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NOTES ON GEOGRAPHIC DISTRIBUTION

Discovery of *Boulenophrys changyangensis* Li, Shi, Liu, Zhao, Gao & Wang, 2025 (Anura, Megophryidae) in Hunan, China, and extension of its distribution southward

Jie Huang[1,2], Yang Xiao[1,2], Wanji Xu[3], Yongxiang Tian[3], Jiapeng Song[4], Jiaxi Zhou[5], Yi Liu[1,2], Tao Wu[1], Wansheng Jiang[1,2]

1 Hunan Engineering Laboratory for Giant Salamander Resource Conservation and Comprehensive Utilization, School of Life Sciences, Jishou University, Zhangjiajie 427000, Hunan, China
2 Key Laboratory of Hunan Forest Products and Chemical Industry Engineering, National and Local United Engineering Laboratory of Integrative Utilization Technology of Eucommia Ulmoides, Jishou University, Zhangjiajie 427000, Hunan, China
3 Xiaoxi National Nature Reserve Administration, Yongshun 416700, Hunan, China
4 School of Life Sciences, South China Normal University, Guangzhou 510000, Guangdong, China
5 Independent researcher, Changsha 410000, Hunan, China

Corresponding authors: Tao Wu (623725242@qq.com); Wansheng Jiang (jiangwschina@163.com)

Abstract. We record *Boulenophrys changyangensis* Li, Shi, Liu, Zhao, Gao & Wang, 2025 from Hunan Xiaoxi National Nature Reserve, Hunan, China. This is the second known locality of this species and the first record in Hunan Province. The new record extends the species' distribution range approximately 200 km to the south. Phylogenetic reconstruction and uncorrected *p*-distances indicated subtle but distinct genetic differentiation between the newly discovered population and the topotypic population, highlighting the potential role of rivers and lowland plains in isolating populations of this species.

Keywords. Genetic differentiation, Hunan Xiaoxi National Nature Reserve, phylogeny

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Introduction

Boulenophrys Fei, Ye & Jiang, 2016 is a genus of frog in the family Megophryidae, subfamily Megophryinae (Amphibia, Anura) primarily distributed in southern China and extending southward to the northern Indochinese Peninsula, including parts of Vietnam, Laos, Myanmar, and Thailand (Fei and Ye 2016; Lyu et al. 2023). Before the systematic revision of the Megophryinae by Lyu et al. (2023), the classification of genera within this subfamily was chaotic, and for a time, all members were collectively referred to the genus *Megophrys* Kuhl & Van Hasselt, 1822 (Chen et al. 2017). Due to morphological conservatism and extensive adaptive radiation, many past identifications based solely on morphology were erroneous (e.g. Lyu et al. 2023; Qian et al. 2023). In recent years, the integration of molecular phylogenetics and acoustic data has enabled multidisciplinary taxonomy to discover numerous new species, and the genus now includes 84 species (Frost 2026).

Li et al. (2025) described a new species of Asian horned toad, *Boulenophrys changyangensis* Li, Shi, Liu, Zhao, Gao & Wang, 2025, from Changyang County, Hubei Province, China (30.5732°N, 110.9404°E, ca. 616 m a.s.l.), which is until now the only known locality for this species. Here we report a new distribution record of *B. changyangensis* from Hunan, based on two specimens collected during a recent survey in Hunan Xiaoxi National Nature Reserve. Molecular phylogenetic analysis and morphological examination confirmed the specimens' identification, and these specimens represent a new provincial record for Hunan and a southward range extension for this species.

Methods

Sampling. On 6 June 2026, two adult male specimens of the genus *Boulenophrys* were collected from the Hunan Xiaoxi National Nature Reserve, located in Yongshun County, Xiangxi Tujia and Miao

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Autonomous Prefecture, Hunan Province, China (Figure 1). Upon collection, morphological characteristics were photographed. The specimens were anesthetised with MS-222 and subsequently euthanised, after which liver tissue was excised for total DNA extraction. The specimens were then fixed in 85% ethanol for two days and subsequently transferred to 75% ethanol for long-term preservation. The specimens (JSU HJ2026073 and JSU HJ2026074) are deposited in the Molecular Ecology Laboratory at the Zhangjiajie Campus, Jishou University (JSU). All sample collections and experimental protocols were approved by the Biomedical Ethics Committee of Jishou University (No. JSDX-2024-0083) and were conducted in accordance with relevant laws and regulations of China.

