as well as by the presence of marbled ventral side scattered with distinct irregular black speckling (vs. immaculate creamy-white belly) and the presence of rounded whitish speckles on surface of cloacal region (vs. absence). *Leptobranchella niveimontis* sp. nov. differs from *L. purpurus* by having a relatively smaller body size in males (SVL 22.5–23.6 mm vs. 25.0–27.5 mm), toes with narrow lateral fringes (vs. wide lateral fringes), dorsum coloration reddish-brown in life (vs. purplish-brown), ventral surfaces marbled and scattered with distinct irregular black speckling (vs. dull-white with indistinct grey dusting), relatively longer hind limbs in males (TIB/SVL 0.48–0.51 vs. 0.43–0.45), black marking on dorsum and flanks without mottling with distinct yellow pigmentation in life (vs. presence of distinct yellow mottling). The new species can be distinguished from *L. eos* by having smaller body size (SVL 22.5–23.6 mm in males, 28.5–28.7 mm in females vs. 33.1–34.7 mm in males, 40.7 mm in female), by reddish-brown dorsal coloration with black markings and spots in life (vs. almost uniformly brown), by narrow and weakly developed lateral fringes on toes (vs. well-developed), by ventral surfaces marbled and scattered with distinct irregular black speckling (vs. immaculate creamy white belly), and by the presence of distinct black markings on flanks (vs. absence). The new species differs from *L. alpina* by having a smaller body size in females (SVL 28.5–28.7 mm vs. 31.7–32.5 mm), by reddish-brown dorsal coloration in life (vs. grey-brown in *alpina*), by ventral sides marbled with distinct irregular black speckling (vs. distinct dark brown spots/blotches), as well as by the absence of white tiny flecks on dorsum (vs. presence). The new species can be diagnosed from *L. oshanensis* by having femoral glands located closer to the knee than to the vent (vs. closer to the vent than to the knee), by pectoral gland larger than femoral gland (vs. the reversed condition), by ventral surfaces marbled with distinct irregular black speckling (vs. dull-white ventral sides scattered with black spots), by the presence of dermal fringes on toes (vs. absence), and by the absence of skin folds on dorsum (vs. presence).

For the remaining known *Leptobranchella* species from Yunnan, *Leptobranchella niveimontis* sp. nov. differs from *L. tengchongensis* by having distinct ventrolateral glands (vs. indistinct), by pectoral gland being larger than femoral gland (vs. the reversed condition), by a bicolored iris (vs. unicolored), and by ventral sides with marbling pattern (vs. whitish, scattered with distinct irregular dark speckling). The new species is distinguished from *L. yingjiangensis* by having narrow dermal fringes on toes (vs. wide), by having pectoral gland larger than femoral gland (vs. the reversed condition), by marbled pattern on ventral surfaces (vs. immaculate creamy-white belly without distinct patterns), by the absence

of dermal fringes on fingers (vs. presence), as well as by the absence of distinct white tiny flecks on dorsum (vs. presence). The new species can be diagnosed from *L. ventripunctatus* by having a relatively larger body size in females (SVL 28.5–28.7 mm vs. 31.5–35.0 mm), by marbled pattern on the ventral surfaces (vs. small black spots on the ventral sides), by the absence of longitudinal skin folds on dorsum (vs. presence), as well as by the presence of lateral fringes on toes (vs. absence).

Lastly, *Leptobranchella niveimontis* sp. nov. differs from *L. pelodytoides*, the only known species from adjoining Myanmar, by having rudimentary toe webbing (vs. one third webbing), by smaller body size in males (SVL 22.5–23.6 mm vs. 27.5–32.3 mm), by marbled whitish and black ventral surfaces of body (vs. immaculate whitish), by dermal ridges under toes distinct (vs. indistinct), and by the absence of longitudinal skin folds on dorsum (vs. presence).

***Leptobranchella flaviglandulosa* sp. nov. Chen, Wang & Che**

**Chresonymy:** *Leptolax* sp. 7 — Chen et al., 2018: 165.

**Holotype:** KIZ032626, adult male, collected from Fadou Station, Xiaoqiaogou Nature Reserve, Xichou County, Wenshan Prefecture, Yunnan Province, China (N104.7818°, E23.3724°, 1 533 m a.s.l.; Figure 1), on 18 April 2018 by Jin-Min Chen.

**Paratypes:** KIZ016064–65, two adult males, from Shangcang Station, Xiaoqiaogou Nature Reserve, Xichou County, Wenshan Prefecture, Yunnan Province, China (N104.7626°, E23.3611°, 1 405 m a.s.l.) collected by Jinmin Chen and Baolin Zhang on 16 April 2014; KIZ016072, KIZ016074, KIZ048751–52, KIZ048754, five adult males and KIZ016076, one adult female from Fadou Station, Xiaoqiaogou Nature Reserve, Xichou County, Wenshan Prefecture, Yunnan Province, China (N104.7818°, E23.3724°, 1 568 m a.s.l.) collected by Jinmin Chen and Baolin Zhang on 12 April 2014; KIZ048793, KIZ048795–96, three adult males from Nanchang Station, Xiaoqiaogou Nature Reserve, Xichou County, Wenshan Prefecture, Yunnan Province, China (N104.8384°, E23.3627°, 1 337 m a.s.l.) collected by Jinmin Chen and Baolin Zhang on 19 April 2014; KIZ032627–28, two adult males, from same locality as holotype, collected by Jin-Min Chen on 18 April 2018.

**Diagnosis:** *Leptobranchella flaviglandulosa* sp. nov. can be distinguished from its congeners by the following combination of morphological characters: (1) body size small (SVL 22.99–26.97 mm in 13 adult males, 29.30 mm in single adult female); (2) tympanum distinct, partially covered by black marking; (3) black supratympanic line present; (4) skin on dorsum shagreened with fine yellowish-brown tubercles; (5) venter whitish with small black speckling on throat, chest and belly margins; (6) supra-axillary gland, femoral, pectoral and ventrolateral glands yellowish; (7) ventrolateral glands forming a discontinuous line; (8) finger webbing and fringes absent; (9) toe webbing poorly developed and lateral fringes narrow; (10) longitudinal ridges distinct under toes and interrupted at the

