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A new species of *Leptobrachella* (Anura, Megophryidae) from Mount Pu Ta Leng, northwest Vietnam

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Abstract

The Hoang Lien Range in northwest Vietnam is known to harbour five species of *Leptobrachella*, a specious genus of terrestrial frogs. We collected specimens of *Leptobrachella* from Mount Pu Ta Leng on the second highest peak in the Hoang Lien Range and use morphological and molecular data to show that this population represents a previously undescribed species which we name *Leptobrachella graminicola* **sp. nov.** after the abundance of calling males on sedge-like plants. This new species is closely related to *L. bourreti* but the new species is distinguished from *L. bourreti* and other congeneric species by a combination of the following characters: (1) a body size range of 23.1–24.6 mm in six adult males and 28.6–32.9 mm in five adult females; (2) skin on dorsum smooth, with many tubercles and lacking dermal ridges; (3) toes with rudimentary webbing and broad lateral fringes; (4) belly white with brown spots; (5) throat dark brown with light grey-brown flecks and spots; (6) a row of large white spots on the outer edge of the tarsus extending from the heel to the inner metatarsal tubercle, sometimes forming a long white stripe; and (7) a bicoloured iris. The new, high-elevation species is likely to be range-restricted and threatened by habitat loss and the activities of tourists.

Tóm tắt tiếng Việt

Dãy núi Hoàng Liên Sơn ở vùng núi Tây Bắc Việt Nam có sự đa dạng cao các loài lưỡng cư trong họ Cóc Mày Megophryidae, trong đó có 5 loài thuộc giống cóc bùn *Leptobrachella* thuộc họ này được ghi nhận. Tại ngọn núi cao thứ hai Việt Nam, núi Pu Ta Leng, chúng tôi đã thu thập một quần thể *Leptobrachella* và kết quả phân tích hình thái cũng như sinh học phân tử cho thấy đây là một loài chưa được mô tả. Từ đó chúng tôi đặt tên loài mới này là cóc lá cỏ *Leptobrachella graminicola* **sp. nov.**, với tên gọi thể hiện tập tính đặc biệt của loài này luôn được tìm thấy trên các lá cỏ ven suối. Loài này có đặc điểm hình thái ngoài gần giống với loài cóc mày bou-re, *Leptobrachella bourreti*, tuy nhiên loài mới có sự khác biệt với loài *L. bourreti* và các loài khác trong giống bởi những đặc điểm sau: (1) cơ thể kích thước trung bình, từ 23.1–24.6 mm ở 6 con đực trưởng thành, và 28.6–32.9 mm ở 5 con cái trưởng thành, (2) da lưng nhẵn với nhiều mụn nhỏ và không có các gờ da, (3) bàn chân có màng bơi vừa phải và rèm da bên các ngón phát triển, (4) bụng màu trắng đục với các đốm nâu, (5) họng màu nâu tối với các vệt và đốm sáng nhạt, (6) một hàng đốm trắng ở mép ngoài gót chân, đôi khi liền thành 1 vệt trắng dài và (7) mắt có hai màu khác biệt. Loài mới được khám phá cho thấy mức độ đa dạng và quan trọng của Dãy Hoàng Liên Sơn. Tuy nhiên, môi trường sống của loài này hiện rất hẹp và đang bị ảnh hưởng bởi sự mất sinh cảnh sống và hoạt động du lịch.

Key words: Amphibian, Frog, Hoang Lien, Leptobrachiinae, *Leptolalax*

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Introduction

The genus *Leptobrachella* Smith, 1925 (Anura Megophryidae) contains 87 species of which 28 are reported to occur in Vietnam (Frost 2021). Frogs in the genus *Leptobrachella* are subject to high rates of species discovery, over 70% of the currently described species have been described since the year 2000. The genus is distributed from northeast India, southern China, and mainland Southeast Asia to the islands of the Sunda Shelf to the south (Frost 2021). *Leptobrachella* are morphologically conserved and morphology alone may be insufficient to delineate species (Rowley *et al*. 2016; Chen *et al*. 2018), therefore an integrative approach is required when assigning populations to species. The Hoang Lien Range in northern Vietnam is continuous with the Ailao Mountains in Yunnan, southern China. Five *Leptobrachella* species have been reported from the Hoang Lien Range in Vietnam; *Leptobrachella botsfordi* (Rowley, Dau & Nguyen 2013)*, L. bourreti* (Dubois 1983)*, L. pluvialis* (Ohler, Marquis, Swan & Grosjean 2000), *L. sungi* (Lathrop, Murphy, Orlov & Ho 1998), and *L. ventripunctata* (Fei, Ye & Li 1990). *Leptobrachella botsfordi* is endemic to the high elevations of Mount Fansipan (IUCN 2015; Nguyen *et al*. 2020), while *L. sungi* is known only from lower elevations (331 m asl) within the Hoang Lien Range in Van Ban District (Bain *et al*. 2007). *Leptobrachella bourreti, L. pluvialis,* and *L. ventripunctata* are known to be sympatric on Mount Fansipan (Ohler *et al*. 2000, 2011; Chen *et al*. 2018).

During field work in the Hoang Lien Range on the southern slope of Vietnam's second highest Mountain, Mount Pu Ta Leng, we encountered a population of *Leptobrachella* that could not be assigned to any of the five species known from the range, nor to any of its congeners from mainland Southeast Asia, and neighbouring provinces of China. We describe this population as a new species on the basis of morphological and genetic differences.

Materials and methods

Eleven specimens were collected at night in forest stream habitats on the southern slope of Mount Pu Ta Leng, Bat Xat District, Lao Cai Province, Vietnam, in October 2019. Geographic coordinates were obtained using a Garmin GPSMAP 60CSx Global Positioning System receiver (Garmin Ltd. Kansas, USA) and recorded as a World Geodetic System 1984 datum. Specimens were photographed in life before being humanely euthanised using a 20% solution of benzocaine applied to the ventral surface of the frog. Tissue samples (liver) for molecular analyses were extracted from freshly euthanised specimens and stored in molecular-grade ethanol prior to fixation of specimens with 6% formalin and storage in 70% ethanol. Type specimens were deposited at the Vietnam National Museum of Nature, Hanoi (VNMN). A referred specimen was deposited at the Hoang Lien National Park headquarters (HLNP) as a reference for National Park scientists. Comparative material was examined at the respective institutions (see Appendix 1).

Molecular data: Total genomic DNA was extracted from ethanol-preserved tissues using a DNeasy® Blood and Tissue Kit (QIAGEN GmbH, Hilden, Germany), following the manufacturer's protocols for purification of genomic DNA from animal tissues. We amplified a section of 16S (mtDNA) using the primers 16sl2021 (5'-CCTACCGAGCTTAGTAATAGCTGGTT-3') modified from Hedges (1994) and Hedges16H1 (5'-CTCCGGTCTGAACTCAGATCACGTAGG-3') by Hedges and Maxson (1993). PCR amplification was carried out in 25-µL reactions volume, including 12.5-µL 2 x Taq Master Mix (CWBIO, China), 0.75-µL of each primer (10pmol/µL), 1-µL of cDNA template, and 10-µL ddH_2O. Negative controls were included in each PCR batch. Thermocycling was performed on an Eppendorf Mastercycler EpS (Eppendorf, Hamburg, Germany) under the following conditions: initial denaturation 94°C (5mins), followed by 35 cycles of 94°C (1 min) denaturation, 55°C (1 min) annealing and 72°C (1 min) extension, followed by a final extension step at 72°C (10 mins). All PCR products were purified using Exo-Sap-ITTM (USB Corporation, Ohio USA), and sequenced in both 5' and 3' directions at Macrogen (Seoul, South Korea). Sequence chromatograms were edited and checked by eye for quality using BioEdit V. 7.0.5.3 (Hall 1999). The new sequences were then checked on BLAST (The National Center for Biotechnology Information) (Altschul *et al*. 1990) to verify their approximate identity and sequences were deposited in GenBank under accession numbers MZ224647–9, MZ224651, MZ224653 and MZ224655 (Table 1).

Phylogenetic analysis: To determine the phylogenetic position of our samples within the genus *Leptobrachella*, we used 115 sequences of 16S available on GenBank (Benson *et al*. 2017) including 111 sequences from *Leptobrachella* and four outgroups in the genera *Megophrys* and *Leptobrachium* following Chen *et al*. (2018, 2020).

Additional sequences from other *Leptobrachella* that are known to occur at similar elevations in the Hoang Lien Range were also included in the phylogenetic analysis.

New and previously published sequences were aligned using MAFFT online service (version MAFFT 7.45) using the default setting (Katoh *et al*. 2019). The final aligned sequences were then trimmed to match the length of new sequences using MEGA 7 (Kumar *et al*. 2016). Locality information and GenBank accession numbers for all sequences included in the analyses can be found in Table 1. Uncorrected *p*-distance (with partial deletion of gaps and missing data) were calculated using MEGA 7.

The matrilineal genealogy was inferred using two methods: Bayesian inference (BI) and maximum likelihood (ML). The optimal evolutionary models for both BI and ML analyses were tested using MODELTEST v. 3.06 (Posada and Crandall 1998), and the best-fit model for both BI and ML were the GTR+G+I model of DNA evolution (suggested by the Akaike information criterion).

BI analysis was conducted in MRBAYES v. 3.2 (Ronquist *et al*. 2012); Metropolis-coupled Markov chain Monte Carlo (MCMCMC) analyses were run for 10 million generations and sampled every 1000 generations. Five independent MCMCMC runs were performed, and the first 25% of trees were discarded as burn-in. The remaining trees were combined, and a 50% majority-rule consensus tree was generated. Confidence in topology was assessed by posterior probability (BPP, Huelsenbeck *et al*. 2001). The ML was performed using IQ-TREE v.2.1.2 (Minh *et al*. 2020) with 10,000 ultrafast bootstrap replications (UFB) (Hoang *et al*. 2018). We considered Bayesian posterior probability (BPP) and ultrafast bootstrap (UFB) support values of ≥0.95 to indicate strong support (Felsenstein 1985; Hoang *et al.* 2018).

Morphological data: Morphological data were recorded to the nearest 0.1 mm from fixed specimens with digital callipers. Morphometric measurements include: snout to vent length (SVL); head length from tip of snout to rear of jaw (HDL); head width at commissure of jaw (HDW); snout length from tip of snout to anterior corner of eye (SNT); diameter of exposed portion of eyeball (EYE); interorbital distance (IOD); horizontal diameter of tympanum (TMP); distance from anterior edge of tympanum to posterior corner of eye (TEY); distance from nostril to anterior edge of eye (EN); distance between nostrils (IN); distance from nostril to tip of snout (NS); tibia length with hindlimb flexed (TIB); femur length (FEL); manus length from tip of third digit to proximal edge of inner metacarpal tubercle (ML); pes length from tip of fourth toe to proximal edge of the inner metatarsal tubercle (PL); maximum diameter of pectoral gland (PEC); maximum diameter of femoral gland (FEM); maximum length of inner metacarpal tubercle (IMCTL); maximum length of outer metacarpal tubercle (OMCTL) and maximum length of inner metatarsal tubercle (IMTTL). Body mass was recorded in life (to the nearest 0.1 g), within 12 hours of specimen collection using a Pesola® spring scales (Pesola AG, Schindellegi, Switzerland).

We obtained comparative morphological data from museum specimens of *Leptobrachella* and photographs of these specimens in life (Appendix 1), and from original descriptions for the following species of *Leptobrachella* reported for Asia north of the Isthmus of Kra: *L. aerea* (Rowley, Stuart, Richards, Phimmachak & Sivongxay 2010a); *L. alpina* (Fei, Ye & Le 1990 in Fei *et al*. 1990); *L. applebyi* (Rowley & Cao 2009); *L. ardens* (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen, and Ziegler 2016); *L. aspera* Wang, Lyu, Qi, & Wang 2020; *L. bashaensis* Lyu, Dai, Wei, He, Yuan, Shi, Zhou, Ran, Kuang, Guo, Wei and Yuan 2020; *L. bidoupensis* (Rowley, Le, Tran, & Hoang 2011); *L. bijie* Wang, Li, Li, Chen & Wang 2019 (in Wang *et al*. 2019); *L. botsfordi*; *L. bourreti* (data from Ohler *et al*. 2011); *L. chishuiensis* Li, Liu, Wei & Wang 2020; *L. crocea* (Rowley, Hoang, Le, Dau & Cao 2010c); *L. dorsospina* Wang, Lyu, Qi & Wang 2020 (in Wang *et al*. 2020); *L. eos* (Ohler, Wollenberg, Grosjean, Hendrix, Vences, Ziegler & Dubois 2011); *L. feii* Chen, Yuan & Che 2020 (in Chen *et al*. 2020); *L. firthi* (Rowley, Hoang, Dau, Le & Cao 2012); *L. flaviglandulosa* Chen, Wang & Che 2020 (in Chen *et al*. 2020); *L. fuliginosa* (Matsui 2006); *L. isos* (Rowley, Stuart, Neang, Hoang, Dau, Nguyen & Emmett 2015a); *L. jinshaensis* Cheng, Shi, Li, Liu, Li, and Wang, 2021; *L. kalonensis* (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen, and Ziegler 2016); *L. khasiorum* (Das, Tron, Rangad, & Hooroo 2010); *L. lateralis* (Anderson 1871; data from Wang *et al*. 2020); *L. laui* (Sung, Yang & Wang 2014); *L. liui* (Fei & Ye 1990 in Fei *et al*. 1990); *L. macrops* (Duong, Do, Ngo, Nguyen & Poyarkov 2018); *L. maculosa* (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler 2016); *L. mangshanensis* (Hou, Zhang, Hu, Li, Shi, Chen, Mo & Wang 2018); *L. maoershanensis* (Yuan, Sun, Chen, Rowley & Che 2017); *L. melanoleuca* (Matsui 2006); *L. melica* (Rowley, Stuart, Neang & Emmett 2010b); *L. minima* (Taylor 1962; data from Ohler *et al*. 2011); *L. nahangensis* (Lathrop, Murphy, Orlov & Ho 1998); *L. namdongensis* Hoang, Nguyen, Luu, Nguyen & Jiang 2019; *L. neangi* Stuart & Rowley 2020; *L. niveimontis* Chen, Poyarkov, Yuan & Che 2020 (in Chen *et al*. 2020); *L. nokrekensis* (Mathew & Sen 2010); *L. nyx* (Ohler, Wollenberg, Grosjean, Hendrix, Vences,

TABLE 1. Specimens, localities, and GenBank accession numbers of *Leptobrachella* used in this study.