Morphological examination. Morphological measurements were taken with digital callipers (Ningbo Deli Tools Co., Ltd.; accuracy 0.1 mm). Eighteen morphometric characters were measured from two adult specimens, following Fei et al. (2009); the characters are defined in Table 1.

DNA extraction and sequencing. Total genomic DNA was isolated from liver tissues using the TIANamp Genomic DNA Kit (TIANGEN Biotech Co., Ltd., Beijing). The primers used for cytochrome c oxidase subunit 1 (COI) gene and 16S rRNA (16S) gene sequence amplification were Chmf4 (5′-TYCWACWAAYCAYAAAGAYATCGG-3′)/Chmr4 (5′-ACYTCRGGRTGRCCRAARAATCA-3′) (Che et al. 2012) and P7 (5′-CGCCTGTTTACCAAAAACAT-3′)/P8 (5′-CCGGTCTGAACTCAGATCACGT-3′) (Simon et al. 1994), respectively. The PCR mixture of 25 µL consisted of 12.5 µL of PCR Master Mix (TIANGEN Biotech Co., Ltd., Beijing), 1 µL of each primer, 1 µL of DNA template, and 9.5 µL of ddH_2O. Thermal cycling was carried out following Huang et al. (2025). PCR products were examined via 1% agarose gel electrophoresis and then sent for Sanger sequencing to a commercial provider (Tongyong Biotechnology Co., Ltd., Quzhou). All newly obtained sequences have been submitted to GenBank (Table 2).