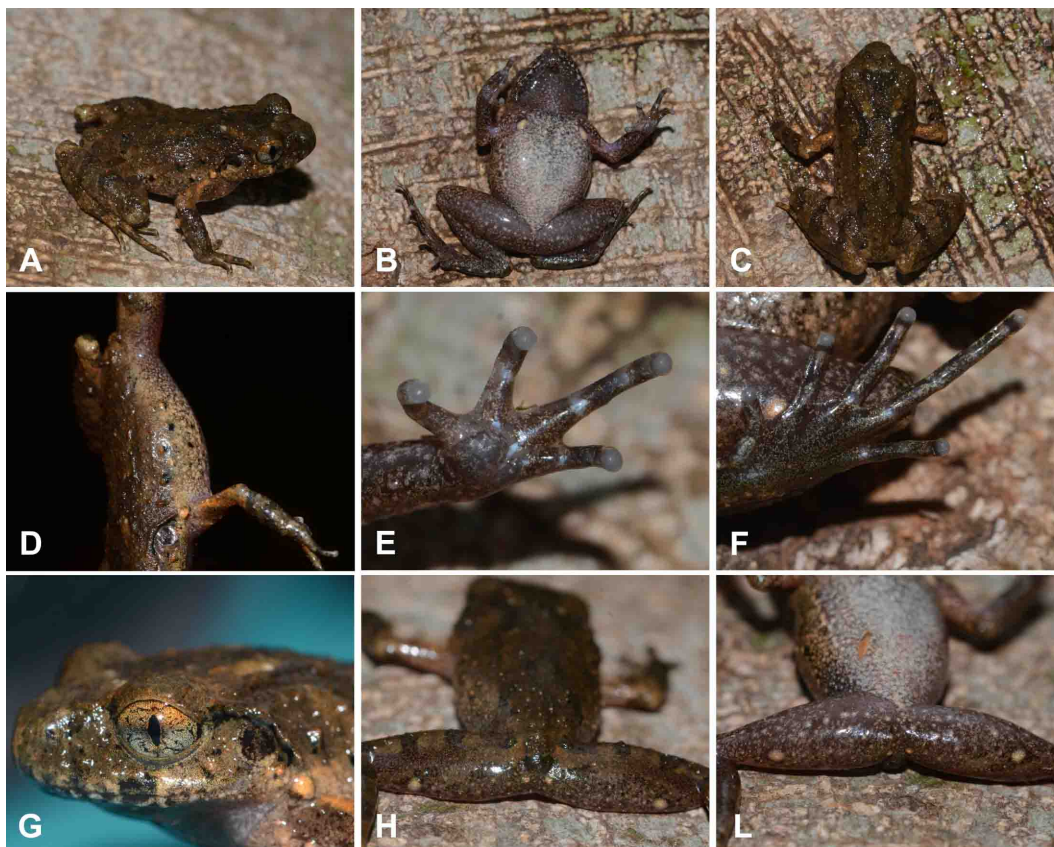


articulations; (11) distinct black blotches scattered on dorsum and flanks; (12) “\ /”-shaped yellow markings on dorsum in scapular region; (13) pectoral glands larger than femoral glands; (14) iris distinctly bicolored, typically golden-orange in upper half, fading to whitish grey in lower half.

**Description of holotype:** Adult male, SVL 26.72 mm; head length almost equal to head width (HDL/HDW=1.03), head triangular in dorsal view; snout rounded in both ventral and lateral views, protruding slightly beyond lower jaw (Figure 7); oval-shaped nostril located closer to tip of snout than to anterior margin of eye; loreal region oblique; canthus rostralis indistinct; eyes large (EYE/HDL=0.36), snout length greater than eye diameter (SNT/EYE=1.12), eyes notably protuberant in both dorsal and lateral views, pupil vertical; tympanum rounded and indistinct in life, but distinct in preservative: tympanum diameter smaller than eye (TMP/EYE=0.59); tympanic annulus slightly elevated; vomerine teeth absent; tongue long, notched at posterior tip; vocal sac openings slit-like, paired, located postero-laterally on mouth floor; supratympanic ridge distinct, running from posterior corner of eye towards axilla, the posterior end of supratympanic ridge not expanded (Figure 7).

Fore-limbs relatively long (FAL/SVL=0.24), fingers long and

slender (ML/SVL=0.25), without webbing or lateral fringing (Figure 7); relative length of fingers:  $II < IV < III$ ; tips of fingers rounded and slightly swollen; subarticular tubercles absent on fingers, inner metacarpal tubercle large and rounded, separated from much smaller outer metacarpal tubercle. Hind limbs relatively long, tibio-tarsal articulation of adpressed limb reaching beyond eye, but not reaching snout; heels meeting when the tibias are located at the right angle to the body axis; tibia length subequal to the half of snout-vent length (TIB/SVL=0.49); relative toe lengths:  $I < II < V < III < IV$ ; toe tips rounded and slightly swollen; rudimentary webbing present between all five toes; moderate lateral fringes present on all toes; subarticular tubercles absent; distinct dermal ridges present under toes except the first toe, the ridges are interrupted at the articulations; moderate, oval inner metatarsal tubercle distinct, outer metatarsal tubercle absent (Figure 7). Dorsal skin relatively rough, with numerous warts on dorsum; smaller warts scattered on flanks; ventral skin smooth; small supra-axillary gland with yellowish tip at fore-limb basis on just above fore-limb insertion; pectoral gland large and oval; round femoral glands small and distinct on rear surfaces of thighs, located closer to knee than to vent; small ventrolateral glands forming discontinuous yellowish lines on



**Figure 7** Holotype of *Leptobranchella flaviglandulosa* sp. nov. (KIZ032626) in life

A: Dorsolateral view; B: Ventral view; C: Dorsal view; D: Lateral view; E: Hand in ventral view; F: Foot in ventral view; G: Iris coloration; H: Dorsal view of thighs; L: Posteroventral view of thigh.



flanks (Figure 7).

**Coloration of holotype:** In life: dorsal surfaces of head and body brown, with a reverse-triangle black marking located between eyes, dorsal surfaces of head dotted with small whitish spots, several dark olive irregularly shaped markings present on trunk and transverse olive bars on dorsal surfaces of fingers and toes, lower arms, tarsus, thighs and tibia (Figure 7); “\ /”-shaped yellow markings on dorsum in scapular region; upper lip barred with dark olive strokes; supratympanic ridge dark olive; elbow to upper arm dorsally light yellow; irregularly shaped black blotches present on flanks from groin to axilla; fine orange tubercles scattered on upper eyelids, snout, dorsal surfaces of head, body and limbs. Ventral surfaces of throat, chest and limbs dark brown, densely covered with white dusting; belly creamy white with dark brown dusting along the margins. Supra-axillary gland, ventrolateral glands, pectoral glands and femoral glands yellow. Iris distinctly bicolored, typically golden orange in upper half and silver white in lower half (Figure 7). In preservative: the dorsal coloration of head and body dark grey with light brown coloration on limbs; irregular dark blotches present on dorsal surface of trunk; dark bars discernable on dorsal surface of fingers and toes, lower arms, tarsus, thighs

and tibia. Ventral surfaces of throat, chest and belly fade to creamy-white; brown dusting present on ventral surfaces of throat, chest, belly and upper arms; ventral surfaces of lower arms and hind limbs brown covered with small white spots (Figure 4B).