No.	Taxa	Voucher No.	GenBank No.	Locality	Reference
1	*Leptobrachella graminicola* **sp. nov.**	VNMN 010910	MZ224655	Mount Pu Ta Leng, Lao Cai, Vietnam	This study
2	*Leptobrachella graminicola* **sp. nov.**	VNMN 010909	MZ224649	Mount Pu Ta Leng, Lao Cai, Vietnam	This study
3	*Leptobrachella graminicola* **sp. nov.**	VNMN 010904	MZ224651	Mount Pu Ta Leng, Lao Cai, Vietnam	This study
4	*Leptobrachella graminicola* **sp. nov.**	VNMN 010905	MZ224648	Mount Pu Ta Leng, Lao Cai, Vietnam	This study
5	*Leptobrachella graminicola* **sp. nov.**	VNMN 010912	MZ224647	Mount Pu Ta Leng, Lao Cai, Vietnam	This study
6	*Leptobrachella graminicola* **sp. nov.**	VNMN 010908	MZ224653	Mount Pu Ta Leng, Lao Cai, Vietnam	This study
7	*L. aerea*	NCSM 76038	MH055809	Vilabuly, Savannakhet, Laos	Chen *et al*. 2018
8	*L. alpina*	KIZ046816	MH055866	Huangcaoling, Yunnan, China	Chen *et al*. 2018
9	*L. alpina*	KIZ049024	MH055867	Caiyanghe, Yunnan, China	Chen *et al*. 2018
10	*L. applebyi*	ZMMU-A-5529-06042	MH055948	Thua Thien-Hue, Vietnam	Chen *et al*. 2018
11	*L. arayai*	BORNEEISIS 22931	AB847558	Liwagu, Kinabalu, Malaysia	Matsui *et al*. 2014
12	*L. ardens*	ZMMU-NAP-06099	MH055949	Kon Ka Kinh National Park, Gia Lai, Vietnam	Chen *et al*. 2018
13	*L. aspera*	SYS a007743	MW046199	Huanglianshan, Lyuchun, Yunnan, China	Wang *et al*. 2020
14	*L. baluensis*	SP 21604	LC056792	Tambunan, Sabah, Malaysia	Eto *et al*. 2015
15	*L. bashaensis*	GIB196403	MW136294	Basha Nature Reserve, Guizhou, China	Lyu *et al*. 2020
16	*L. bidoupensis*	ZMMU-A-4797-01454	MH055945	Bidoup-Nui Ba National Park, Lam Dong, Vietnam	Chen *et al*. 2018
17	*L. bijie*	SYSa007320	MK414538	Zhaozishan Nature Reserve, Guizhou, China	Wang *et al*. 2019
18	*L. bijie*	SYSa007319	MK414539	Zhaozishan Nature Reserve, Guizhou, China	Wang *et al*. 2019
19	*L. botsfordi*	AMS R 176540	MH055952	Fansipan, Lao Cai, Vietnam	Chen *et al*. 2018
20	*L. bourreti*	1999.566	KR827860	Sapa, Lao Cai, Vietnam	Grosjean *et al.* 2015
21	*L. bourreti*	VNMN 010916	MZ209167	Mount Pu Ta Leng, Lao Cai, Vietnam	This study
22	*L. bourreti*	ZMMU-A5636-02280	MH055872	Bat Xat District, Lao Cai, Vietnam	Chen *et al*. 2018
23	*L. bourreti*	AMS R 177673	KR018124	Sapa, Lao Cai Province, Vietnam	Rowley *et al*. 2015b
24	*L. bourreti*	AMS R.188515	MZ208835	Ky Quan San, Lao Cai, Vietnam	This study
25	*L. bourreti*	AMS R.188516	MZ224652	Ky Quan San, Lao Cai, Vietnam	This study
26	*L. bourreti*	HLNP2017 0908 00021	MZ224650	Ky Quan San, Lao Cai, Vietnam	This study
27	*L. brevicrus*	KUHE 55935	LC137810	Mulu National Park, Sarawak, Malaysia	Eto *et al*. 2016
28	*L. chishuiensis*	CIBCS20190518047	MT117053	Chishui National, Chishui City, Guizhou, China	Li *et al.* 2020
29	*L. chishuiensis*	CIBCS20190518048	MT330119	Chishui National, Chishui City, Guizhou, China	Li *et al.* 2020

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TABLE 1. (Continued)

No.	Taxa	Voucher No.	GenBank No.	Locality	Reference
30	*L. crocea*	ZMMU-NAP-02274	MH055955	Thua Thien-Hue, Vietnam	Chen *et al*. 2018
31	*L. dorsospina*	SYS a004962	MW046195	Yushe Forest Park, Shuicheng, Guizhou, China	Wang *et al*. 2020
32	*L. dorsospina*	SYS a004974	MW046197	Yushe Forest Park, Shuicheng, Guizhou, China	Wang *et al*. 2020
33	*L. dringi*	NMBE1056372	KJ831298	Gunung Mulu National Park, Sarawak, Malaysia	Oberhummer *et al.* 2014
34	*L. eos*	NCSM 80551	MH055887	Boun Tay, Phongsaly, Laos	Chen *et al*. 2018
35	*L. eos*	NCSM79810	MH055881	Xaysomboun, Vientiane, Laos	Chen *et al*. 2018
36	*L. feii*	KIZ048894	MT302634	Xiaoqiaogou Nature Reserve, Yunnan, China	Chen *et al*. 2020
37	*L. firthi*	ZMMU-NAP-02264	MH055962	Kon Ka Kinh National Park, Gia Lai, Vietnam	Chen *et al*. 2018
38	*L. flaviglandulosa*	KIZ032626	MT302633	Xiaoqiaogou Nature Reserve, Yunnan, China	Chen *et al*. 2020
39	*L. fritinniens*	FMNH 244800	MH055971	Danum Valley Field Center, Sabah, Malaysia	Chen *et al*. 2018
40	*L. fuliginosa*	KUHE 20197	LC201988	Phetchaburi, Thailand	Matsui *et al.* 2017b
41	*L. gracilis*	FMNH 273682	MH055972	Bukit Kana, Sarawak, Malaysia	Chen *et al*. 2018
42	*L. hamidi*	KUHE 17545	AB969286	Bukit Lanjan, Selangor, Malaysia	Matsui *et al.* 2014
43	*L. heteropus*	KUHE 15487	AB530453	Larut, Perak, Malaysia	Matsui *et al*. 2010
44	*L. isos*	ZMMU-NAP-02266	MH055966	Chu Mom Ray National Park, Kon Tum, Vietnam	Chen *et al*. 2018
45	*L. itiokai*	KUHE 55898	LC137806	Mulu National Park, Sarawak, Malaysia	Eto *et al*. 2016
46	*L. jinshaensis*	CIBJS20200516001	MT814014	Jinsha County, Guizhou Province, China	Cheng *et al.* 2021
47	*L. jinshaensis*	CIBJS20200516002	MT814015	Jinsha County, Guizhou Province, China	Cheng *et al.* 2021
48	*L. juliandringi*	KUHE 17557	LC056784	Lanjak Entimau, Sarawak, Borneo, Malaysia	Eto *et al*. 2015
49	*L. kajangensis*	LSUHC:4431	LC202001	Tioman, Malaysia	Matsui *et al.* 2017b
50	*L. kalonensis*	IEBR A.2014.15	KR018114	Binh Thuan, Vietnam	Rowley *et al*. 2015b
51	*L. kecil*	KUHE:52440	LC202004	Cameron, Malaysia	Matsui *et al.* 2017b
52	*L. khasiorum*	SDBDU 2009.329	KY022303	Khasi Hills, Meghalaya, India	Mahony *et al.* 2017
53	*L. laui*	SYS a002450	MH055904	Shenzhen, Guangdong, China	Chen *et al*. 2018
54	*L. liui*	ZYCA907	MH055908	Wuyi Shan, Fujian, China	Chen *et al*. 2018
55	*L. macrops*	ZMMU A-5823	MG787993	Hon Den Mt. Phu Yen, Vietnam	Duong *et al.* 2018
56	*L. maculosa*	AMS R 177660	KR018119	Ninh Thuan, Vietnam	Rowley *et al*. 2015b
57	*L. mangshanensis*	MSZTC201703	MG132198	Mangshan, Hunan, China	Hou *et al*. 2018
58	*L. maoershanensis*	KIZ07614	MH055927	Mao'er Shan, Guangxi, China	Chen *et al*. 2018

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TABLE 1. (Continued)

No.	Taxa	Voucher No.	GenBank No.	Locality	Reference
59	*L. marmorata*	KUHE:53204	AB969288	Mount Penrissen, Sarawak, Malaysia	Matsui *et al.* 2014
60	*L. maura*	SP 21450	AB847559	Kinabalu, Malaysia	Matsui *et al.* 2014
61	*L. melanoleuca*	KIZ018032	MH055968	Kapoe, Ranong, Thailand	Chen *et al*. 2018
62	*L. melica*	MVZ 258199	HM133601	Virachey National Park, Ratanakiri, Cambodia	Rowley *et al*. 2010
63	*L. minima*	NCSM 79277	MH055847	Parklai, Sainyabuli, Laos	Chen *et al*. 2018
64	*L. mjobergi*	KUHE 47872	LC056787	Gading National Park, Sarawak, Malaysia	Eto *et al*. 2015
65	*L. nahangensis*	ZMMU-NAP-02259	MH055854	Na Hang, Tuyen Quang, Vietnam	Chen *et al*. 2018
66	*L. namdongensis*	VNUF A.2017.37	MK965389	Nam Dong, Thanh Hoa, Vietnam	Hoang *et al*. 2019
67	*L. neangi*	CBC 1624	MT644613	Veal Veng District, Pursat, Cambodia	Stuart & Rowley 2020
68	*L. niveimontis*	KIZ015734	MT302618	Daxueshan Nature Reserve, Yunnan, China	Chen *et al*. 2020
69	*L. niveimontis*	KIZ028277	MT302621	Daxueshan Nature Reserve, Yunnan, China	Chen *et al*. 2020
70	*L. nyx*	AMNH A163810	DQ283381	Mount Tay Conn Linh, Ha Giang, Vietnam	Frost *et al*. 2006
71	*L. oshanensis*	KIZ025776	MH055895	Emei Shan, Sichuan, China	Chen *et al*. 2018
72	*L. oshanensis*	KIZ-YPX37492	MH055896	Emei Shan, Sichuan, China	Chen *et al*. 2018
73	*L. pallida*	ZMMU-A-4717-01740	MH055946	Bidoup-Nui Ba National Park, Lam Dong, Vietnam	Chen *et al*. 2018
74	*L. parva*	KUHE 55308	LC056791	Mulu NP, Sarawak, Borneo, Malaysia	Eto *et al*. 2015
75	*L. petrops*	ROM 13483	MH055901	Ba Vi National Park, Ha Tay, Vietnam	Chen *et al*. 2018
76	*L. picta*	AE102/S32	DQ642120	Kinabalu, Malaysia	Veith *et al*. 2006
77	*L. pluvialis*	ZMMU-A-5222-02262	MH055844	Sapa, Lao Cai, Vietnam	Chen *et al*. 2018
78	*L. pluvialis*	VNMN 010918	MZ208923	Sapa, Lao Cai, Vietnam	This study
79	*L. puhoatensis*	AMS R 184852	KY849588	Pu Hoat Nature Reserve, Nghe An, Vietnam	Rowley *et al*. 2017b
80	*L. purpuraventra*	SYSa007283	MK414523	Wujing Nature Reserve, Guizhou, China	Wang *et al*. 2019
81	*L. purpuraventra*	SYSa007284	MK414524	Wujing Nature Reserve, Guizhou, China	Wang *et al*. 2019
82	*L. purpurus*	SYS a006530	MG520354	Jinzhuzhai Village, Yunnan, China	Yang *et al*. 2018
83	*L. purpurus*	SYS a006531	MG520355	Jinzhuzhai Village, Yunnan, China	Yang *et al*. 2018
84	*L. pyrrhops*	ZMMU-A-4873-00158	MH055950	Loc Bac, Lam Dong, Vietnam	Chen *et al*. 2018
85	*L. rowleyae*	ITBCZ 2783	MG682552	Son Tra, Da Nang, Vietnam	Nguyen *et al*. 2018
86	*L. sabahmontana*	BORNEENSIS 12454	AB847550	Mahua, Crocker, Malaysia	Matsui *et al*. 2014
87	*L. shangsiensis*	NHMG1704003	MK095463	Guangxi Prov. China	Chen *et al*. 2019