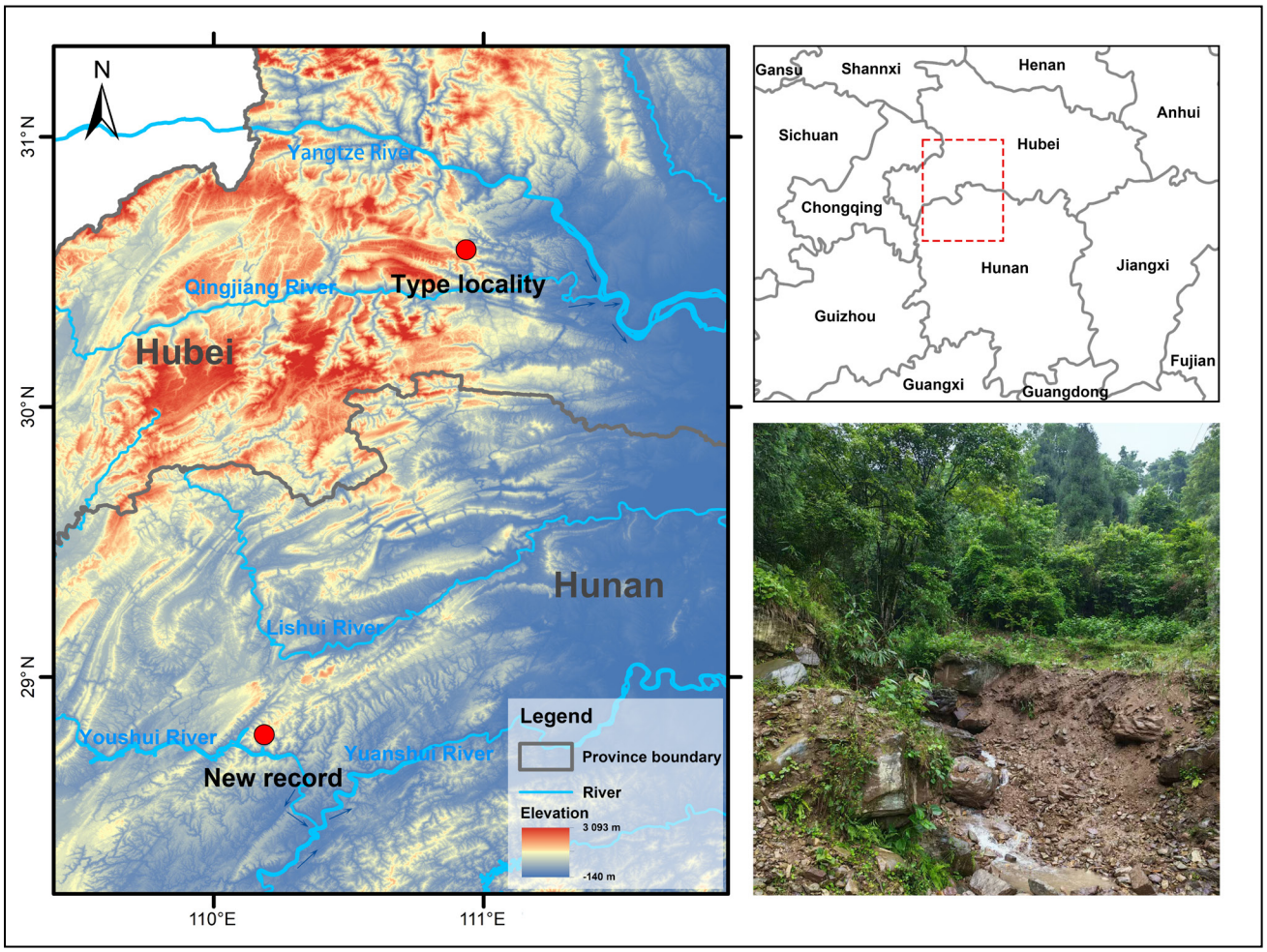

Figure 1. Localities and habitat of *Boulenophrys changyangensis*. Type locality of *B. changyangensis* was derived from Li et al. (2025); elevation data were obtained from the SRTM-DEM 90 m resolution digital elevation product (Geospatial Data Cloud, Computer Network Information Center, Chinese Academy of Sciences, http://www.gscloud.cn); the map (approval no. GS(2019)1674) shows part of China, where the locality of *B. changyangensis* is highlighted by a red dashed box; habitat of *B. changyangensis* in Hunan was photographed by Jie Huang.

Table 1. Morphometric characters used in this study.

Abbreviation	Measurement	Definition
SVL	Snout–vent length	Distance from the tip of the snout to the posterior edge of the vent
HDL	Head length	Distance from the tip of the snout to the articulation of the jaw
HDW	Maximum head width	Greatest width between the left and right articulations of the jaw
SL	Snout length	Distance from the tip of the snout to the anterior corner of the eye
ED	Eye diameter	Distance from the anterior corner to the posterior corner of the eye
IND	Internasal distance	Minimum distance between the inner margins of the external nares
IOD	Interorbital distance	Minimum distance between the inner edges of the upper eyelids
UEW	Upper eyelid width	Greatest width of the upper eyelid margins measured perpendicular to the anterior-posterior axis
TYD	Maximal tympanum diameter	Maximum diameter of the tympanum
LAL	Length of lower arm and hand	Distance from the elbow to the distal end of Finger IV
LW	Lower arm width	Maximum width of the lower arm
ML	Manus length	Distance from the tip of the third digit to the proximal edge of the inner palmar tubercle
HLL	Hindlimb length	Maximum length from the vent to the distal tip of Toe IV
THL	Thigh length	Distance from the vent to the knee
TL	Tibia length	Distance from the knee to the tarsus
TW	Maximal tibia width	Maximum width of the tibia
TFL	Length of foot and tarsus	Distance from the tibiotarsal articulation to the distal end of Toe IV
FL	Foot length	Distance from the tarsus to the tip of the fourth toe

Table 2. Information for samples used in molecular phylogenetic analyses and genetic distance calculation in this study.