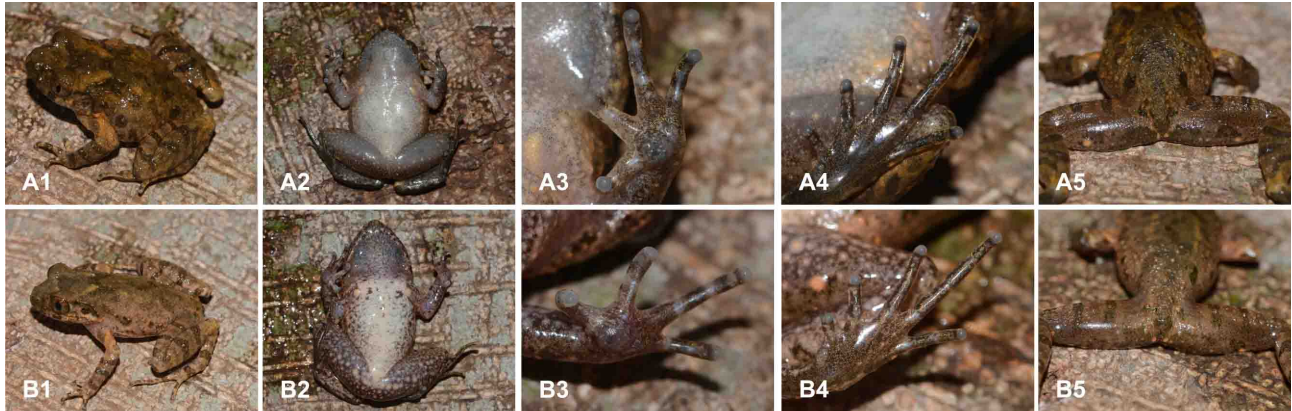
**Morphological variation:** All paratypes match the overall characters of the holotype (see Table 3). Representative photographs of paratypes in life are shown in Figure 8. Females are relatively larger than males (SVL 22.99–26.97 mm in 13 adult males and 29.30 mm in a single adult female). All males have small slit-like vocal sac openings, as well as the indistinct nuptial spines on the dorsal surfaces of the first and second fingers; The size of dark blotches on flanks is variable: KIZ016072, KIZ016074, KIZ048754 and KIZ048793 have small blotches on flanks; KIZ032627, KIZ032628, KIZ016064, KIZ048796, KIZ048752, KIZ048795, KIZ048751 and KIZ016076 have larger blotches on flanks. The dusting pattern on belly also varies: KIZ032626, KIZ016064, KIZ016072, KIZ016074, KIZ048752 and KIZ048754 with dusting being denser in the center of belly; while KIZ032627, KIZ032628, KIZ048796, KIZ048795, KIZ048793, KIZ048751 and KIZ016076 have the center of belly immaculate white.

**Etymology:** The species epithet, “*flaviglandulosa*”, is a Latin

**Table 3** Measurements of type series of *Leptobranchella flaviglandulosa* sp. nov. (All measurements are in mm)

Species	<i>Leptobranchella flaviglandulosa</i> sp. nov.													
	Holotype	Paratype	Paratype	Paratype	Paratype	Paratype	Paratype	Paratype	Paratype	Paratype	Paratype	Paratype	Paratype	Paratype
Voucher	KIZ	KIZ	KIZ	KIZ	KIZ	KIZ	KIZ	KIZ	KIZ	KIZ	KIZ	KIZ	KIZ	KIZ
Nos.	032626	016064	016065	016072	016074	032627	032628	048751	048754	048793	048795	048796	048752	016076
Sex	M	M	M	M	M	M	M	M	M	M	M	M	M	F
EYE	3.65	3.10	3.84	3.43	3.19	3.78	3.75	3.58	3.19	3.42	3.35	3.92	3.92	3.59
FAL	6.51	6.63	5.81	6.15	6.41	6.30	6.59	6.33	5.63	6.00	5.80	5.56	5.63	7.86
FEM	1.31	0.75	0.73	0.84	1.24	1.26	1.22	0.79	0.75	0.98	0.89	1.03	0.82	1.10
FIIL	4.25	3.52	4.21	4.27	3.71	4.60	4.57	4.01	3.94	3.12	3.73	3.08	3.83	4.84
FOT	12.45	11.12	12.02	11.20	11.14	11.90	12.85	11.53	10.02	10.81	9.78	10.62	12.05	13.81
HDL	10.23	9.74	10.27	9.87	10.48	10.08	10.49	10.41	9.67	9.80	10.22	9.14	10.06	11.79
HDW	9.90	9.26	9.30	8.60	10.32	9.87	10.08	10.06	9.54	9.21	9.55	8.83	9.64	11.03
HLL	43.61	41.31	42.70	39.54	43.69	44.10	43.86	42.74	39.58	38.93	42.65	37.53	41.64	46.51
IMT	1.38	1.21	1.29	1.17	1.55	1.36	1.33	1.37	1.11	1.34	1.24	1.33	1.30	1.33
IND	2.89	2.55	2.91	2.62	2.45	2.63	2.47	2.62	2.29	2.39	2.59	2.54	2.65	3.10
IOD	3.51	2.43	3.09	2.40	2.45	3.15	2.73	3.33	2.90	2.59	2.58	2.88	2.65	3.10
ML	6.66	6.30	7.00	6.22	6.46	7.34	7.10	7.13	6.56	5.93	6.44	6.69	6.99	7.42
NEL	2.33	2.18	2.13	2.03	2.74	2.16	2.26	2.54	2.06	2.16	2.31	2.15	2.10	3.18
NSL	1.79	1.69	1.52	1.51	1.48	1.54	1.53	1.80	1.20	1.55	1.47	1.74	1.56	1.63
PEC	1.76	1.66	1.63	1.57	1.77	1.75	1.54	1.53	1.72	1.45	1.25	1.33	1.47	1.22
SNT	4.07	3.85	3.96	3.90	3.97	4.10	4.02	4.30	3.29	3.62	3.95	3.64	4.08	4.56
SVL	26.72	25.11	25.33	24.73	25.72	26.97	26.77	26.39	25.57	23.45	25.35	22.99	26.05	29.30
TEY	1.33	0.86	0.81	0.86	1.17	1.34	1.24	1.67	1.22	1.17	1.40	0.94	1.02	1.53
THL	12.94	12.67	13.12	11.54	13.83	13.22	12.93	13.38	11.31	11.82	12.84	11.12	13.42	15.58
TIB	13.13	12.59	12.58	12.25	13.08	13.29	12.64	13.49	12.29	11.65	12.85	12.25	12.13	15.01
TMP	2.16	2.08	2.13	2.16	2.03	2.23	2.12	2.00	1.69	1.54	1.85	1.69	2.08	2.25
UEW	2.98	2.57	2.98	2.64	2.94	2.49	2.73	2.44	2.81	2.17	2.74	2.58	2.77	2.78

For abbreviations see Material and Methods. M: Male; F: Female.



**Figure 8** Paratypes of *Leptobrachella flaviglandulosa* sp. nov. in life  
A: KIZ032627; B: KIZ032628.

adjective in feminine gender, derived from Latin “*flavus*” for “yellow color” and “*glandula*” for “gland”, in reference to characteristic life coloration of femoral, pectoral and ventrolateral glands in the new species. For the common name, we recommend “Yellow-gland Leaf Litter Toad” (English) and “Huang Xian Zhang Tu Chan” (黄腺掌突蟾) (Chinese).