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TABLE 1. (Continued)

No.	Taxa	Voucher No.	GenBank No.	Locality	Reference
88	*L. sola*	KU RMB20973	MH055973	Gunung Stong, Kelantan, Malaysia	Chen *et al*. 2018
89	*L. suiyangensis*	GZNU20180606006	MK829650	Suiyang County, Guizhou, China	Luo *et al*. 2020
90	*L. suiyangensis*	GZNU20180606003	MK829649	Suiyang County, Guizhou, China	Luo *et al*. 2020
91	*L. sungi*	ZMMU-NAP-02268	MH055862	Na Hang, Tuyen Quang, Vietnam	Chen *et al*. 2018
92	*L. tadungensis*	UNS00515	KR018121	Dak Nong, Vietnam	Rowley *et al*. 2015b
93	*L. tengchongensis*	SYS a003766	MH055897	Gaoligong Shan, Yunnan, China	Chen *et al*. 2018
94	*L. tuberosa*	ZMMU-NAP-02275	MH055959	Kon Ka Kinh National Park, Gia Lai, Vietnam	Chen *et al*. 2018
95	*L. ventripunctata*	SYS a004536	MH055831	Zhushihe, Yunnan, China	Chen *et al*. 2018
96	*L. ventripunctata*	ROM28378	MH055829	Sapa, Lao Cai, Vietnam	Chen *et al*. 2018
97	*L. wuhuangmontis*	SYSa003486	MH605578	Mt. Wuhuang, Guangxi, China	Wang *et al*. 2018
98	*L. wulingensis*	CSUFT177	MT530315	Tianzishan Nature Reserve, Zhangjiajie, Hunan	Qian *et al*. 2020
99	*L. wulingensis*	CSUFT 203	MT530318	Tianzishan Nature Reserve, Zhangjiajie, Hunan	Qian *et al*. 2020
100	*L. yingjiangensis*	SYS a006535	MG520357	Jinzhuzhai Village, Yunnan, China	Yang *et al*. 2018
101	*L. yunkaiensis*	SYS a004663	MH605584	Dawuling Forest Station, Guangdong, China	Wang *et al*. 2018
102	*L. zhangyapingi*	KIZ07460	MH055865	Pang Kom Pang Hin, Chiang Mai, Thailand	Chen *et al*. 2018
103	*Leptobrachella* sp. 1	KIZ025777	MH055874	Emei Shan, Sichuan, China	Chen *et al*. 2018
104	*Leptobrachella* sp. 1	SYS a001830	KM014810	Emei Shan, Sichuan, China	Sung *et al*. 2014
105	*Leptobrachella* sp. 2	–	NC020610	Emei Shan, Sichuan, China	Xiang *et al.* 2013
106	*Leptobrachella* sp. 3	KIZ-YPX14561	MH055873	Xiaocaoba Township, Yunnan, China	Chen *et al*. 2018
107	*Leptobrachella* sp. 4	Tissue ID: YPX32063	MH055893	Guangwu Shan, Sichuan, China	Chen *et al*. 2018
108	*Leptobrachella* sp. 4	Tissue ID: YPX32062	MH055892	Guangwu Shan, Sichuan, China	Chen *et al*. 2018
109	*Leptobrachella* sp. 5	K3427	JN848455	Doi Chiang Dao, Chiangmai, Thailand	Ohler *et al*. 2011
110	*L. wulingensis*	YPX11012	MH055891	Badagongshan Nature Reserve, Hunan, China	Chen *et al*. 2018
111	*L. wulingensis*	ZYC799	AY526215	Nanchuan, Chongqing, China	Zheng *et al*. 2004
112	*Leptobrachium huashen*	KIZ049025	KX811931	Mengyang, Yunnan, China	Chen *et al.* 2017
113	*Leptobrachium boringii*	KIZ-YPX37539	KX811930	Sichuan, China	Chen *et al.* 2017
114	*Megophrys glandulosa*	KIZ048439	KX811762	Yunnan, China	Chen *et al.* 2017
115	*Megophrys glandulosa*	KIZ046978	KX811759	Yunnan, China	Chen *et al.* 2017

Ziegler & Dubois 2011); *L. oshanensis* (Liu 1950; data from Ohler *et al*. 2011); *L. pallida* (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler 2016); *L. pelodytoides* (Boulenger 1893; Ohler *et al*. 2011); *L. petrops* (Rowley, Dau, Hoang, Le, Cutajar & Nguyen 2017a); *L. pluvialis*; *L. puhoatensis* (Rowley, Dau & Cao 2017b); *L. purpuraventra* Wang, Li, Li, Chen & Wang 2019 (in Wang *et al*. 2019); *L. purpurus* (Yang, Zeng & Wang, 2018); *L. pyrrhops* (Poyarkov, Rowley, Gogoleva, Vassilieva, Galoyan & Orlov 2015); *L. rowleyae* (Nguyen, Poyarkov, Le, Vo, Phan, Duong, Murphy & Nguyen 2018)*; L. shangsiensis* Chen, Liao, Zhou & Mo 2019); *L. suiyangensis Luo, Xiao, Gao & Zhao 2020; L. sungi*; *L. tadungensis* (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler 2016); *L. tamdil* (Sengupta, Sailo, Lalremsanga, Das & Das 2010); *L. tengchongensis* Yang, Wang, Chen & Rao 2016; *L. tuberosa* (Inger, Orlov & Darevsky 1999; Rowley *et al*. 2010c); *L. ventripunctata* (Fei, Ye & Li 1990 in Fei *et al*. 1990; data from Ohler *et al*. 2011); *L. wuhuangmontis* Wang, Yang & Wang 2018 (in Wang *et al*. 2018); *L. wulingensis* Qian, Xia, Cao, Xiao & Yang 2020; *L. yeae* Shi, Hou, Song, Jiang & Wang 2021; *L. yingjiangensis* Yang, Zeng & Wang 2018; *L. yunkaiensis* Wang, Li, Lyu & Wang (in Wang *et al*. 2018), and *L. zhangyapingi* (Jiang, Yan, Suwannapoom, Chomdej & Che 2013). Terminology for species comparison follows Rowley *et al*. (2016).

Results

Molecular data: The aligned 16S mtDNA dataset contained 1043 characters and 86 taxa including gaps and missing data. There were 453 conserved sites, 578 variable sites, and 441 parsimony-informative characters in the dataset. In the BI run, the standard deviation of split frequencies was 0.004987. Monophyly of the genus *Leptobrachella* was supported in both BI and ML analysis (BPP/UFB 1.0/99 respectively; Fig. 1).

The six samples collected from the Mount Pu Ta Leng population formed a clade (BPP/UFB 1.0/98%) (here after *Leptobrachella* sp.) which is a sister clade to *Leptobrachella* sp.1 (Chen *et al*. 2018; Sung *et al*. 2014) from Emei Shan, Sichuan, China (BPP/UFB 0.98/92%). *Leptobrachella* sp. collected from Mount Pu Ta Leng, is nested within *Leptobrachella* Clade A1 (after Chen *et al*. 2018), a radiation of *Leptobrachella* associated with southern China and Indo-Burma (Chet *et al*. 2018) which includes other *Leptobrachella* from the Hoang Lien Range (*L. bourreti, L. pluvialis, L. sungi* and *L. ventripunctata*).

Genetic diversity was low within sequences of *Leptobrachella* sp. from Mount Pu Ta Leng (*p*-distance 0.0–0.8%, N=6) which supports the hypothesis that sequenced specimens represent a single operational taxonomic unit. Uncorrected *p*-distance between *Leptobrachella* sp. and other taxa in the genus *Leptobrachella* ranged from 3.10–4.02% (*L. bourreti*) to 19.17% (*L. kecil*). A value of 3.0% is the threshold proposed for candidate species at the 16S gene (Vences *et al*. 2005) and exceeds that of other named taxa in the genus (e.g. Li *et al*. 2020; Wang *et al*. 2020; Shi *et al*. 2021). Uncorrected *p*-distance between *Leptobrachella* sp. and its closest relatives, *Leptobrachella* sp.1 (Chen *et al*. 2018; Sung *et al*. 2014) and a sympatric species, *L. bourreti,* ranged from 3.36–4.41% (N=2) for *Leptobrachella* sp.1 and from 3.10–4.02 % for *L. bourreti* (including new sequences from this study). Uncorrected *p*-distance between *Leptobrachella* sp. and the four other sympatric species from the Hoang Lien Range were 9.61% for *L. sungi* (N=1), 10.80% for *L. pluvialis* (N=2, including one new sequence from this study), 11.65–12.17% for *L. ventripunctata* (N=2), and 12.69% for *L. botsfordi* (N=1).

Molecular data combined with morphological differences (below) provide additional support that the newly collected specimens of *Leptobrachella* sp. from Mount Pu Ta Leng represent a new species.

Leptobrachella graminicola sp. nov.

Figs. 3–5 & 7–8

Holotype: VNMN 010904 (field tag Fan119), adult male on grass 0.1 m from ground, 1 m from 2–3 m wide rocky stream in evergreen broadleaf forest, Mount Pu Ta Leng, Bat Xat Nature Reserve, Lao Cai Province, Vietnam (N22.40751° E103.60984°; 2386 m elevation, Fig. 2 & 6). Collected at night on 04 October 2019, 21:11h by Luan Thanh Nguyen, Luong Van Nong, and Tam Van Ha.

Paratypes: Ten specimens, all collected at night in the same 2–3 m wide rocky stream in evergreen broadleaf forest on Mount Pu Ta Leng, Bat Xat Nature Reserve, Lao Cai Province, Vietnam by Luan Thanh Nguyen, Luong

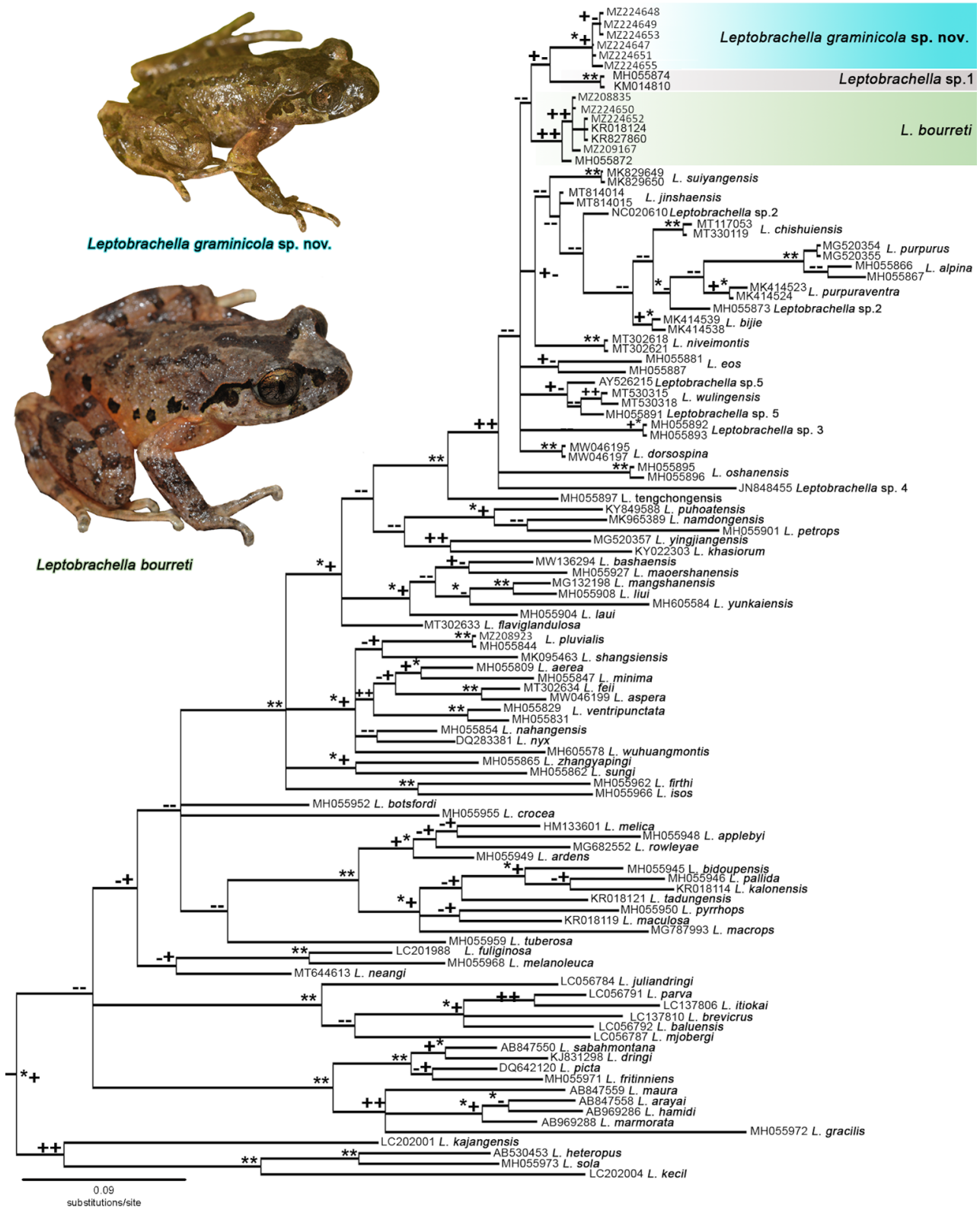

FIGURE 1. A BI phylogenetic tree for a 1043 bp section of 16S (mtDNA) gene for all *Leptobrachella* which comparative available sequences. Bayesian posterior probability and ML utral bootstrap support for nodes indicated on the tree; 1.0 BPP or 100% UFB asterisk, ≥0.95 BPP or ≥95% UFB plus, and <0.95 BPP or ≤95% UFB hyphen. Outgroups (*Leptobrachium huashen, L. boringii,* and *Megophrys glandulosa*) are not shown.