ID	Species	Voucher no.	Locality	GenBank accession no.	
				16S	COI
1	*B. changyangensis*	JSU HJ2026073	China: Hunan: Yongshun	PZ618604	PZ618606
2	*B. changyangensis*	JSU HJ2026074	China: Hunan: Yongshun	PZ618605	PZ618607
3	*B. changyangensis*	MT CY20220806005	China: Hubei: Changyang	PV768530	PV768629
4	*B. changyangensis*	MT CY20220806004	China: Hubei: Changyang	PV768529	PV768628
5	*B. changyangensis*	MT CY20220806006	China: Hubei: Changyang	PV768531	PV768630
6	*B. changyangensis*	MT CY20220806002	China: Hubei: Changyang	PV768527	PV768626
7	*B. changyangensis*	MT CY20220806003	China: Hubei: Changyang	PV768528	PV768627
8	*B. acuta*	SYS a002266	China: Guangdong: Fengkai	KJ579119	MH406122
9	*B. angka*	KIZ 040591	Thailand: Chiang Mai	MN508052	—
10	*B. anlongensis*	CIB AL20190531018	China: Guizhou: Anlong	MT823184	MT823261
11	*B. baishanzuensis*	CIB QY20200719002	China: Zhejiang: Qingyuan	MW001151	MT998292
12	*B. baolongensis*	KIZ 019216	China: Chongqing: Wushan	KX811813	KX812093
13	*B. binchuanensis*	KIZ 019441	China: Yunnan: Mt. Jizu	KX811849	KX812112
14	*B. binlingensis*	SYSa005314	Wawu Shan: Sichuan: China	MH406893	MH406355
15	*B. boettgeri*	KIZ YPXJK033	China: Fujian: Mt. Wuyi	KX811814	KX812104
16	*B. brachykolos*	SYS a002258	China: Hong Kong	KJ560403	MH406120
17	*B. caobangensis*	IEBR 4385	Vietnam: Cao Bang: Nguyen Binh	LC483945	—
18	*B. caudoprocta*	SYS a004293	China: Hunan: Sangzhi	MH406796	MH406258
19	*B. cheni*	SYS a004050	China: Jiangxi: Mt. Jinggang	MF667873	MH406241
20	*B. chishuiensis*	SYS a004953	China: Guizhou: Chishui	MH406867	MH406329
21	*B. congjiangensis*	GZNU 20200706003	China: Guizhou: Congjiang	MW959773	MW959761
22	*B. daiyunensis*	SYS a007711	China: Fujian: Tong'an	MW367054	MW365504
23	*B. daoji*	SYS a006212	China: Zhejiang: Mt. Tiantai	MW367047	MW365497
24	*B. daweimontis*	KIZ 048997	China: Yunnan: Mt. Dawei	KX811867	KX812125
25	*B. dongguanensis*	SYS a002007	China: Guangdong: Dongguan	MH406654	MH406090
26	*B. elongata*	GEPa155	China: Guangdong: Huizhou	OR601595	OR597101
27	*B. fanjingmontis*	SYS a004350	China: Guizhou: Mt. Fanjing	MH406808	MH406270
28	*B. fansipanensis*	AMS R186115	Vietnam: Lao Cai: Sapa	MH514887	MW086548
29	*B. fengshunensis*	SYS a004724	China: Guangdong: Fengshun	MH406848	MH406310
30	*B. frigida*	AMS R186131	Vietnam: Lao Cai: Bat Xat	MT364279	MW086550
31	*B. gaolanensis*	SYS a009225	China: Guangdong: Zhuhai	PQ217847	PQ218778
32	*B. gutu*	JHUN SSC24005	China: Hunan: Hengyang: Mt. Hengshan	PX369581	PX367376
33	*B. hengshanensis*	JHUN SSC24180	China: Hunan: Hengyang: Mt. Hengshan	PX369596	PX367390
34	*B. hoanglienensis*	VNMN 2018.02	Vietnam: Lao Cai: Sapa	MH514889	MW086551
35	*B. hungtai*	SYS a007577	China: Guangdong: Jiexi	OL635594	OL634861
36	*B. insularis*	SYS a002171	China: Guangdong: Nan'ao	MH406665	MH406105
37	*B. jiangi*	CIB KKS20180722006	China: Guizhou: Kuankuoshui	MN107743	MN107748
38	*B. jingdongensis*	SYS a003928	China: Yunnan: Mt. Wuliang	MH406773	MH406232
39	*B. jinggangensis*	SYS a004028	China: Jiangxi: Mt. Jinggang	MH406780	MH406239
40	*B. jiulianensis*	SYS a004218	China: Jiangxi: Mt. Jiulian	MH406790	MH406252
41	*B. kuatunensis*	SYS a003449	China: Jiangxi: Mt. Wuyi	MF667881	MH406206
42	*B. leishanensis*	KIZ 049172	China: Guizhou: Mt. Leigong	KX811825	KX812102
43	*B. liboensis*	GNUG 20150813001	China: Guizhou: Libo	MF285253	—

Table 2. Continued.