**Distribution and habits:** *Leptobrachella flaviglandulosa* sp. nov. is currently known only from Xiaoqiaogou Nature Reserve, Xichou County, Wenshan City, Yunnan Province, China. The species was found in the environs of the Fadou, Shangchang and Nanchang stations of the nature reserve. The new species was recorded along small seeps or clear-water rocky mountain streams in well-preserved montane evergreen broadleaf forest at elevations between 1000 and

1400 m a.s.l. (Figure 9). The new species was recorded in sympatry with *L. bourreti* and *L. nyx*. During our field surveys, calling males were found mostly while sitting on vegetation, and some were calling from rocky crevices. An insect-like calls could be heard along the stream from March to June; gravid female was collected in March bearing pure-white eggs.

**Comparison:** Compared with the 26 known congeners occurring south of the Isthmus of Kra, *Leptobrachella flaviglandulosa* sp. nov. can be easily distinguished from *L. arayai*, *L. dringi*, *L. fritinniensis*, *L. gracilis*, *L. hamidi*, *L. heteropus*, *L. kajangensis*, *L. kecil*, *L. marmorata*, *L. melanoleuca*, *L. maura*, *L. picta*, *L. platycephala*, *L. sabahmontana*, and *L. sola* by the presence of supra-axillary and ventrolateral glands (vs. absence); and from the following species by having a rounded digit tips (vs. pointed digit tips in



**Figure 9** Breeding habitat and general aspect in field of *Leptobrachella* spp. in Xiaoqiaogou Nature Reserve

A1: Breeding stream in Fadou Station, which harbors three co-occurring species: *Leptobrachella bourreti*, *L. flaviglandulosa* sp. nov., and *L. nyx*. A2: Stream in Nanchang Station, which harbors two co-occurring sympatric species: *Leptobrachella flaviglandulosa* sp. nov. and *L. nyx*. A3: Stream in Xiaoqiaogou Station, which harbors two sympatric species: *L. bourreti* and *Leptobrachella feii* sp. nov. B1: *Leptobrachella bourreti* in the field. B2: *Leptobrachella flaviglandulosa* sp. nov. in the field. B3: *Leptobrachella feii* sp. nov. in the field.

all species listed below) and obviously larger body size (SVL 22.5–23.6 mm in males): *L. baluensis* (14.9–15.9 mm in males), *L. brevicrus* (17.1–17.8 mm in males), *L. bondangensis* (17.8 mm in males), *L. fusca* (16.3 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. natunae* (17.6 mm in males), *L. parva* (15.0–16.9 mm in males), *L. palmata* (14.4–16.8 mm in males) and *L. serasanae* (16.9 mm in female).

*Leptobranchella flaviglandulosa* **sp. nov.** differs from all other species of *Leptobranchella* occurring north of the Isthmus of Kra by a combination of body size, distinct tympanum, black supratympanic line, dorsal skin texture, ventral and gland coloration, degree of webbing and fringing on the fingers and toes, presence of longitudinal ridges under toes interrupted at the articulations, distinct black blotches scattered on dorsum and flanks, presence of “\ /”-shaped yellow markings on dorsum, and a bicolored iris (Table 2).

In particular, *Leptobranchella flaviglandulosa* **sp. nov.** can be easily distinguished from its phylogenetically close congeners. The new species can be diagnosed from *L. liui* by having narrow lateral fringes on toes (vs. wide), by yellow femoral, pectoral and ventrolateral glands (vs. white), by ventrolateral glands forming a discontinuous line (vs. continuous), and by black speckling on the throat, chest and margins of belly (vs. immaculate white belly), as well as by the presence of “\ /”-shaped yellow marking on the dorsum above the shoulder (vs. absence) and the absence of glandular folds (vs. presence). The new species differs from *L. mangshanensis* by having black speckling on throat and belly (vs. white throat and belly), by distinct longitudinal ridges under toes interrupted at the articulations (vs. ridges indistinct), by femoral, pectoral and ventrolateral glands yellow (vs. white), and by ventrolateral glands forming a discontinuous line (vs. continuous). *Leptobranchella flaviglandulosa* **sp. nov.** is distinguished from *L. laui* by having yellow femoral, pectoral and ventrolateral glands (vs. pale copper), narrow lateral fringes on toes (vs. wide), longitudinal ridges under toes interrupted at the articulations (vs. not interrupted), and the absence of dermal fringes on fingers (vs. presence). The new species differs from *L. aerea*, by having a bicoloured iris (vs. uniform coloration), yellow femoral, pectoral and ventrolateral glands (vs. white), narrow lateral fringes on toes (vs. wide), as well as the presence of black spots on flanks (vs. absence) and the presence of distinct supratympanic fold (vs. absence).

For *Leptobranchella* species presently known to occur in Yunnan, *Leptobranchella flaviglandulosa* **sp. nov.** differs from *L. ventripunctata* by having yellow femoral, pectoral and ventrolateral glands (vs. white); by ventrolateral glands forming a discontinuous line (vs. continuous), by longitudinal ridges distinct under toes and interrupted at the articulations (vs. uninterrupted), by sparse black spots on belly (vs. dense black spotting), and by the absence of longitudinal skin folds on dorsal surfaces of body (vs. presence in *ventripunctata*). The new species can be distinguished from *L. alpina* by having yellow femoral, pectoral and ventrolateral glands (vs.

white), by ventrolateral glands forming a discontinuous line (vs. continuous), by narrow lateral fringes on toes (vs. wide fringes on toes), by longitudinal ridges under toes distinct and interrupted at the articulations (vs. uninterrupted in *alpina*), and by the absence of distinct white tiny flecks on dorsum (vs. presence). *Leptobranchella flaviglandulosa* **sp. nov.** can be distinguished from *L. purpurus* by having narrow lateral fringes on toes (vs. wide), by yellow coloration of femoral, pectoral and ventrolateral glands (vs. white), ventrolateral glands forming discontinuous line (vs. distinct ventrolateral glands forming continuous line), by dorsum coloration dark brown or brown in life (vs. purplish brown), by a relatively longer hind limbs (males TIB/SVL ratio 0.48–0.53 in *flaviglandulosa* **sp. nov.** vs. 0.43–0.45 in *purpurus*), and by the absence of distinct yellow pigmentation on dorsum in life (vs. presence). The new species differs from *L. tengchongensis* by having a bicolored iris (vs. uniform), by pectoral gland larger than femoral gland (vs. the reversed condition), by yellow femoral, pectoral and ventrolateral glands (vs. white), by a relatively longer head (HDL/SVL=0.37–0.42 vs. HDL/SVL=0.34–0.35), and the absence of whitish spots on ventral sides (vs. presence). The new species can be diagnosed from *L. yingjiangensis* by having narrow lateral fringes on toes (vs. wide), by pectoral gland larger than femoral gland (vs. the reversed condition), by ventrolateral glands discontinuous (vs. continuous), by yellow femoral, pectoral and ventrolateral glands (vs. white), by the absence of tiny white flecks on edges of dark brown markings on dorsum (vs. presence), and by the absence of dermal fringes on fingers (vs. the presence of narrow to moderate dermal fringes on 2nd to 4th fingers).