Van Nong, and Tam Van Ha: VNMN 010910 (field tag Fan117), VNMN 010909 (field tag Fan118) adult females on the blade of a sedge-like plant 0.1 m from ground, 1 m from stream, collection details the same as the holotype; VNMN 010905 (field tag Fan120) adult male and VNMN 010907 (field tag Fan124) adult females on the blade of a sedge-like plant 0.1–0.2 m from ground, middle of stream (N22.40787° E103.60900°; 2048 m elevation), collected on 04 October 2019; VNMN 010906 (field tag Fan126) adult male on the blade of a sedge-like plant 0.2 m from ground, nearby stream (N22.40820° E103.60934°; 2400 m elevation), collected on 04 October 2019; VNMN 010913 (field tag Fan128) adult female on the blade of a sedge-like plant 0.2 m from ground nearby stream (N22.40780° E103.60925°; 2405 m), collected on 04 October 2019; VNMN 010912 (field tag Fan166) and VNMN 010914 (field tag Fan172) two adult males and VNMN 010908 (field tag Fan170) adult female on the blade of a sedge-like plant 0.1–0.4 m from ground, nearby stream (N22.40700° E103.61219°; 2343 m), all collected on 05 October 2019.

Referred specimens: HLNP 20191005 00017 male on the blade of a sedge-like plant 0.1–0.4 m from ground, nearby stream (N22.40700° E103.61219°; 2343 m), collected on 05 October 2019. This specimen is not included in the type series due to it being deposited in a local collection. Its taxonomic identity is not in question.

Etymology: Specific epithet "*graminicola*" is a noun in apposition, composed from Latin *gramineus* (grass) and *incola* (inhabitant) as all individuals were encountered on graminoid plants with grass-like morphology.

Suggested vernacular name: Mount Pu Ta Leng leaf-litter frog (English), Cóc mày lá cỏ (Vietnamese).

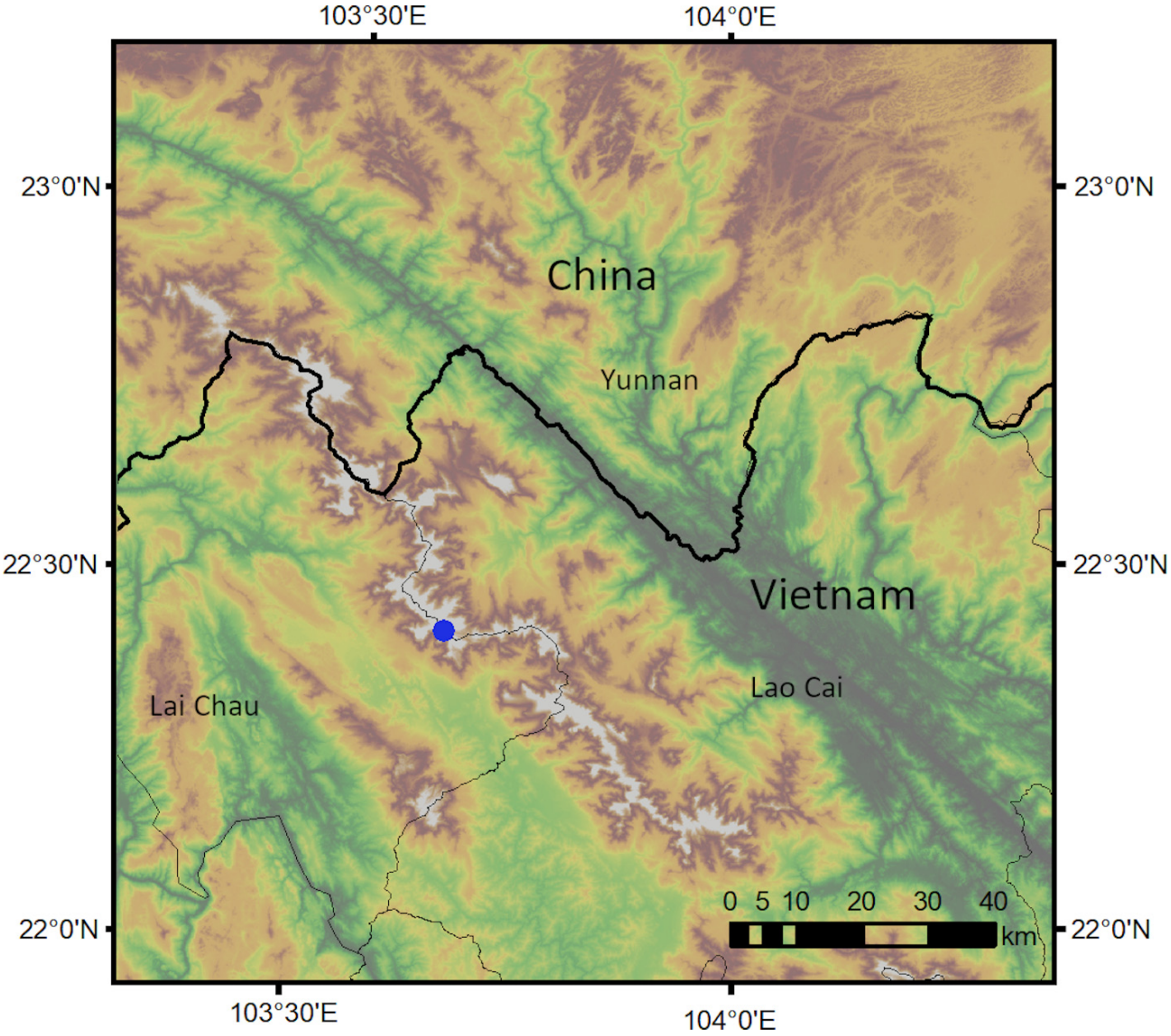

FIGURE 2. Collection site of *Leptobrachella graminicola* **sp. nov.** in the Hoang Lien Range, northwest Vietnam. Blue point represents the collection site. Pale grey areas indicative of higher elevation, dark green indicative of lowest elevation.

FIGURE 3. Dorsolateral view of *Leptobrachella graminicola* **sp. nov.** in life (A) adult male holotype VNMN 010904, (B) adult female paratype VNMN 010910, (C) adult female paratype VNMN 010913, (D) adult male paratype VNMN 010906.

Diagnosis: Assigned to the genus *Leptobrachella* on the basis of the following characters: small size, presence of macroglands on body (including supra–axillary, pectoral, femoral and ventrolateral glands), the absence of vomerine teeth, the presence of tubercles on eyelids, anterior tip of snout with pale vertical bar and fingers free of webbing (Smith 1925; Dubois 1983; Lathrop *et al*. 1998; Delorme *et al*. 2006). *Leptobrachella graminicola* **sp. nov.** is distinguished from its congeners by a combination of: (1) a body size range of 23.1–24.6 mm in six adult males and 28.6–32.9 mm in five adult females; (2) skin on dorsum smooth, with many tubercles and lacking dermal ridges; (3) toes with rudimentary webbing and broad lateral fringes; (4) belly white with brown spots; (5) throat dark brown with light grey-brown flecks and spots; (6) a row of large white spots on the outer edge of the tarsus extending from the heel to the inner metatarsal tubercle, sometimes forming a long white stripe; and (7) a bicoloured iris.

Description of holotype: Head length greater than head width; snout truncate in dorsal and lateral view, protruding slightly beyond margin of lower jaw; distance from nostril to eye subequal to nostril to snout; canthus rostralis distinct; lores sloping; pupil vertical; eye diameter greater than snout length; tympanum distinct, round, diameter half the diameter of eye; tympanic rim slightly elevated; vomerine teeth absent; pineal ocellus absent; vocal sac openings small, located posterolaterally on floor of mouth; tongue wide with broad, shallow notch at posterior tip; supratympanic ridge broad and distinct, running from posterior edge of eye to axillary gland. Tips of fingers rounded, very slightly swollen; relative finger lengths I < II < IV < III; nuptial pads absent; subarticular tubercle present at the base of finger IV, absent on finger I, II and III; large, round inner palmar tubercle present, distinctly separated from small, outer palmar tubercle; fingers without interdigital webbing and lateral fringes. Tips of toes rounded, very slightly swollen; relative toe length I < II < V < III < IV; subarticular tubercles absent; oval shaped inner metatarsal tubercle present, outer metatarsal tubercle absent; interdigital toe webbing rudimentary; distinct and wide lateral fringes on toes II, III, IV and V. Tibia 48.8% of snout to vent length; tibiotarsal articulation reaching to anterior edge of eye. Small tubercles on upper eyelid and on dorsal surfaces of body, forearms and hindlimbs; small tubercles present on throat becoming larger and more dense along the outer margin of the lower jaw; skin on dorsum smooth, with many tubercles; dermal ridges absent; one pair of large round tubercles on the dorsal surface,

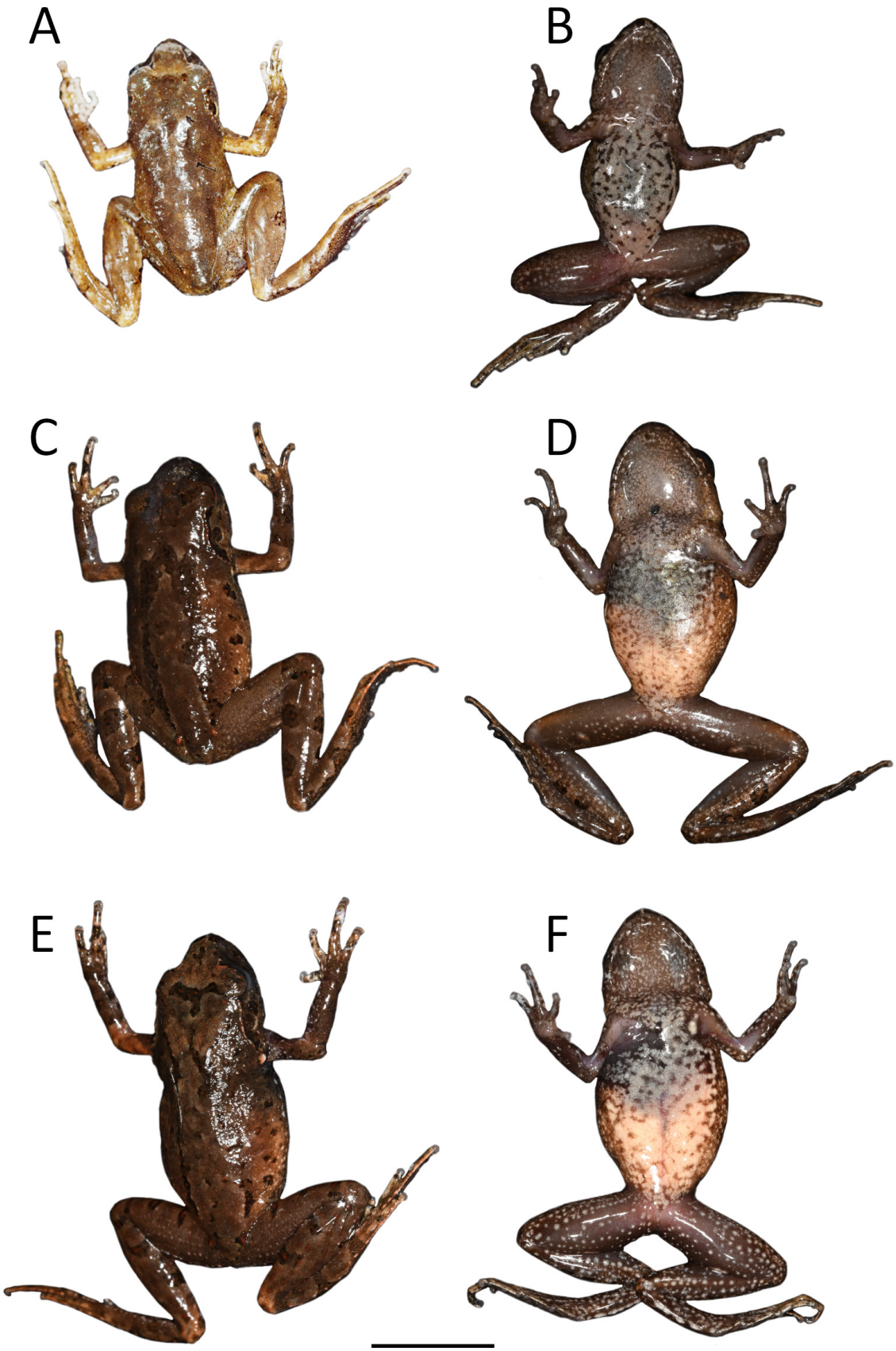

FIGURE 4. *Leptobrachella graminicola* **sp. nov.** in life under sedation. (A) dorsal view of adult male holotype VNMN 010904, (B) ventral view of adult male holotype VNMN 010904, (C) dorsal view of adult female paratype VNMN 010910, (D) ventral view of adult female paratype VNMN 010910, (E) dorsal view of adult female paratype VNMN 010908, (F) ventral view of adult female paratype VNMN 010908. 10 mm scale bar.

one on either side of vent; ventral skin smooth; pectoral gland oval, 1.7 mm maximum diameter; femoral gland oval, approximately 1.3 mm maximum diameter, on posteroventral surface of thigh, closer to knee than to vent; supra–axillary gland oval, raised, 1.1 mm diameter at widest point. Ventrolateral glands present.