ID	Species	Voucher no.	Locality	GenBank accession no.	
				16S	COI
44	*B. lichun*	CIB 121428	China: Fujian: Ningde	PQ309137	PQ300665
45	*B. lini*	KIZ 07053	China: Jiangxi: Mt. Jinggang	KX811842	KX812110
46	*B. lishuiensis*	SYS a008445	China: Zhejiang: Liandu	OQ180984	OQ180872
47	*B. lushuiensis*	CIB YN201909289	China: Yunnan: Lushui	MW001226	MW000913
48	*B. minor*	KIZ YPX37545	China: Sichuan: Dujiangyan	KX811895	KX812144
49	*B. mirabilis*	SYS a002289	China: Guangxi: Lingui	MH406681	MH406127
50	*B. mufumontana*	SYS a006419	China: Hunan: Mt. Mufu	MK524107	MK524138
51	*B. nankunensis*	SYS a004503	China: Guangdong: Mt. Nankun	MH406824	MH406286
52	*B. nanlingensis*	SYS a001962	China: Guangdong: Ruyuan	MH406645	MH406081
53	*B. obesa*	SYS a002275	China: Guangdong: Fengkai	KJ579123	MH406125
54	*B. ombrophila*	CIB WY18082308	China: Fujian: Mt. Wuyi	MW001159	MT998300
55	*B. omeimontis*	KIZ 025765	China: Sichuan: Mt. Emei	KX811884	KX812136
56	*B. palpebralespinosa*	KIZ 011650	Vietnam: Thanh Hoa: Pu Hu	KX811889	KX812138
57	*B. pepe*	GEP a207	China: Guangdong: Qingyuan	PQ131151	PQ130479
58	*B. puningensis*	SYS a005770	China: Guangdong: Puning	OL635585	OL634853
59	*B. qianbeiensis*	CIBTZ20190608015	China:Guizhou:Tongzi	MT651553	MT654520
60	*B. rubrimera*	AMS R177676	Vietnam: Lao Cai: Sapa	MF536419	MW086542
61	*B. sangzhiensis*	SYS a004313	China: Hunan: Sangzhi	MH406802	MH406264
62	*B. sanmingensis*	SYS a007057	China: Jiangxi: Mt. Magu	MW367051	MW365501
63	*B. shimentaina*	SYS a002077	China: Guangdong: Yingde	MH406655	MH406092
64	*B. shuichengensis*	JSU HJ2025005	China: Chongqing: Nanchuan	PX124142	PX122102
65	*B. shunhuangensis*	HNNU 16SH04	China: Hunan: Mt. Shunhuang	MK836027	—
66	*B. spinata*	CIBLS20190801002	China: Guizhou: Mt. Leigong	MT651551	MT654518
67	*B. tongboensis*	SYS a003227	China: Jiangxi: Mt. Tongbo	MH406744	MH406201
68	*B. tuberogranulata*	KIZ YPX10987	China: Hunan: Sangzhi	KX811823	KX812095
69	*B. wugongensis*	SYS a004801	China: Jiangxi: Anfu	MH406854	MH406316
70	*B. wuliangshanensis*	SYS a003924	China: Yunnan: Mt. Wuliang	MH406771	MH406230
71	*B. wushanensis*	KIZ YPX47799	China: Chongqing: Wushan	KX811835	—
72	*B. xiangnanensis*	SYS a002874	China: Hunan: Shuangpai	MH406713	MH406165
73	*B. xianjuensis*	CIB XJ190503	China: Zhejiang: Xianju	MN563758	MN563774
74	*B. xuefengmontis*	SYS a007227	China: Hunan: Mt. Xuefeng	OQ180973	OQ180861
75	*B. yangmingensis*	SYS a002888	China: Hunan: Shuangpai	MH406719	MH406171
76	*B. yaoshanensis*	SYS a004878	China: Guangxi: Jinxiu	MH406863	MH406325
77	*B. yingdeensis*	SYS a004721	China: Guangdong: Yingde	MH406846	MH406308
78	*B. youran*	HUNL XB25061603	China: Hunan: Nanshan	PX601289	PV363620
79	*B. yunkaiensis*	SYS a004694	China: Guangdong: Xinyi	MH406845	MH406307
80	*Xenophrys mangshanensis*	SYS a002177	China: Guangdong: Huaiji	MH406666	MH406106