In addition, *Leptobranchella flaviglandulosa* **sp. nov.** differs from the newly described species *L. niveimontis* (see above) by having yellow femoral, pectoral and ventrolateral glands (vs. white), by yellowish-brown tubercles scattered on the dorsum (vs. reddish), by ventrolateral glands discontinuous (vs. distinct and continuous), by longitudinal ridges under toes interrupted at the articulations (vs. uninterrupted), by supratympanic ridges black (vs. reddish), as well as by the presence of “\ /”-shaped yellow marking on the dorsum (vs. absence), by the absence of marbling pattern on the ventral surfaces (vs. presence), and the absence of rounded whitish speckles around cloacal region (vs. presence).

***Leptobranchella feii* sp. nov. Chen, Yuan & Che**

**Chresonymy:** *Leptolalax* sp. 9 — Chen et al., 2018: 165.

**Holotype:** KIZ032625, adult male, collected from Pingzhai Station, Xiaoqiaogou Nature Reserve, Xichou County, Wenshan Prefecture, Yunnan Province, China (N104.6849°, E23.3610°, 1 577 m a.s.l.; Figure 1), on 22 April 2014 by Jin-Min Chen.

**Paratypes:** KIZ048973, KIZ048921, two adult males, and KIZ048972, one adult female from hillside of Daweishan Nature Reserve, Pingbian County, Honghe, Yunnan Province, China (N103.6958°, E22.9546°, 1 850 m a.s.l.; Figure 1) collected by Jinmin Chen and Bao-Lin Zhang on 22 April 2014; KIZ048893–94, two adult males, from the same locality



as the holotype, collected by Jin-Min Chen and Bao-Lin Zhang on 22 April 2014.

**Diagnosis:** *Leptobranchella feii* sp. nov. can be distinguished from its congeners by a combination of the following morphological characters: (1) body size small (SVL 21.51–22.75 mm in 5 adult males, 25.67 mm in a single adult female); (2) tympanum distinct, partially covered by black marking; (3) distinct black supratympanic line; (4) skin on dorsum shagreened with small tubercles and unequal dermal ridges; (5) distinct black blotches scattered on the creamy white chest and belly; (6) small black spots densely covering the throat; (7) ventrolateral glands present, forming a distinct continuous white line; (8) finger webbing and fringing absent; (9) toe webbing rudimentary with narrow lateral fringes; (10) longitudinal ridges under toes distinct and uninterrupted at the articulations; (11) small white pectoral glands; (12) black patches scattered on lateral sides of the body; (13) relatively long head (HDL/SVL=0.38–0.43 in males); and (14) iris distinctly bicolored, typically golden orange in upper half, fading to silver white in lower half.

**Description of holotype:** Adult male, SVL 22.75 mm; head slightly longer than wide (HDL/HDW=1.16), triangular in dorsal view; snout rounded in both ventral and lateral views, slightly

protruding beyond lower jaw (Figure 10); oval-shaped nostril located closer to tip of snout than to anterior margin of eye; loreal region oblique; canthus rostralis indistinct; eyes moderate (EYE/HDL=0.32), eye diameter slightly shorter than snout length (EYE/SNT=0.92); eyes notably protuberant in both dorsal and lateral aspects; pupil vertical; tympanum distinct in life, rounded, tympanum diameter smaller than eye (TMP/EYE=0.61); tympanic annulus distinctly elevated; vocal sac openings slit-like, paired, located posteriolaterally on mouth floor; vomerine teeth absent; tongue long, notched at posterior tip; supratympanic ridge distinct, running from posterior eye corner towards axilla, supratympanic ridge not expanded posteriorly (Figure 10).

Fore-limbs relatively long (FAL/SVL=0.26), fingers long and slender (ML/SVL=0.26), lacking webbing and lateral fringes (Figure 10); relative length of fingers: II<I≤IV<III; tips of fingers rounded, slightly swollen; subarticular tubercles absent on fingers, inner metacarpal tubercle large and rounded, separated from much smaller outer metacarpal tubercle. Hind limbs relatively long, tibio-tarsal articulation of adpressed limb reaching beyond eye, but not reaching snout; heels meeting when limbs located at right angle to body axis; tibia length shorter than a half of snout-vent length (TIB/SVL=0.46);



**Figure 10** Holotype of *Leptobranchella feii* sp. nov. (KIZ032625) in life

A: Dorsolateral view; B: Ventral view; C: Dorsal view; D: Lateral view; E: Hand in ventral view; F: Foot in ventral view; G: Iris coloration; H: Dorsal view of thighs; L: Posteroventral view of thigh.



relative toe lengths: I<II<V<III<IV; toe tips rounded and slightly swollen; rudimentary webbing present between all five toes; narrow lateral dermal fringes present on all toes; subarticular tubercles absent; distinct dermal ridges present under all toes except the first toe, dermal ridges interrupted at the articulations; moderate in size, oval-shaped inner metatarsal tubercle distinct, outer metatarsal tubercle absent (Figure 10). Skin on dorsum relatively rough; small warts scattered on flanks; ventral skin smooth; distinct supra-axillary gland with yellowish tip at fore-limb insertion; pectoral gland small and round; femoral glands small, distinct on rear surfaces of thighs, located closer to knee than to vent; small ventrolateral glands forming a distinct white line on flanks (Figure 10).

**Coloration of holotype:** In life, dorsal surfaces of head and trunk olive brown, with a distinct reverse-triangle dark marking between eyes and a W-shaped marking in scapular region; some dark irregularly shaped markings on dorsum and transverse dark-brown bars on dorsal surfaces of fingers and toes, lower arms, tarsus, thighs and tibia (Figure 10); upper lip barred with dark brown lines; supratympanic ridge dark; dorsal surfaces of elbow to upper arm light yellow; large irregularly shaped black blotches present on flanks from groin to axilla. Ventral surfaces of throat and limbs dark brown, densely covered with white dusting; belly creamy white with dark

brown spots along the margins. Supra-axillary gland orange; ventrolateral glands, pectoral glands and femoral glands white (Figure 10). Iris bicolored, golden orange in upper half and silver white in lower half. In preservative, dorsal surface turns relatively smooth, and the coloration of head and trunk fades to dark grey, limbs fade to light brown (Figure 4C); irregular dark markings present on dorsum; dark bars present on dorsal surfaces of fingers and toes, lower arms, tarsus, thighs and tibia. Ventral surfaces of throat, chest and belly fade to creamy white; brown dusting present on throat; bigger brown spots present on ventral surfaces of chest, belly and fore-limbs; ventral surfaces of lower arms and hind limbs brown covered with numerous white spots (Figure 4C).