Colour of holotype in life: Dorsal surface of body brown with darker brown spots, spots level with the scapula bordered with cream; dark brown interorbital bar; canthus rostralis brown; upper lip light grey with dark brown spots. Tympanum dark brown with light brown flecks in the centre, lower border of tympanum cream; lower half of supratympanic ridge dark brown, upper half reddish-brown. Tubercles on upper eyelid and body reddish-brown; flanks olive green, small light grey tubercles on flanks, large orange-coloured tubercles on the dorsal surface either side of vent; dorsal surfaces of hindlimbs, forelimbs, hands and feet light brown, forearm bicoloured lighter brown on the humerus; tubercles on hind limbs ringed with dark brown. Ventral surface of belly white with very dark blackish brown spots; throat dark brown with light grey-brown flecks and spots, spots becoming denser nearer outer margin of lower jaw; ventral surface of forearms unpigmented; ventral surface of the thighs reddish brown with lighter grey-brown spots, ventral surface of tarsus and shank reddish brown with lighter grey-brown flecks, row of large white spots on the outer edge of the tarsus extending from the heel to the inner metatarsal tubercle (Fig. 5D). Supra–axillary gland white, femoral glands white, ventrolateral glands white; iris bicolored, upper half copper with black reticulations, lower half silver with black reticulations.

Colour of holotype in preservative: Dorsum dark grey-brown; dorsal surface of limbs light brown. Ventral surface of throat and forearms cream with dark brown flecks; ventral surfaces of body and hindlimbs cream with dark brown spots. Row of large creamy-white spots on the outer edge of the tarsus extending from the heel to the inner metatarsal tubercle. Palmar aspects of hands cream with brown flecks, fingertips grey. Plantar aspect of feet dark grey-brown with grey finger tips, lateral fringes and webbing. Pectoral glands, femoral glands, supra-axillary glands and ventrolateral glands creamy white.

Variation: VNMN 010910 and VNMN 010909 with several large dark brown spots on the flank; all specimens, except holotype with dark brown transverse bars present on thigh, shank, tarsus, lower arms fingers and toes, those on thigh and shank with orange makings in the centre of each bar. Supra–axillary gland white with orange tip (VNMN 010910, VNMN 010909, VNMN 010913 and HLNP 20191005 00017). VNMN 010907, VNMN 010906, VNMN 010912 and VNMN 010914 with three pairs of large round, orange-coloured tubercles on the dorsal surface (three tubercles on either side of vent), all other specimens with one pair of orange tubercles, one on either side of the vent. The row of large creamy-white spots on the outer edge of the tarsus joined to form a white stripe extending from the heel to the inner metatarsal tubercle in VNMN 010912, HLNP 20191005 00017 and VNMN 010908. See Table 2 for measurements of all individuals in the type series.

Natural history: All specimens of *Leptobrachella graminicola* **sp. nov.** were associated with disturbed evergreen broadleaf forest with a relatively open canopy of bamboo (Fig. 6). All individuals were encountered at night from 19:00–22:00h in October 2019 and observed on sedge-like plants found growing alongside or in the middle of 2–3 m wide streams. The streams were clear, shallow and with a rocky bed. Males were sometimes found in close proximity to each other on the sedge-like plants (Fig. 6C). During the surveys, males were not heard calling although all females of this species observed were gravid. There was no rain during surveys and the air temperature ranged from 12.8–13.2°C at night. Tadpoles were not observed. Within the genus *Leptobrachella*, *Leptobrachella graminicola* **sp. nov.** is syntopic with *L. bourreti*. Within Megophryidae, the new species is syntopic with *Megophrys gigantica* (Liu, Hu, and Yang 1960), *M. frigida* Tapley, Cutajar, Nguyen, Portway, Mahony, Nguyen, Harding, Luong and Rowley, 2021 and *Leptobrachium ailaonicum* (Yang, Chen, and Ma, 1983). The presence of gravid females and presence of multiple males on the blades of the sedge-like plants in the middle of the stream indicates that the breeding season could be October.

Distribution and conservation status: This species is only known from the recently designated Bat Xat Nature Reserve on Mount Pu Ta Leng at elevations between 2383 and 2437 m asl (Fig. 2). The site where *Leptobrachella graminicola* **sp. nov.** is found faces the immediate threat of habitat loss and the activities of tourists. *Leptobrachella graminicola* **sp. nov.** was not encountered during other surveys on Mount Pu Ta Leng at different times of year and at different elevations (October 2019, 1568 m elevation on the same side of the mountain, 1.7 km in a straight line from the collection site; April 2018 and October 2019, 2300 m elevation on the opposite side of the mountain, 2.2 km straight line from the collection site; April 2018 and October 2019, 1900 m elevation at the on the opposite side of the mountain, 3.7 km straight line from the collection site). Most species of *Leptobrachella* are associated with forests and it is likely that they are forest dependent. Lao Cai Province has been subject to some of the most intense

levels of forest degradation and deforestation in Vietnam (Khuc *et al*. 2018). *Leptobrachella graminicola* **sp. nov.** likely qualifies for being assessed as Data Deficient in accordance with the IUCN Red List of Threatened Species categories and criteria (see IUCN 2012).

Comparisons: *Leptobrachella graminicola* **sp. nov.** can be distinguished from all reported *Leptobrachella* from Asia north of the Isthmus of Kra on the basis of morphology. Comparisons with each species can be seen in Table 3. The following comparison is based on six male and five female *Leptobrachella graminicola* **sp. nov.**

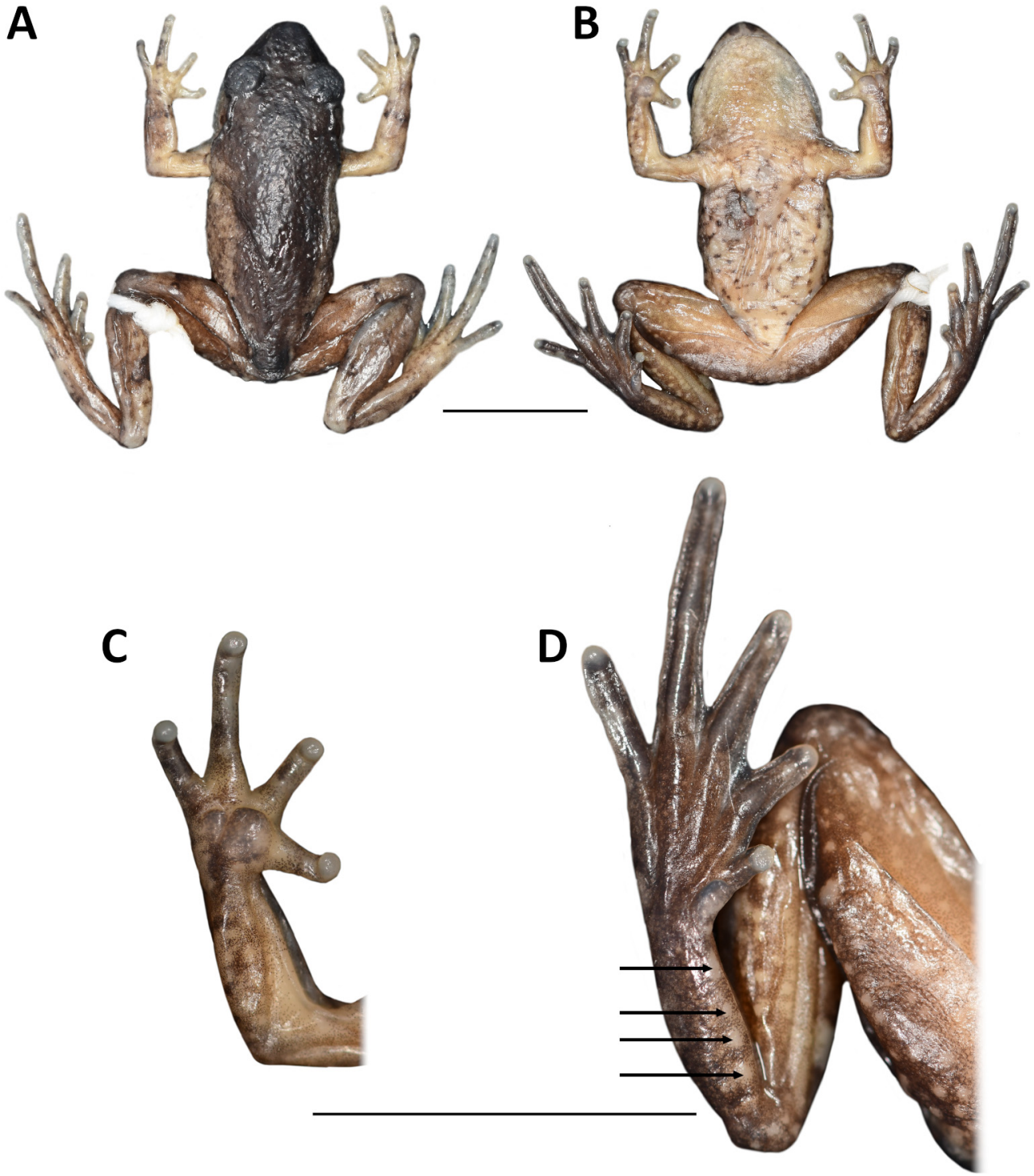

FIGURE 5. *Leptobrachella graminicola* **sp. nov.** adult male holotype VNMN 010904 in preservative. (A) Dorsal view, (B) ventral view, (C) palmar surface of right hand, and (D) plantar surface of right foot, black arrows highlight the row of large white spots on the outer edge of the tarsus extending from the heel to the inner metatarsal tubercle. 10 mm scale bar.

TABLE 2. Measurements (mm) of *Leptobrachella graminicola* **sp. nov.** Abbreviations defined in Materials and methods section. *Holotype, **paratypes. ^referred specimen.

Specimens	**VNMN 010904***	**VNMN 010905 ****	**VNMN 010906 ****	**VNMN 010912****	**VNMN 010914****	**HLNP 20191005 00017 ^**			
Sex	**M**	**M**	**M**	**M**	**M**	**M**	Mean	Min	Max
SVL	24.6	24.6	23.1	24.5	23.7	23.9	24.1	23.1	24.6
HDL	9.2	9.9	9.0	9.1	8.7	9.2	9.2	8.7	9.9
HDW	8.6	8.7	8.0	8.5	8.3	8.3	8.4	8.0	8.7
SNT	3.1	4.8	4.0	4.0	4.0	3.7	3.9	3.1	4.8
TEY	1.0	1.0	1.0	1.0	1.1	1.0	1.0	1.0	1.1
IN	3.0	3.5	2.7	3.1	3.0	3.1	3.1	2.7	3.5
NS	1.8	1.7	1.7	1.8	2.2	2.2	1.9	1.7	2.2
EN	1.7	1.8	2.1	2.0	2.0	2.0	1.9	1.7	2.1
EYE	3.4	3.5	3.2	3.0	3.2	3.3	3.3	3.0	3.5
IOD	2.7	2.8	3.0	2.8	3.1	3.1	2.9	2.7	3.1
TMP	1.7	1.6	1.4	1.4	1.3	1.9	1.6	1.3	1.9
FEL	12.2	12.7	13.8	12.5	12.0	12.7	12.7	12.0	13.8
TIB	12.0	12.5	12.4	12.3	12.5	12.4	12.4	12.0	12.5
ML	6.0	7.1	6.2	6.6	7.1	6.3	6.6	6.0	7.1
PL	12.2	12.7	12.2	13.0	13.1	12.2	12.6	12.2	13.1
IMCTL	1.8	1.5	1.7	1.4	1.1	1.6	1.5	1.1	1.8
OMCTL	1.0	1.3	1.0	1.0	1.0	1.3	1.1	1.0	1.3
IMTTL	1.3	1.6	1.5	1.3	1.4	1.7	1.5	1.3	1.7
PEC	1.7	1.5	1.5	1.5	1.4	1.7	1.6	1.4	1.7
FEM	1.3	1.4	1.4	1.7	1.0	1.8	1.4	1.0	1.8
Mass in life (g)	1.9	1.0	0.8	1.0	1.3	1.3	1.2	0.8	1.9

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TABLE 2. (Continued)