Phylogenetic analysis and genetic calculation. We performed sequence assembly using DNAMAN v. 10.3.6.158. A total of 78 16S sequences and 72 COI sequences, representing 73 species, were downloaded from GenBank for subsequent analyses. As a close relative of *Boulenophrys*, *Xenophrys mangshanensis* (Fei & Ye, 1990) was included in the final dataset as the outgroup (Table 1) (Lyu et al. 2023). Phylogenetic analyses were conducted using Phylosuite v. 1.2.3 (Zhang et al. 2020), with maximum-likelihood (ML) trees inferred using IQ-TREE v. 2.2.0 (Nguyen et al. 2015) and Bayesian inference (BI) trees estimated using MrBayes v. 3.2.7a (Ronquist et al. 2012), based on concatenated 16S (525 bp) and COI (557 bp) gene sequences. The ML tree was constructed under the TVM+F+I+I+R4 model selected by Modelfinder v. 2.2.0 (Kalyaanamoorthy et al. 2017) using the BIC criterion, with 50,000 ultrafast bootstraps (Minh et al. 2013). BI analysis was performed under the GTR+F+I+G4 model (two parallel runs, 2×10^6 generations), also selected by Modelfinder v. 2.2.0 and BIC criterion, with the first 25% of samples discarded as burn-in. Additionally, uncorrected *p*-distances for 16S and COI genes were estimated in MEGA v. 11.0.13 (Tamura et al. 2021).

Results

Boulenophrys changyangensis **Li, Shi, Liu, Zhao, Gao & Wang, 2025**
Figure 2, Table 3

New record. CHINA — Hunan Province • Xiangxi Tujia and Miao Autonomous Prefecture, Yongshun County, Hunan Xiaoxi National Nature Reserve; 28.79707°N, 110.21106°E; 824 m alt; 6 June 2026; Jie Huang leg.; mitochondrial 16S rRNA gene barcodes (PZ618604, PZ618605), mitochondrial COI gene barcodes (PZ618606, PZ618607); 2 ♂ in ethanol, JSU HJ2026073, JSU HJ2026074.