**Morphological variation:** Paratypes generally match the overall characters of the holotype (see Table 4). Females are larger than males (SVL 21.51–22.75 mm in five adult males and 25.67 mm in a single adult female). All males have small slit-like vocal sac openings, and indistinct nuptial spines on the dorsal surfaces of first and second fingers, which are absent in females. The number and size of blotches on flanks is variable.

**Etymology:** The specific epithet “*feii*” is a patronymic noun in the genitive singular; derived from the name of Prof. Liang Fei of the Chengdu Institute of Biology, CAS, China, in recognition

**Table 4** Measurements of type series of *Leptobranchella feii* sp. nov. (All measurements are in mm)

Species	<i>Leptobranchella feii</i> sp. nov.					
	Holotype	Paratype	Paratype	Paratype	Paratype	Paratype
Voucher Nos.	KIZ 032625	KIZ 048893	KIZ 048894	KIZ 048921	KIZ 048972	KIZ 048973
Sex	M	M	M	M	M	M
EYE	3.09	3.01	2.96	3.27	3.83	3.21
FAL	6.01	6.10	5.06	4.82	6.22	5.83
FEM	1.03	1.07	1.12	1.07	1.43	1.11
FIIL	3.84	3.31	3.31	3.75	3.96	3.97
FOT	9.94	9.96	9.40	9.90	10.31	11.35
HDL	9.70	9.67	8.96	8.36	9.84	9.32
HDW	8.37	8.54	7.57	8.65	9.12	9.00
HLL	37.03	37.42	35.32	34.49	40.16	37.40
IMT	1.01	1.09	1.16	1.09	0.77	0.97
IND	2.52	2.70	2.15	2.29	2.47	2.13
IOD	2.64	2.69	2.81	2.29	2.51	3.00
ML	5.96	6.49	6.07	5.87	5.70	6.03
NEL	2.20	2.12	2.26	1.24	2.20	1.78
NSL	1.36	1.22	1.37	1.21	1.70	1.39
PEC	1.19	0.66	0.80	1.07	1.37	1.21
SNT	3.36	3.63	3.44	2.98	3.85	3.56
SVL	22.75	22.48	21.51	22.01	25.67	22.33
TEY	1.03	1.02	1.30	0.73	0.75	0.74
THL	10.36	11.29	11.47	10.78	13.00	12.29
TIB	10.42	10.95	10.57	10.98	12.73	11.91
TMP	1.88	1.67	1.78	1.59	1.69	1.68
UEW	1.88	1.85	2.43	2.28	2.56	2.04

For abbreviations see Material and Methods. M: Male; F: Female.

of his long-term and significant contribution to the advancement of amphibian research in China, particularly his research on the family Megophryidae. For the common name, we recommend “Fei’s Leaf Litter Toad” (English) and “Fei Shi Zhang Tu Chan” (费氏掌突蟾) (Chinese).

**Distribution and habits:** *Leptobrachella feii* sp. nov. is currently known to occur at two disjunct localities: Xiaoqiaogou Nature Reserve, Xichou County, Wenshan City, Yunnan Province, China, and Daweishan Nature Reserve, Pingbian County, Yunnan Province, China (Figure 1); these two sites are separated by a flying distance about 150 km. The new species was found along small seeps in well-preserved montane evergreen broadleaf forest at elevations between 1 577 m and 1 850 m a.s.l. (Figure 9). The new species is sympatric with *L. bourreti*. During our field surveys, males were usually recorded while calling sitting on vegetation (usually on the members of the family Acanthaceae species; see Figure 9). Advertisement calls of the new species, resembling calling of orthopterans, could be heard along the streams from March to June; gravid female collected in April were swollen with pure white eggs.

**Comparison:** When compared with the 26 known congeners occurring south of the Isthmus of Kra, *Leptobrachella feii* sp. nov. can be easily distinguished from *L. arayai*, *L. dringi*, *L. fritinniensis*, *L. gracilis*, *L. hamidi*, *L. heteropus*, *L. kajangensis*, *L. kecil*, *L. marmorata*, *L. melanoleuca*, *L. maura*, *L. picta*, *L. platycephala*, *L. sabahmontana*, and *L. sola* by the presence of supra-axillary and ventrolateral glands (vs. absence); and from the following miniaturized species it can be distinguished by presence of rounded digit tips (vs. pointed digit tips in all species listed below) and by having an obviously larger body size (SVL 22.5–23.6 mm in males of the new species): *L. baluensis* (14.9–15.9 mm in males), *L. brevicrus* (17.1–17.8 mm in males), *L. bondangensis* (17.8 mm in males), *L. fusca* (16.3 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. natunae* (17.6 mm in males), *L. parva* (15.0–16.9 mm in males), *L. palmata* (14.4–16.8 mm in males) and *L. serasanae* (16.9 mm in female).

*Leptobrachella feii* sp. nov. differs from all other species of *Leptobrachella* occurring north of the Isthmus of Kra by a combination of small body size, distinct tympanum and supratympanic line, dorsal skin texture, ventral coloration, distinct ventrolateral glands, degree of webbing and fringing on the toes, relatively long head, distinct black blotches scattered on the flanks, longitudinal ridges under the toes distinct and uninterrupted at the articulations, and a bicolored iris (Table 2).

Specifically, *Leptobrachella feii* sp. nov. can be easily distinguished from its phylogenetically closely related congeners. The new species differs from *L. aerea* by having a bicolored iris (vs. uniform), relatively smaller body size in males (SVL 21.5–22.5 vs. 25.1–28.9), narrow fringes on toes (vs. wide), as well as the presence of black blotches on flanks (vs. absence) and the presence of distinct supratympanic fold (vs. absence). The new species also differs from *L. nyx* by

having a relatively smaller body size (SVL 21.51–22.75 mm in males, 25.67 mm in female vs. SVL 26.7–32.6 mm in males, 37.0–41.0 mm in females), by a golden-orange upper iris coloration and a silvery-white lower iris coloration (vs. iris copper above, golden below), as well as the presence of distinct black blotches on the flank (vs. indistinct), and the presence of lateral fringes on toes (vs. absence). *Leptobrachella feii* sp. nov. differs from *L. minima* by having a relatively smaller body size (SVL 21.51–22.75 mm in males, 25.67 mm in female vs. SVL 25.7–31.4 mm in males, 31.6–37.3 mm in females), by shagreened dorsal surfaces (vs. smooth); by distinct black blotches scattered on the ventral surfaces (vs. indistinct), by distinct pectoral glands (vs. indistinct), as well as by the presence of lateral fringes on toes (vs. absence). The new species differs from *L. ventripunctatus* by having a relatively smaller body size (SVL 21.51–22.75 mm in males, 25.67 mm in female vs. SVL 25.5–28.0 mm in males, 31.5–35.0 mm in females), by iris coloration with golden orange above and silver white below (vs. copper above and grey-brown below), by sparse black spots on belly (vs. dense), as well as by the presence of dermal fringes on toes (vs. absence) and by the absence of longitudinal skin folds on dorsum (vs. presence).