Specimens	VNMN 010907 **	VNMN 010908 **	VNMN 010909 **	VNMN 010910 **	VNMN 010913**			
Sex	**F**	**F**	**F**	**F**	**F**	**Mean**	**Min**	**Max**
SVL	32.9	29.8	31.0	28.7	28.6	29.3	28.6	32.9
HDL	11.5	11.0	11.0	10.0	11.9	10.9	10.0	11.9
HDW	10.9	10.6	10.3	9.5	10.0	10.0	9.5	10.9
SNT	4.3	4.7	4.7	4.2	4.0	4.5	4.0	4.7
TEY	1.9	1.4	1.2	1.2	1.2	1.3	1.2	1.9
IN	3.2	3.3	3.6	3.4	3.2	3.4	3.2	3.6
NS	2.3	2.4	2.7	2.1	2.0	2.3	2.0	2.7
EN	2.0	2.3	3.5	2.6	2.3	2.5	2.0	3.5
EYE	3.5	3.6	3.8	3.4	3.6	3.6	3.4	3.8
IOD	3.2	3.2	3.2	3.0	2.5	3.0	2.5	3.2
TMP	2.0	1.6	2.0	1.7	2.1	1.9	1.6	2.1
FEL	15.7	14.8	16.0	14.9	14.1	14.9	14.1	16.0
TIB	16.0	14.9	14.9	13.5	14.1	14.3	13.5	16.0
ML	8.3	8.0	7.3	7.1	7.1	7.5	7.1	8.3
PL	15.5	14.4	14.2	13.3	14.6	14.2	13.3	15.5
IMCTL	1.9	1.5	2.0	1.5	1.8	1.8	1.5	2.0
OMCTL	1.3	1.0	1.2	1.3	1.1	1.2	1.0	1.3
IMTTL	2.0	1.4	1.7	1.4	1.4	1.6	1.4	2.0
PEC	1.6	1.4	2.0	1.7	2.0	1.7	1.4	2.0
FEM	1.8	3.1	1.7	1.7	3.0	2.2	1.7	3.1
Mass in life (g)	2.5	2.5	2.5	1.6	2.5	2.3	1.6	2.5

TABLE 3. Selected diagnostic characters for species in the genus *Leptobrachella* occurring north of the Isthmus of Kra. Grey shading indicates non-overlapping characters when compared to *Leptobrachella graminicola* **sp. nov.**

Species	SVL male	SVL female	**Interdigital toe webbing**	**Lateral fringes on toes**	**Belly pattern**	**Dorsal skin texture**	**Bicolored iris**	**Reference**
Leptobrachella graminicola **sp. nov.**	23.1–24.6 (N=6)	28.6–32.9 (N=5)	Rudimentary	Wide	White with very dark blackish brown spots	Smooth, with many tubercles and lacking dermal ridges	Yes	This study
L. aerea	25.1–28.9 (N=28)	27.1–38.6 (N=12)	Rudimentary	Narrow	Nearly immaculate creamy white, brown speckling on margins	Finely tuberculate	No	Rowley *et al*. 2010a
L. alpina	24.0–26.4	31.7–32.5	Rudimentary	Wide (in males)	Creamy white with dark spots	Relatively smooth, some with small warts	No	Ohler *et al*. 2011; Wang *et al*. 2020
L. applebyi	19.6–20.8 (N = 5)	21.7 (N=1)	Rudimentary	Absent	White with dark speckling or brown with white speckling	Smooth	No	Rowley & Cao, 2009
L. ardens	21.3–24.7 (N=15)	25.4 (N=1)	Absent	Absent	Reddish brown with white speckling	Smooth – finely shagreened	No	Rowley *et al*. 2016
L. aspera	22.4 (N=1)	25.0–26.4 (N=3)	Rudimentary	Narrow	Creamy white with distinct dark spots	Rough with dense conical granules, tubercles, and dermal ridges	Yes	Wang *et al*. 2020
L. bashaensis	22.9–25.6 (N=6)	27.1 (N=1)	Rudimentary	Narrow	Off-white with dark spots	Shagreened with small tubercles	Yes	Lyu *et al*. 2020
L. bidoupensis	23.6–24.6 (N=4)	29.2–29.4 (N=2)	Rudimentary	Narrow	Reddish brown with white speckling or marbling	Smooth	Yes	Rowley *et al*. 2011
L. bijie	29.0–30.4 (N=8)	Unknown	Rudimentary	Narrow	White with distinct nebulous greyish speckling	Shagreened and granular with short longitudinal dermal ridges	Yes	Wang *et al*. 2019
L. botsfordi	29.1–32.6 (N=7)	30.0–31.8 (N=2)	Rudimentary	Narrow	Reddish brown with white speckling	Shagreened	No	Rowley *et al*. 2013
L. bourreti	27.4–36.2 (N=26)	39.5–45.0 (N=7)	Rudimentary	Narrow	Creamy white, light yellow and orange in some specimens.	Relatively smooth, some with small warts	Yes	Bourret, 1937; Ohler *et al*. 2011; this study

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TABLE 3. (Continued)

Species	SVL male	SVL female	**Interdigital toe webbing**	**Lateral fringes on toes**	**Belly pattern**	**Dorsal skin texture**	**Bicolored iris**	**Reference**
L. chishuiensis	30.8–33.4 (N=7)	34.2 (N=1)	Rudimentary	Narrow	White with grey speckling on margins	Shagreened and granular	Yes	Li *et al*. 2020
L. crocea	22.2–27.3 (N=16)	Unknown	Rudimentary	Absent	Bright orange	Highly tuberculate	No	Rowley *et al*. 2010b
L. dorsospina	28.7–30.5 (N=2)	32.1–39.8 (N=5)	Rudimentary	Narrow	Greyish white with dark spots	Rough with dense conical granules, tubercles, dermal ridges, and conical spines	Yes	Wang *et al*. 2020
L. eos	33.1–34.7 (N=6)	40.7 (N=1)	Rudimentary	Wide	Creamy white	Shagreened	Yes	Ohler *et al*. 2011
L. feii	21.51–22.75 (N=5)	25.67 (N=1)	Rudimentary	Narrow	Creamy white with small dark spots	Shagreened with small tubercles and unequal dermal ridges	Yes	Chen *et al*. 2020
L. firthi	26.4–29.2 (N=21)	25.7–36.9 (N=14)	Rudimentary	Wide	Creamy white	Shagreened	Variable	Rowley *et al*. 2012
L. flaviglandulosa	23.0–27.0 (N=13)	29.3 (N=1)	Rudimentary	Narrow	White with black speckling	Shagreened with yellowish brown tubercles	Yes	Chen *et al*. 2020
L. fuliginosa	28.2–30.0 (N=4)	Unknown	Rudimentary	Narrow	White with brown speckling	Nearly smooth, few tubercles	Yes	Matsui, 2006
L. isos	23.7–27.9 (N=38)	28.6–31.5 (N=9)	Rudimentary	Wide	Creamy white with white dusting on margins	Mostly smooth, females more tuberculate	Yes	Rowley *et al*. 2015a
L. jinshaensis	29.7–31.2 (N=5)	Unknown	Absent	Narrow	Creamy white	Shagreened, some of the granules forming longitudinal short skin ridges	Yes	Cheng *et al*. 2021
L. kalonensis	25.8–30.6 (N=16)	28.9–30.6 (N=3)	Absent	Absent	Dark brown with white speckling	Smooth	Yes	Rowley *et al*. 2016
L. khasiorum	24.5–27.3 (N=4)	31.2–33.4 (N=3)	Rudimentary	Wide	Creamy white	Isolated speckled tubercles	Yes	Das *et al*. 2010

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TABLE 3. (Continued)

Species	SVL male	SVL female	**Interdigital toe webbing**	**Lateral fringes on toes**	**Belly pattern**	**Dorsal skin texture**	**Bicolored iris**	**Reference**
L. lateralis	26.9–28.25 (N=NR)	36.6 (N=NR)	Rudimentary	Absent	Creamy white	Rough and granular	Unknown	Anderson, 1871; Wang *et al*. 2020
L. laui	24.8–26.7 (N=1)	28.1 (N=1)	Rudimentary	Wide	Creamy white with white dusting on margins	Round, granular tubercles	No	Sung *et al*. 2014
L. liui	22.5–26.5 (NR)	24.5–27.8 (NR)	Rudimentary	Wide	Creamy white with tiny grey spots	Round, granular tubercles, dermal ridges present	No	Ohler *et al*. 2011; Wang *et al*. 2020
L. macrops	28.0–29.3 (N=3)	30.3 (N=1)	Rudimentary	Absent	Greyish-violet with some white speckling	Dorsal skin roughly granular with larger tubercles	Yes	Duong *et al*. 2018
L. maculosa	24.2–26.6 (N=3)	27.0 (N=1)	Absent	Absent	Brown with white speckling	Mostly smooth	Yes	Rowley *et al*. 2016
L. mangshanensis	22.2–27.8 (N=27)	30.2 (N=1)	Rudimentary	Narrow	Reddish brown with white speckling	Nearly smooth, scattered tubercles	Yes	Hou *et al*. 2018
L. maoershanensis	25.2–30.4 (N=8)	29.1 (N=1)	Rudimentary	Narrow	Creamy white with irregular black spots	Shagreened with small tubercles and longitudinal ridges	Yes	Yuan *et al*. 2017
L. melanoleuca	26.6–29.0 (N=11)	32.7	Rudimentary	Absent	Large black spots on a white background	Skin nearly smooth, scattered with small tubercles	Yes	Matsui, 2006
L. melica	19.5–22.7 (N=7)	Unknown	Rudimentary	Absent	Pale pink with greyish spots and white speckling	Smooth	No	Rowley *et al*. 2010b
L. minima	25.7–31.4 (N=46)	31.6–37.3 (N=14)	Rudimentary	Absent	Creamy white	Smooth	Yes	Ohler *et al*. 2011; Wang *et al*. 2020
L. nahangensis	40.8 (N=1)	Unknown	Rudimentary	Absent	Creamy white	Smooth	No	Lathrop *et al*. 1998
L. namdongensis	30.9 (N=1)	32.1–35.3 (N=3)	Rudimentary	Absent	Creamy white with brown dusting on margins	Finely tuberculate	Yes	Hoang *et al*. 2019
L. neangi	NR	35.4–36.3 (N=2)	Rudimentary	Absent	Belly transparent, immaculate purplish grey in life	Dorsal skin with small, irregular bumps and ridges	No	Stuart & Rowley, 2020

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TABLE 3. (Continued)

Species	SVL male	SVL female	**Interdigital toe webbing**	**Lateral fringes on toes**	**Belly pattern**	**Dorsal skin texture**	**Bicolored iris**	**Reference**
L. niveimontis	22.5–23.6 (N=5)	28.5–28.7 (N=2)	Rudimentary	Narrow	Black and bluish-white marbling	Relatively smooth with small tubercles	Yes	Chen *et al*. 2020
L. nokrekensis	26.0–33.0 (N=5)	34.0–35.0 (N=2)	Rudimentary	Unknown	Creamy white	Tubercles and longitudinal dermal ridges	Yes	Mathew and Sen, 2010
L. nyx	26.7–32.6 (N=7)	37.0–41.0 (N=3)	Rudimentary	Absent	Creamy white, with brown margins	Rounded tubercles	Unknown	Ohler *et al*. 2011 ; Chen *et al*. 2020
L. oshanensis	26.6–30.7 (N=NR)	31.6 (N=1)	Absent	Absent	Whitish with small grey spots	Smooth with few dermal ridges	Yes	Ohler *et al*. 2011
L. pallida	24.5–27.7 (N=8)	Unknown	Absent	Absent	Brown with white speckling	Tuberculate	Yes	Rowley *et al*. 2016
L. pelodytoides	27.5–32.3 (N=NR)	Unknown	One third webbed	Narrow	Whitish	Small, smooth warts	Unknown	Chen *et al*. 2020
L. petrops	23.6–27.6 (N=21)	30.3–47.0 (N=17)	Absent	Narrow	White	Highly tuberculate	Yes	Rowley *et al*. 2017a
L. pluvialis	21.3–27.52 (N=32)	25.5–33.5 (N=9)	Absent	Absent	Dirty white with brown marbling or dark spots	Smooth, flattened tubercles on flanks	Yes	Ohler *et al*. 2000; This study
L. puhoatensis	24.2–28.1 (N=8)	27.3–31.5 (N=3)	Rudimentary	Narrow	Deep reddish brown with faint white speckling	Longitudinal skin ridges	Yes	Rowley *et al*. 2017b
L. purpuraventra	27.3–29.8 (N=12)	33.0–35.3 (N=4)	Rudimentary	Narrow	Greyish white with nebulous dark speckling	Shagreened and granular with dermal ridges	Yes	Wang *et al*. 2019
L. purpurus	25.7–27.5 (N=2)	Unknown	Rudimentary	Wide	Dull white with indistinct grey dusting to dull grey	Shagreened with small tubercles	Yes	Yang *et al*. 2018
L. pyrrhops	30.3–33.9 (N=2)	30.8–34.3 (N=7)	Rudimentary	Absent	Opaque pinkish to light bluish-purple with bluish-white speckles	Slightly shagreened	Yes	Poyarkov *et al*. 2015
L. rowleyae	23.4–25.4 (N=11)	27.0–27.8 (N=2)	Absent	Absent	Pinkish milk-white to lightly brown with numerous white speckles	Mostly smooth	Yes	Nguyen *et al*. 2018

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TABLE 3. (Continued)