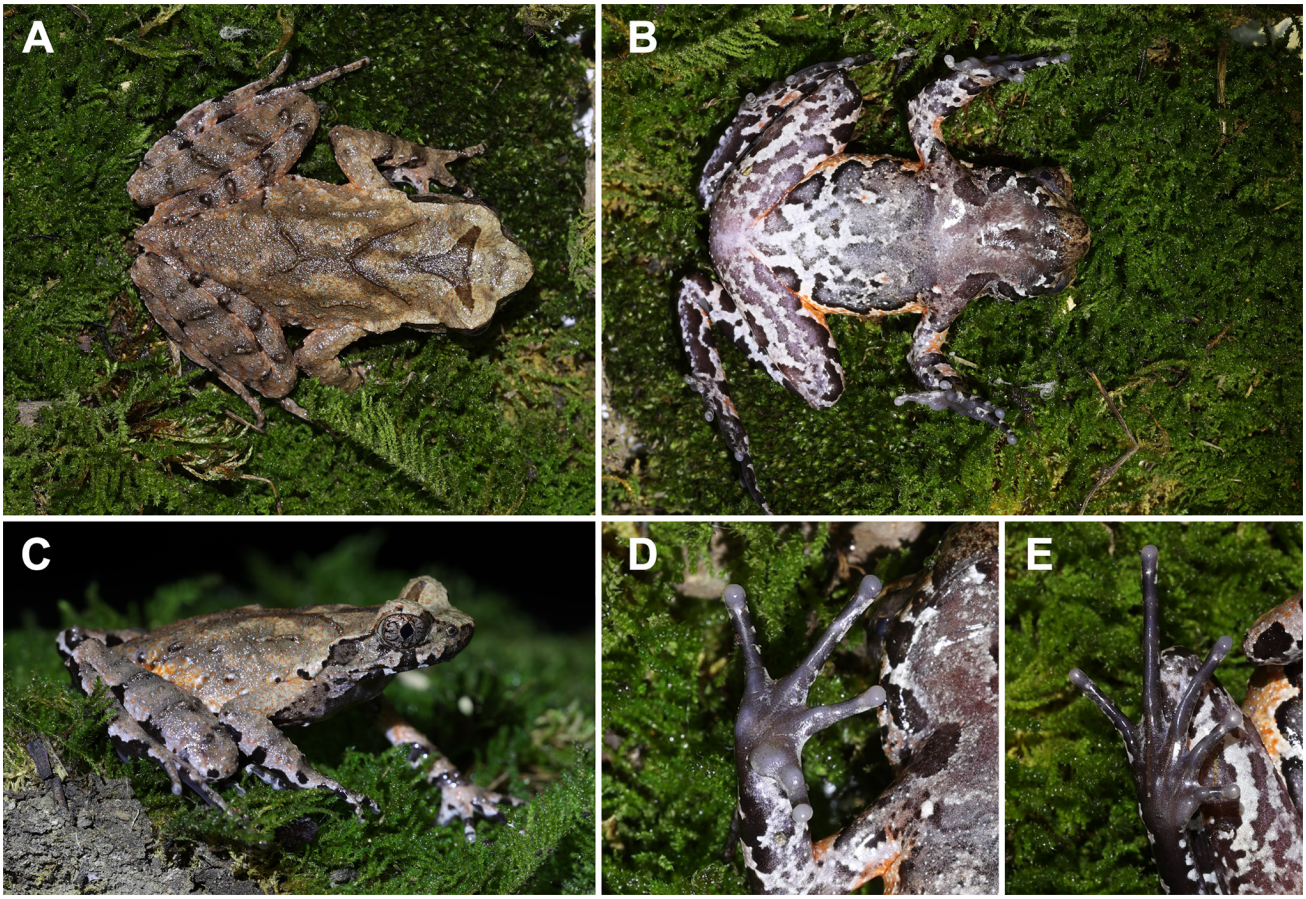

Figure 2. Adult male specimen of *Boulenophrys changyangensis* (JSU HJ2026073) collected in Hunan. **A.** Dorsal view. **B.** Ventral view. **C.** Lateral left view. **D.** Ventral view of the right hand. **E.** Ventral view of the right foot. Photographs by Jie Huang and Yi Liu.

Geographic range extension. The new collection locality (28.79707°N, 110.21106°E) lies approximately 210 km south of the type locality in Changyang County, Hubei (110.9404°E, 30.5732°N; Li et al. 2025), with a latitudinal span of roughly 197 km, thus substantially extending the known range of *B. changyangensis* southward.

Morphological identification. Morphometric measurements of the newly collected specimens are summarised in Table 3 and are largely consistent with those of the type specimens of *B. changyangensis*. Photographs of the live specimen (JSU HJ2026073) are shown in Figure 2. These specimens were identified based on the following combination of morphological characters from Li et al. (2025): body size moderate, with SVL 36–38.7 mm in males; a small horn-like tubercle present on the edge of each upper eyelid; vomerine ridges weak and vomerine teeth absent; tongue without a posterior notch; no large, pale-white, circular spots on the shoulders; toes lacking webbing or lateral fringes; heels overlapping when thighs are positioned at right angles to the body; tibiotarsal articulation reaching the level of the middle of the eye when the leg is stretched forward; males with a single internal subgular vocal sac.

Molecular identification. Bayesian MCMC chains converged reliably, with all parameters having effective sample size >7,000 and potential scale reduction factor ≈1.0. Both ML and BI analyses based on the combined 16S and COI gene sequences strongly supported the monophyly of specimens JSU HJ2026073 and JSU HJ2026074 from Hunan Xiaoxi National Nature Reserve (Hunan) with the type series of *B. changyangensis* (MT CY20220806005, MT CY20220806004, MT CY20220806006, MT CY20220806002, MT CY20220806003), with robust nodal support (BS = 100%, BPP = 1.00; Figure 3). The uncorrected *p*-distances between the Hunan and Hubei populations ranged from 0.012 to 0.014 for 16S and from 0.023 to 0.025 for COI (Tables 4, 5). For 16S, these values are slightly higher than the minimum interspecific distance reported within *Boulenophrys* (0.006, between *B. fanjingmontis* and *B. youran*); for COI, however, they remain below the minimum interspecific distance (0.031, between *B. fanjingmontis* and *B. sangzhiensis*) (Supplemental file: Tables S1, S2). Collectively, the molecular evidence unequivocally identifies the specimens from Hunan (JSU HJ2026073 and JSU HJ2026074) as *B. changyangensis*.