For the remaining known *Leptobrachella* species from Yunnan, *Leptobrachella feii* sp. nov. differs from *L. purpurus* by having a relatively smaller body size in males (SVL 21.51–22.75 mm vs. SVL 25.0–27.5 mm), a relatively longer hind limbs (males HLL/SVL ratio 1.56–1.67 vs. 1.41–1.48), by presence of narrow lateral fringes on the toes (vs. wide lateral fringes on toes), by olive brown dorsum coloration in life (vs. purplish-brown), by dorsal skin shagreened with brown tubercles (vs. reddish tubercles), as well as by the absence of black markings on dorsum and flanks mottled with distinct yellow pigmentation in life (vs. presence). The new species further differs from *L. yingjiangensis* by having a relatively smaller body size in males (SVL 21.51–22.75 mm vs. SVL 25.7–27.6 mm), by narrow lateral fringes on the toes (vs. wide), by throat covered with dense small black spots (vs. throat pinkish and transparent), as well as by the absence of distinct tiny white flecks on edges of dark brown markings on dorsum (vs. presence) and the absence of dermal fringes on fingers (vs. presence on 2nd to 4th fingers). *Leptobrachella feii* sp. nov. differs from *L. tengchongensis* by having a relatively smaller body size (SVL 21.51–22.75 mm in males, 25.67 mm in female vs. SVL 23.9–26.0 mm in males, 28.8–28.9 mm in females), by a relatively longer head in males (HDL/SVL=0.38–0.43 vs. 0.34–0.35), by presence of brown tubercles on dorsum in life (vs. reddish tubercles), by distinct ventrolateral glands (vs. indistinct), by a bicolored iris (vs. iris uniformly colored). The new species differs from *L. alpina* by having a relatively smaller body size (SVL 21.51–22.75 mm in males, 25.67 mm in female vs. SVL 24.0–26.4 mm in males, 31.7–32.5 mm in females), by relatively longer hind limbs (mean HLL/SVL ratio 1.57–1.67 vs. 1.55 for males), by having head longer than wide (vs. approximately equal), and by presence of narrow dermal fringes on toes (vs. wide), as well

as the absence of distinct white tiny flecks on dorsum (vs. presence).

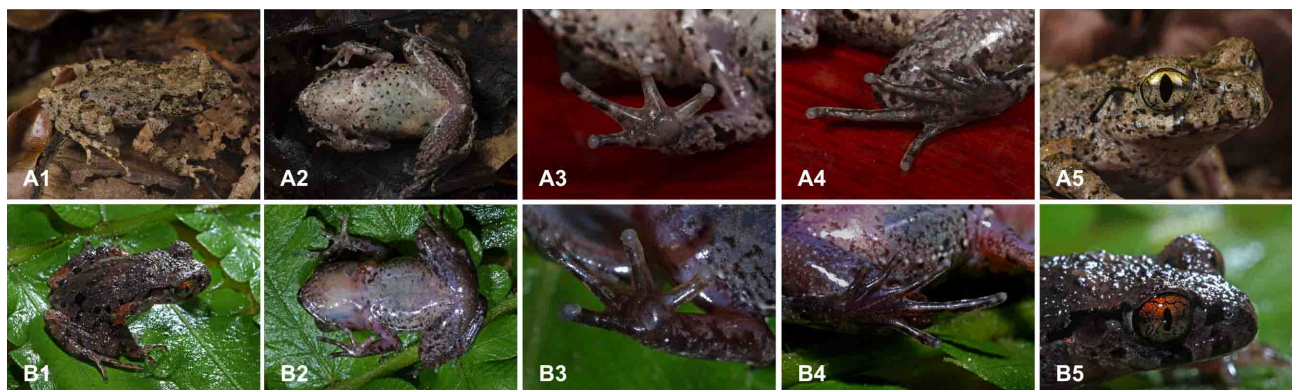
Lastly, *Leptobranchella feii* sp. nov. differs from the newly described and sympatrically co-occurring species *Leptobranchella flaviglandulosa* (see above) by having a relatively smaller body size (SVL 21.51–22.75 mm in males, 25.67 mm in female vs. SVL 22.99–26.97 mm in males, 29.30 mm in female), by having distinct ventrolateral glands forming a continuous line (vs. discontinuous), by longitudinal ridges uninterrupted at the articulations on toes (vs. interrupted), by white femoral, pectoral and ventrolateral glands (vs. yellow), by dorsal skin scattered with brown tubercles (vs. yellowish-brown). In addition, *Leptobranchella feii* sp. nov. differs from the newly described species *L. niveimontis* (see above) by having a relatively longer head (mean HDL/SVL=0.41 vs. 0.35 in males), by black supratympanic ridge (vs. reddish), by dorsal skin scattered with brown tubercles (vs. reddish), as well as by the absence of rounded whitish speckles on cloacal region (vs. presence), and by the absence of marbled pattern on the ventral surfaces (vs. presence).

## DISCUSSION

Recent large-scale phylogenetic analysis showed that there are at least 15 genetic lineages of *Leptobranchella* that may represent new species (Chen et al., 2018). Combining morphological and molecular lines of evidence, our present work confirms three of them as new species. Our results bring the total number of *Leptobranchella* to 78, and the number of *Leptobranchella* known from China to 23. In addition to the three new taxa described herein, the following species of *Leptobranchella* are known from China: *L. alpina*, *L. bourreti*, *L. bijie*, *L. eos*, *L. laui*, *L. liui*, *L. mangshanensis*, *L. maoershanensis*, *L. nyx*, *L. oshanensis*, *L. pelodytoides*, *L. purpurus*, *L. purpuraventra*, *L. shangsiensis*, *L. sungi*, *L. tengchongensis*, *L. ventripunctata*, *L. wuhuangmontis*, *L. yingjiangensis* and *L. yunkaiensis* (AmphibiaChina, 2019; Chen et al., 2018; Frost, 2019).

In addition, one lineage from Longchuan County (*L. cf. yingjiangensis*, sample 27; Figures 2, 11, Supplementary Table S2) may also represent another putative new species. This sample from Longchuan County showed some distinct morphological characters when compared with *L. yingjiangensis* s. str., including a dorsal coloration, ventral markings, and different iris coloration (upper half red, lower half bluish-white vs. upper half orange yellow, lower half silver white in *L. yingjiangensis* s. str.) (Figure 11). In addition, the sequence divergence ( $p$ -distance=2.9% in 16S rRNA gene) between the Longchuan sample and *L. yingjiangensis* s. str. almost reached the threshold of species-level genetic divergence in anurans (3.0%; Vences et al., 2005). However, given that we had only a single specimen from Longchuan lineage, we tentatively assign the Longchuan sample as *L. cf. yingjiangensis*, pending further taxonomic works following collection of additional material. Our description of the female of *L. yingjiangensis* (Figure 11, Supplementary Table S2) should aid that future comparison.