Species	SVL male	SVL female	**Interdigital toe webbing**	**Lateral fringes on toes**	**Belly pattern**	**Dorsal skin texture**	**Bicolored iris**	**Reference**
L. shangsiensis	24.9–29.4 (N=11)	30.8–35.9 (N=5)	Rudimentary	Narrow	yellowish creamy-white	Mostly smooth with numerous tiny tubercles	Yes	Chen *et al*. 2019
L. suiyangensis	28.7–29.7 (N=5)	30.5–33.5 (N=3)	Rudimentary	Narrow	Yellowish creamy-white with brown speckling	Shagreened with small granules	Yes	Luo *et al*. 2020
L. sungi	48.3–52.7 (N=3)	56.7–58.9	Rudimentary	Narrow	White	Granular	No	Lathrop *et al*. 1998; Fei *et al*. 2012
L. tadungensis	23.5–26.0 (N=8)	32.1 (N=1)	Absent	Absent	Dark brown with fine white speckling	Smooth	No	Rowley *et al*. 2016
L. tamdil	32.3 (N=1)	32.3 (N=1)	Rudimentary	Narrow	Pale grey	Weakly tuberculate	Yes	Sengupta *et al*. 2010
L. tengchongensis	23.9–26.0 (N=5)	28.8–28.9 (N=2)	Rudimentary	Narrow	White with brown spots	Longitudinal dermal ridges	No	Yang *et al*. 2016
L. tuberosa	24.4–29.5 (N=16)	30.2 (N=1)	Rudimentary	Absent	White with small grey spots / streaks	Highly tuberculate	No	Inger *et al*. 1999
L. ventripunctata	23.7–27.7 (N=19)	31.5–35.0 (N=2)	Rudimentary	Absent	Creamy white with dark brown spots	Longitudinal dermal ridges	Yes	Ohler *et al*. 2011
L. wuhuangmontis	25.6–30.0 (N=9)	33.0–36.0 (N=4)	Rudimentary	Narrow	Greyish white with tiny white and black spots	Rough, scattered with dense conical tubercles	Yes	Wang *et al*. 2018
L. wulingensis	24.5–32.8 (N=4)	29.9–38.5 (N=3)	Rudimentary	Narrow	Creamy white	Shagreened with sparse large warts, sometimes with longitudinal dermal ridges	Yes	Qian *et al*. 2020
L. yeae	25.8–31.2 (N=10)	33.7–34.1 (N=10)	Rudimentary	Narrow	Cream white with brown specking on sides and upper abdomen	Relatively smooth with tiny granules	Yes	Shi *et al*. 2021
L. yingjiangensis	25.7–27.6 (N=6)	Unknown	Rudimentary	Wide	Creamy white with dark brown flecks on margin	Shagreened with small tubercles	Yes	Yang *et al*. 2018
L. yunkaiensis	25.9–29.3 (N=6)	34.0–35.3 (N=2)	Rudimentary	Wide	Pinkish, with brown speckling	Shagreened with short skin ridges and raised warts	Yes	Wang *et al*. 2018
L. zhangyapingi	45.8−52.5 (N=7)	Unknown	Rudimentary	Wide	Creamy white with brown margins	Mostly smooth with distinct tubercles	yes	Jiang *et al*. 2013

FIGURE 6. (A) Macrohabitat of of *Leptobrachella graminicola* **sp. nov.** Mount Pu Ta Leng, Bat Xat District, Lao Cai Province, (B) microhabitat of *Leptobrachella graminicola* **sp. nov.** Mount Pu Ta Leng, Bat Xat District, Lao Cai Province, and (C) specimens of *Leptobrachella graminicola* **sp. nov.** *in situ*.

Leptobrachella graminicola **sp. nov.** is readily distinguished from its closest relative *L. bourreti*. *Leptobrachella graminicola* **sp. nov.** differs from *L. bourreti* (Fig. 7 & Table 4) by having a smaller adult male size SVL 23.1–24.6 mm (versus 27.4–36.2 mm, N=26, in *L. bourreti*; Ohler *et al*. 2011; material examined), a smaller adult female size SVL 28.6–32.9 mm (versus 39.5–45.0 mm, N=5, in *L. bourreti*; Ohler *et al*. 2011; material examined), wide lateral fringes on toes (versus narrow in *L. bourreti*; Ohler *et al*. 2011; material examined) and a white ventral surface with brown spots (versus creamy white, yellow or orange in *L. bourreti*; Bourret 1937; Ohler *et al*. 2011; material examined).

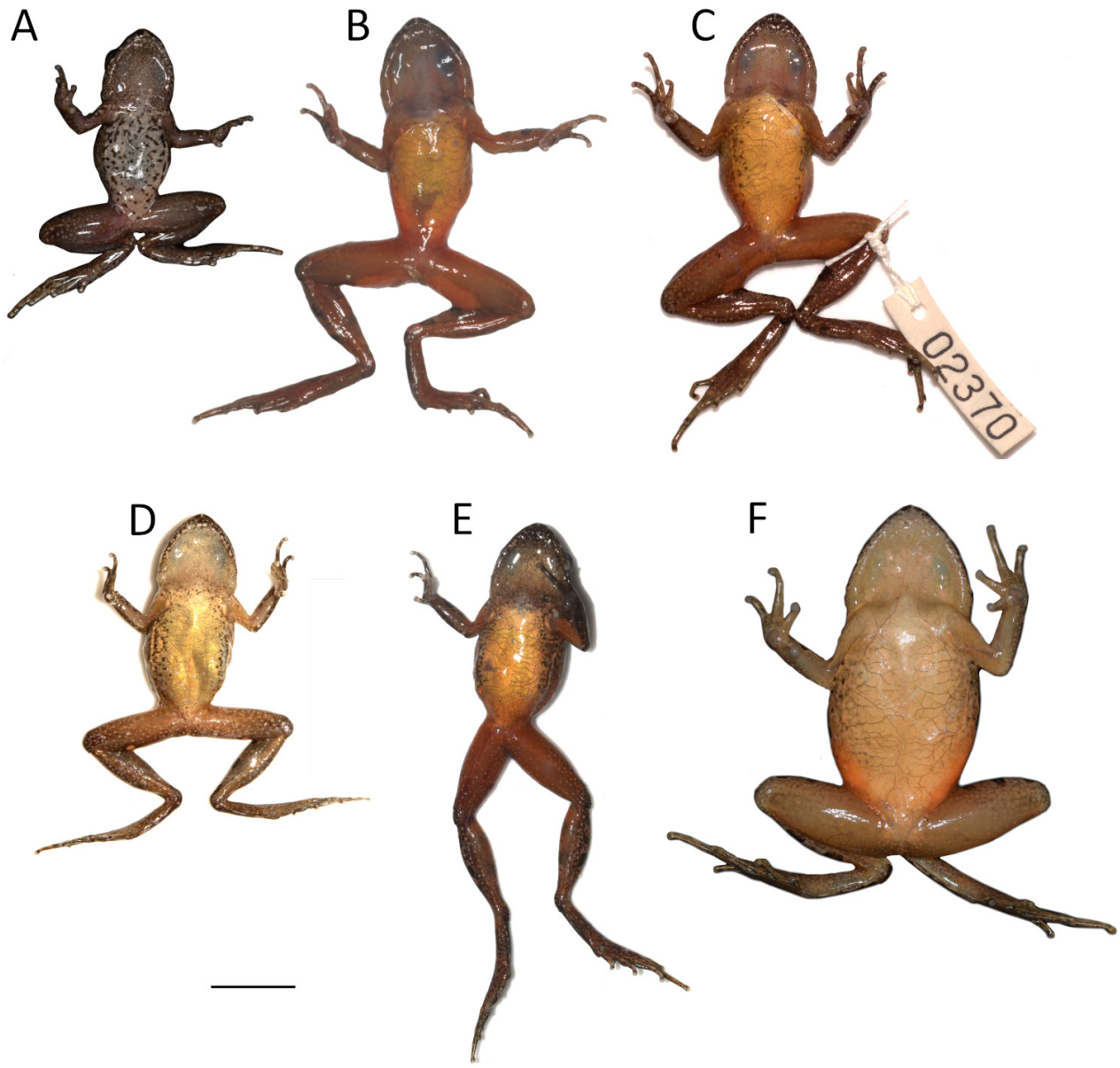

FIGURE 7. Ventral surfaces of *Leptobrachella graminicola* **sp. nov.** from Bat Xat District compared with *Leptobrachella bourreti* (A) ventral view of *Leptobrachella graminicola* **sp. nov.** adult male holotype VNMN 010904, (B) ventral view of adult male *L. bourreti* VNMN 010916, (C) ventral view of adult male *L. bourreti* AMS R.186825 from Sa Pa District, (D) ventral view of adult male *L. bourreti* HLNP2017 0908 00021 from Bat Xat District, (E) ventral view of adult female *L. bourreti* AMS R.188515 from Bat Xat District, (F) ventral view of adult female *L. bourreti* VNMN 10917 from Bat Xat District. 10 mm scale bar.

Leptobrachella graminicola **sp. nov.** can be distinguished from other *Leptobrachella* species known from the Hoang Lien Range. *Leptobrachella graminicola* **sp. nov.** differs from *L. botsfordi* by having a smaller adult male size SVL 23.1–24.6 mm (versus 29.1–32.6 mm, N=7, in *L. botsfordi*; Rowley *et al*. 2013), wide lateral fringes on toes (versus narrow in *L. botsfordi*; Rowley *et al*. 2013), a smooth dorsal surface with many tubercles (versus shagreened dorsal surface in *L. botsfordi*; Rowley *et al*. 2013) and a bicolored iris (versus a brownish gold iris with black reticulations, Rowley *et al*. 2013); from *L. pluvialis* (Fig. 8 & Table 4) by the presence of rudimentary webbing and wide lateral fringes on toes (versus absence of webbing and lateral fringes in *L. pluvialis*; Ohler *et al*. 2000) and dark brown throat with light grey-brown flecks and spots, spots becoming denser nearer outer margin of lower jaw, no large white spots on lower jaw (versus throat white without grey-brown flecks and two white spots on the lower jaw in *L. pluvialis;* material examined); from *L. sungi* by having a smaller adult male size SVL 23.1–24.6

mm (versus 48.3–52.7 mm, N=3, in *L. sungi*; Lathrop *et al*. 1998), a smaller adult female size SVL 28.6–32.9 mm (versus 56.7–58.9 mm, N=3, in *L. sungi*; Lathrop *et al*. 1998), wide lateral fringes on toes (versus narrow in *L. sungi*; Lathrop *et al*. 1998), a white ventral surface with brown spots (versus white ventral surface in *L. sungi*; Lathrop *et al*. 1998), a smooth dorsal surface with many tubercles (versus granular dorsal surface in *L. sungi*; Lathrop *et al*. 1998) and a bicolored iris (versus iridescent gold green iris in *L. sungi*; Lathrop *et al*. 1998) and from *L. ventripunctata* by having wide lateral fringes on toes (versus absence of lateral fringes on toes in *L. ventripunctata;* Ohler *et al*. 2000) dorsal surface lacking dermal ridges (versus dermal ridges present on the dorsal surface in *L. ventripunctata*; Ohler *et al*. 2000; Yang *et al*. 2018).

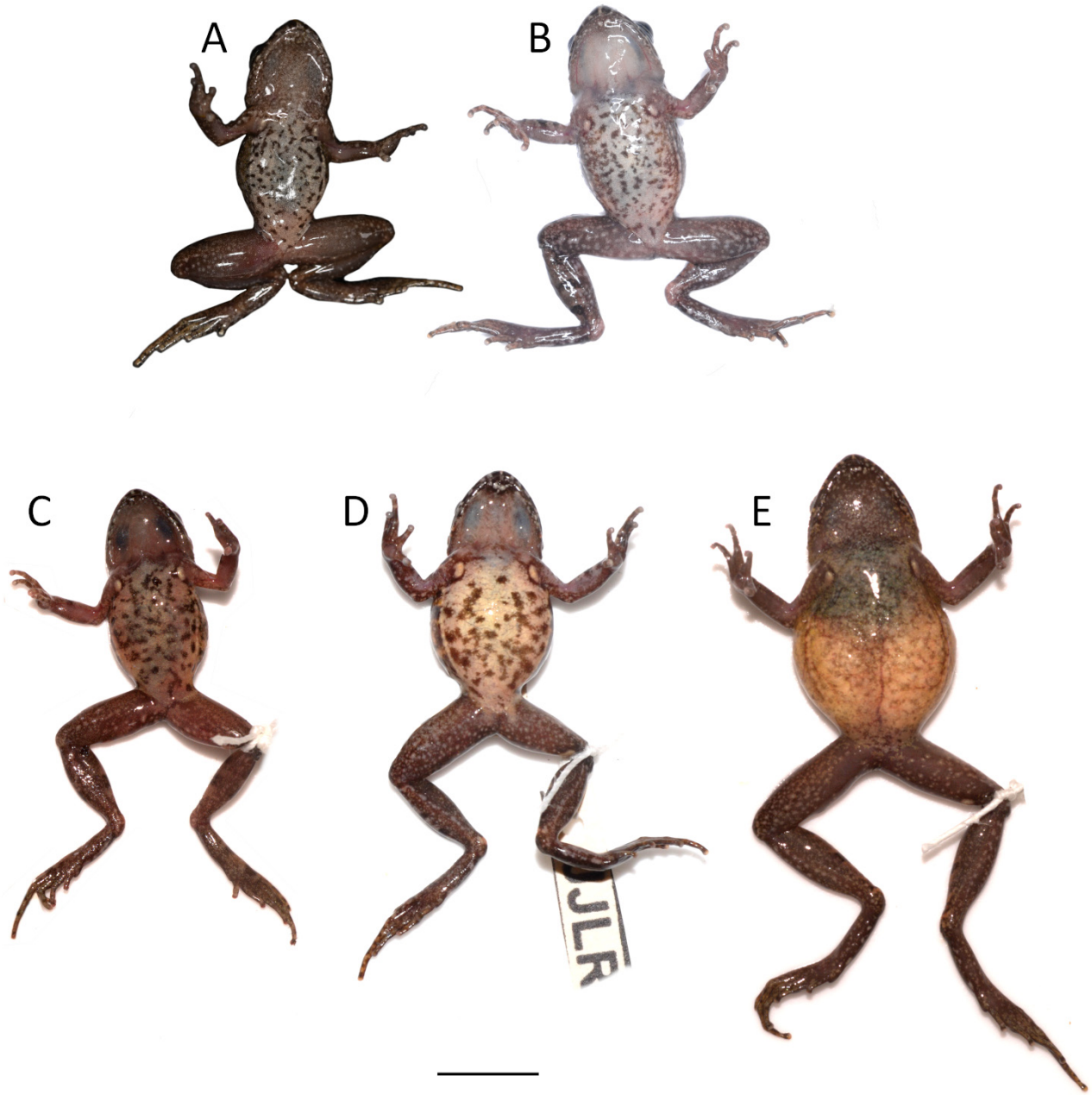

FIGURE 8. Ventral surfaces of *Leptobrachella graminicola* **sp. nov.** from Bat Xat District compared with *Leptobrachella pluvialis* collected from Sa Pa District (A) ventral view of *Leptobrachella graminicola* **sp. nov.** adult male holotype VNMN 010904, (B) ventral view of adult male *L. pluvialis* VNMN 010918, (C) ventral view of adult male *L. pluvialis* AMS R.186793 (D), ventral view of adult male *L. pluvialis* AMS R.186797 and (E) ventral view of adult female *L. pluvialis* AMS R.186798. 10 mm scale bar.