Our results reinforce the finding that the micro-endemic nature and sympatric distributions of different highly divergent lineages of *Leptobranchella* complicates taxonomy of the group, as revealed previously (e.g., Rowley et al., 2015b). Based on our field work in Xiaoqiaogou Nature Reserve, adults and tadpoles of multiple species of *Leptobranchella* (*L. flaviglandulosa*, *L. nyx*, *L. feii*, and *L. bourreti*) were found in the same stream at the same time. These four species in Xiaoqiaogou Nature Reserve were all previously mistaken with *L. pelodytoides* (Yang & Rao, 2008), which was until recently considered to be a notoriously “widespread” species in *Leptobranchella*. Recent studies have demonstrated, however, that *L. pelodytoides* represents a complex of numerous distinct species, and that the real extent of distribution of *L. pelodytoides* s. str. is poorly understood (e.g., Chen et al., 2018; Ohler et al., 2011; Yang et al., 2016). Therefore, future taxonomic studies need to be cautious about the possibility of confusing multiple morphologically similar species that co-



**Figure 11** Comparisons of samples from Longchuan County and *L. yingjiangensis* s. str. in life

A: Female of *L. yingjiangensis* (KIZ027521) from Yingjiang County at type locality; B: *L. cf. yingjiangensis* (KIZ048513) from Longchuan County, Yunnan. 1: Dorsolateral view; 2: Ventral view; 3: Right hand in volar view; 4: Foot in plantar view; 5: Head in lateral view showing distinct iris coloration.

occur at the same microhabitat. Further evolutionary studies are needed to explore the mechanisms of the sympatric coexistence of multiple lineages alongside with wide distribution of point-endemic species in *Leptobranchella*. In addition, regarding the new species *Leptobranchella niveimontis*, our field surveys demonstrated that this species occurs above 2 500 m of elevation in the Daxueshan Mountains, higher than any other *Leptobranchella* species reported from China to date, which is the second known species of *Leptobranchella* occurring above 2 500 m (the other is *L. botsfordi* from northern Vietnam). *Leptobranchella niveimontis* is vulnerable to climate change as it is restricted to a narrow zone of high-elevation habitat. The differential niche requirements might be one of the factors that lead to the isolation and speciation among these montane species.

Lastly, our study further highlights the lack of knowledge on amphibian taxonomy and the underestimated biodiversity along the border areas in southern China. Considering the continuous forests habitats that stretch across the international borders in this region, we expect that the new species found in this study may occur in the adjacent Southeast Asian countries (in particular, in northern Myanmar and Vietnam). We call for focal biodiversity surveys and international collaboration along these international borders in Southeast Asia (e.g., [Chen et al., 2017](#); [Stuart and Bain, 2008](#)), and propose collaborative conservation efforts across these habitats for better estimation and preserve of the rich biodiversity in these politically sensitive areas.

#### Key to species of *Leptobranchella* Smith, 1925 from Yunnan Province, China

- 1a) Black spots on flanks absent; distinct supratympanic fold absent ..... *L. eos*
- 1b) Black spots on flanks present; distinct supratympanic fold present ..... 2
- 2a) Toes with one third webbing ..... *L. pelodytoides*
- 2b) Toes with rudimentary or poorly developed webbing ... 3
- 3a) Lateral fringes on toes absent ..... 4
- 3b) Lateral fringes on toes present ..... 5
- 4a) Dorsal skin with rounded tubercles; the lower half of iris golden in life; black blotches on flanks indistinct; ventral side whitish with brown edges ..... *L. nyx*
- 4b) Dorsal skin with longitudinal skin ridges; the lower half of iris grey-brown in life; black blotches on flanks distinct; ventral side densely covered with small black spots ..... *L. ventripunctata*
- 5a) Iris uniformly colored ..... *L. tengchongensis*
- 5b) Iris bicolored ..... 6
- 6a) Fringes on toes wide ..... 7

- 6b) Fringes on toes narrow ..... 8
- 7a) Distinct white tiny flecks on dorsum absent, dorsal surfaces purplish brown in life ..... *L. purpureus*
- 7b) Distinct white tiny flecks on dorsum present, dorsal surfaces brown or grey brown in life ..... 9
- 8a) Dermal ridges under toes indistinct; body size medium (SVL  $\geq$ 28.0 mm in males, >42.0 mm in females); dorsal skin relatively smooth ..... *L. bourreti*
- 8b) Dermal ridges under toes distinct; body size small (SVL < 27.0 mm in male; <30.0 mm in female); dorsal skin shagreened with small tubercles ..... 10
- 9a) Dermal fringes on fingers absent; pectoral gland larger than tips of fingers and femoral gland; distinct dark brown spots/blotches present on belly ..... *L. alpina*
- 9b) Dermal fringes on fingers present; pectoral gland smaller than tips of fingers and femoral gland; medial belly immaculate creamy white ..... *L. yingjiangensis*
- 10a) Femoral, pectoral and ventrolateral glands yellow in life; longitudinal ridges under toes interrupted at the articulations; ventrolateral glands discontinuous ..... *L. flaviglandulosa*
- 10b) Femoral; pectoral and ventrolateral glands white; longitudinal ridges under toes uninterrupted at the articulations; ventrolateral glands continuous ..... 11
- 11a) Supratympanic ridge black; rounded whitish speckles on cloacal region absent; creamy white chest and belly scattered with black blotches ..... *L. feii*
- 11b) Supratympanic ridge reddish; rounded whitish speckles on cloacal region present; marbling venter scattered with black speckling ..... *L. niveimontis*

#### NOMENCLATURAL ACTS REGISTRATION

The electronic version of this article in portable document format will represent a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new names contained in the electronic version are effectively published under that Code from the electronic edition alone (see Articles 8.5–8.6 of the Code). This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information can be viewed through any standard web browser by appending the LSID to the prefix <http://zoobank.org/>.

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*Leptobranchella niveimontis* LSID:

urn:lsid:zoobank.org:act:16C89C0C-26A7-47DC-BC7E-F7B98444D825.

*Leptobranchella flaviglandulosa* LSID:

urn:lsid:zoobank.org:act:7853EFEC-52C9-41C2-9831-



269F91AB8180.

*Leptobranchella feii* LSID:

urn:lsid:zoobank.org:act:60A73137-DBD1-43D4-AFBC-1EB7927A30BA.

## SCIENTIFIC FIELD SURVEY PERMISSION INFORMATION

Permission for field surveys in Yunnan Province was granted by the Forestry Department and National Reserves of China. Collecting permit (Y601741431) was issued by the Forestry Department of Yunnan Province.

## SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

## COMPETING INTERESTS

The authors declare that they have no competing interests.

## AUTHORS' CONTRIBUTIONS

J.C. and J.M.C. designed the study. J.M.C., Z.Y.Y., and M.H. collected specimens in the field. J.M.C., S.C. and K.X. performed molecular experiments. K.X. measured the specimens. J.M.C. performed data analyses. J.M.C., N.A.P., K.W., and J.C. wrote the manuscript. All authors read and approved the final version of the manuscript.

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