TABLE 4. Measurements (mm) of *Leptobrachella pluvialis* and *Leptobrachella bourreti* collected in the Hoang Lien Range. Abbreviations defined in Materials and methods section.

	Leptobrachella bourreti				***Leptobrachella pluvialis***			
	Males (N=19)		Females (N=5)		Males (N=39)		Females (N=9)	
	Range	**Mean; SD**	**Range**	**Mean; SD**	**Range**	**Mean; SD**	**Range**	**Mean; SD**
SVL	27.4–34.3	31.20; 1.89	39.5–44.7	41.80; 2.07	22.7–27.5	25.05; 1.17	25.5–33.6	30.55; 2.44
HDL	10.7–12.8	12.00; 0.66	15.4–17.4	16.14; 0.83	8.8–14.4	9.90; 1.15	10.2–12.5	11.51; 0.74
HDW	10.0–12.3	11.30; 0.59	14.7–16.5	15.35; 0.73	8.6–14.5	9.65; 1.19	9.6–12.0	11.11; 0.78
SNT	4.2–5.7	4.61; 0.36	5.8–6.9	6.20; 0.51	3.5–4.7	3.96; 0.27	4.1–5.1	4.65; 0.32
EYE	3.2–4.6	3.90; 0.36	4.5–5.2	4.64; 0.30	2.7–3.7	3.21; 0.22	3.1–4.5	3.62; 0.40
IOD	2.4–3.8	3.27; 0.31	3.5–4.9	4.09; 0.64	2.2–3.6	3.10; 0.29	2.9–3.6	3.19; 0.22
TMP	1.7–2.4	2.05; 0.18	2.6–2.8	2.76; 0.12	1.6–2.2	1.84; 0.15	1.9–2.2	2.04; 0.13
TEY	1.0–1.6	1.30; 0.19	1.6–2.6	2.06; 0.40	0.7–1.8	1.04; 0.20	1.1–1.7	1.28; 0.18
TIB	14.8–17.6	16.04; 0.80	19.8–21.7	20.57; 0.77	10.6–15.3	12.32; 0.81	12.9–15.1	14.54; 0.68
EN	1.9–4.4	2.61; 0.49	2.6–3.5	3.15; 0.35	1.8–2.4	2.04; 0.16	2.3–3.4	2.60; 0.31
IN	2.6–4.4	3.20; 0.51	3.7–4.1	3.86; 0.17	2.2–3.5	2.68; 0.31	2.4–3.3	2.77; 0.27
NS	1.7–2.3	2.05; 0.18	2.5–3.3	2.81; 0.32	1.5–2.1	1.84; 0.18	1.7–2.3	2.02; 0.19
ML	7.9–9.7	8.77; 0.52	10.0–11.8	11.04; 0.76	5.6–7.9	6.87; 0.47	6.9–8.2	7.82; 0.53
PL	14.4–17.8	15.51; 0.89	17.7–21.7	19.88; 1.50	10.5–14.0	11.95; 0.85	8.4–14.9	13.26; 1.97
PEC	1.2–1.8	1.48; 0.20	2.0–2.0	2.00; 0.00	1.0–1.9	1.46; 0.22	1.3–2.3	1.79; 0.33
FEM	0.5–1.7	1.34; 0.30	1.9–2.3	2.10; 0.15	0.7–2.7	1.41; 0.41	0.1–2.7	1.59; 0.71
Weight (g)	2.0–2.8 (N=5)	2.38; 0.26	4.8–7.2 (N=3)	5.73; 1.29	0.9–1.8 (N=39)	1.36; 0.20	1.5–3.2	2.44; 0.46

Leptobrachella graminicola **sp. nov.** differs from other congeneric species north of the Isthmus of Kra by having a white belly with very dark blackish brown spots *(L. aerea, L. chishuensis, L. eos, L. firthi, L. isos, L. jinshaensis*, *L. khasiorum*, *L. lateralis*, *L. laui, L. minima, L. nahangensis, L. namdongensis, L. nokrekensis, L. nyx, L. pelodytoides*, *L. petrops*, *L. purpurus*, *L. tamdil, L. wulingensis, L. yeae*, *L. yingjiangensis,* and *L. zhangyapingi* have a mostly white, to pale grey belly, with or without dark spots around the margin); *L. applebyi* has a white belly with dark speckling or brown a brown belly with white speckling; *L. ardens; L. bidoupensis, L. kalonensis, L. maculosa, L. mangshanensis, L. pallida, L. puhoatensis,* and *L. tadungensis* have brown or reddish-brown bellies with white speckling; *L. crocea* has a bright orange belly; *L. shangsiensis* and *L. suiyangensis* have a yellowish, cream-white belly; *L. flaviglandulosa* and *L. fuliginosa* have a white belly with black or brown speckling; *L. liui*, *L. oshanensis,* and *L.tuberosa* have a whitish belly with grey spots, *L. macrops* has a greyish-violet belly with white speckling; *L. melica* has a pale pink belly with greyish spots and white speckling*; L. neangi* has a transparent, immaculate purplish grey belly in life; *L. wuhuangmontis* has a greyish white belly with tiny white and black spots; and *L. yunkaiensis* has a pinkish belly with brown speckling. *Leptobrachella graminicola* **sp. nov.** differs from *L. melanoleuca, L. pyrrhops,* and *L. rowleyae* by the presence of wide lateral fringes on toes (lateral fringes are absent in *L. melanoleuca*, *L. pyrrhops* and *L. rowleyae*). *Leptobrachella graminicola* **sp. nov.** differs from *L. alpina* and *L. tengchongensis* by the presence of a bicoloured iris which is absent in *L. alpina* and *L. tengchongensis*. *Leptobrachella graminicola* **sp. nov.** differs from *L. aspera*, *L. bashaensis*, and *L. feii*, by having a larger adult female size SVL 28.6–32.9 mm (versus SVL ≤ 27.1 mm in adult female *L. aspera*, *L. bashaensis* and *L. feii*; Table 3), and further from *L. bashaensis* by having a dark brown throat with light grey-brown flecks and spots (versus an off-white throat with faint spots) and further from both *L. aspera* and *L. feii* by the absence of dermal ridges on the dorsum (versus presence of unequal dermal ridges on the dorsum of *L. feii*; and dermal ridges on the dorsum of *L. aspera*). *Leptobrachella graminicola* **sp. nov.** differs from *L. bijie*, *L. dorsospina, L. jinshaensis,* and *L. purpuraventra* by having a smaller adult male size SVL 23.1–24.6 mm (versus SVL ≥ 27.3 mm in adult male *L. bijie*; *L. dorsospina*, *L. jinshaensis* and *L. purpuraventra*; Table 3) and further from *L. bijie*; *L. dorsospina*, *L. jinshaensis* and *L. purpuraventra* by the absence of dermal ridges on the dorsum (versus presence of dermal ridges on the dorsum of *L. bijie*, *L. dorsospina*, *L. jinshaensis* and *L. purpuraventra*; Table 3). *Leptobrachella graminicola* **sp. nov.** differs from *L. maoershanensis* by having a dark brown throat with light grey-brown flecks and spots (versus a creamy white throat which may be immaculate or have irregular black spots) and further from *L. maoershanensis* by the absence of dermal ridges on the dorsum (versus presence of dermal ridges on the dorsum in *L. maoershanensis*). *Leptobrachella graminicola* **sp. nov.** differs from in *L. niveimontis* by having a dark brown throat with small white spots (versus marbled with bluish white and black in *L. niveimontis*).

Discussion

The amphibian fauna of the Hoang Lien Range is comprised of more than 80 species (Bourret 1942; Ohler *et al*, 2000; Nguyen *et al*. 2009; Nguyen *et al*. 2013; Rowley *et al*. 2013; Hoang *et al*. 2016; Matsui *et al*. 2017a; Kropachev *et al*. 2019; Luong *et al*. 2019; Tapley *et al*. 2021). *Leptobrachella graminicola* **sp. nov.** is the ninth new species of amphibian described from the Hoang Lien Range in the past decade (Nguyen *et al*. 2013; Rowley *et al*. 2013; Matsui *et al*. 2017a; Kropachev *et al*. 2019; Tapley *et al*. 2021) and it is highly likely that there are many more amphibians to be described from these mountains.

Leptobrachella graminicola **sp. nov.** is likely to be range restricted, forest dependent and threatened due to ongoing habitat loss and degradation and it is important that additional research is undertaken to describe the call and the larvae of this species. Future work should focus on determining the elevational range and distribution of this species as a priority. It is almost certain that *Leptobrachella graminicola* **sp. nov.** occurs in neighbouring Lai Chau Province as the site where all specimens were collected is just 200 m away from the provincial boundary between Lao Cai and Lai Chau provinces.

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APPENDIX 1. Specimens examined.

Abbreviations: AMS = Australian Museum, ITBCZ = Institute of Tropical Biology Zoological Collection (Vietnam), HLNP = Hoang Lien National Park Headquarters (Vietnam), HUS = Hue University of Science (Vietnam).

Leptobrachella aerea: 6 specimens: ITBCZ 4003, ITBCZ 4053, Vietnam, Thua Thien-Hue Province, Bach Ma National Park; ITBCZ 4391–2, ITBCZ 4426–7, Vietnam, Quang Tri Province, Bac Huong Hoa Nature Reserve.

Leptobrachella applebyi: 10 specimens: HUS 0156, HUS 1629–34, ITBCZ 3736, ITBCZ 5257, ITBCZ 5270, Vietnam, Thua Thien-Hue Province, Bach Ma National Park.

Leptobrachella bourreti: 24 specimens: AMS R.186824–7, AMS R.186831–33, AMS R.186835–6, AMS R.186839–41, AMS R.186829, AMS R.186834, AMS R.186843, VNMN 010915, HLNP2018 0612 00018, HLNP2017 1230 00019, Vietnam, Lai Cao Province, Sa Pa District, Mount Fansipan. 2 specimens HLNP2017 1003 00020, VNMN 010916, Vietnam, Lai Cao Province, Bat Xat District, Mount Pu Ta Leng. 1 specimen VNMN 10917, Vietnam, Lai Cao Province, Van Ban District, Mount Nam Kang Ho Tao. AMS R.188515–6 Vietnam, Lai Cao Province, Bat Xat District, Mount Ky Quan San. HLNP2017 0908 00021 Vietnam, Lai Cao Province, Bat Xat District, Mount Ky Quan San.

Leptobrachella crocea: 5 specimens: Vietnam, Thua Thien-Hue Province, Phu Loc District (HUS 00456, ITBCZ 3745). Vietnam, Thua Thien-Hue Province, Bach Ma National Park (HUS 0398, ITBCZ 3974, ITBCZ 3978)

Leptobrachella firthi: 1 specimen: ITBCZ 0546, Vietnam, Gia Lai Province, Kon Ka Kinh National Park.

Leptobrachella cf. *firthi*: 3 specimens: HUS 0155, ITBCZ 3735, ITBCZ 3737, Vietnam, Thua Thien-Hue Province, Bach Ma National Park.

Leptobrachella pluvialis: 48 specimens: AMS R.186786, AMS R.186792, AMS R.186794, AMS R.186798, AMS R.186801, AMS R.186807, AMS R.186809, AMS R.186815, AMS R.186818, AMS R.186785, AMS R.186787–91, AMS R.186793, AMS R.186795–97, AMS R.186799, AMS R.186800. AMSR.186802–06, AMS R.186808, AMS R.186810–14, AMS R.186816–17, AMS R.186819, AMS R.186821–23, VNMN 010918, VNMN 010919–22, HLNP2019 0618 00022–26, Vietnam, Lai Cao Province, Sa Pa District, Mount Fansipan.

Leptobrachella sungi: 1 specimen: ITBCZ 3956, Vietnam, Bac Kan Province, Na Ri District, Na Tha Commune.

Leptobrachella tuberosa: 3 specimens: ITBCZ 4887–8, Vietnam, Quang Ngai Province; ITBCZ 5044, Vietnam, Gia Lai Province, Kon Ka Kinh National Park.

Leptobrachella ventripunctata: 2 specimens: ITBCZ 5083–4, Vietnam, Vinh Phuc Province, Tam Dao National Park.