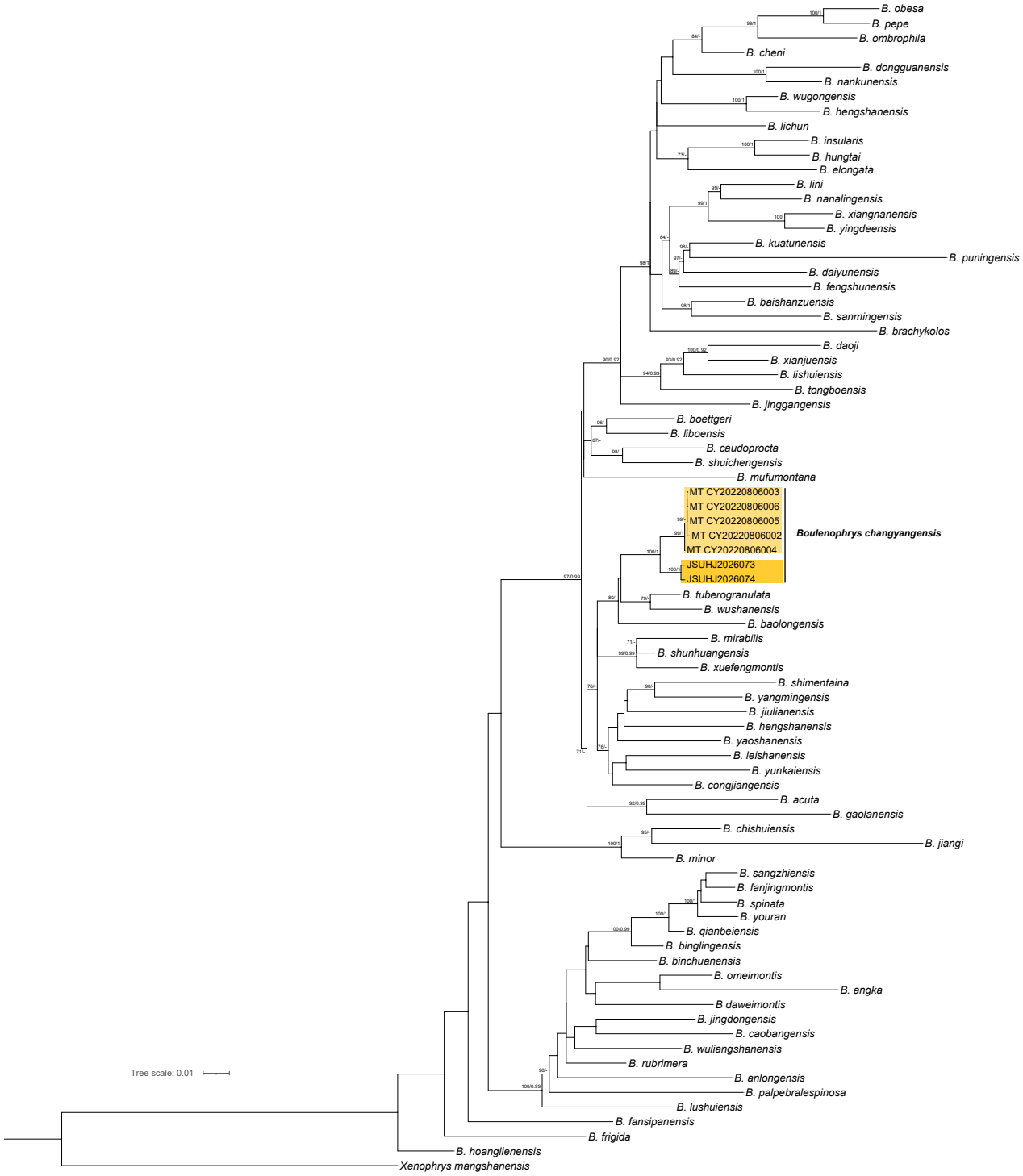

Figure 3. Maximum-likelihood (ML) tree of the genus *Boulenophrys* reconstructed based on combined 16S and COI gene sequences. ML bootstrap (BS) values and Bayesian posterior probabilities (BPP) are indicated at each node; values below 70% (BS) or 0.9 (BPP) are not shown.

Discussion

Both morphological examination and molecular phylogenetic analyses supported the identification of the two *Boulenophrys* specimens collected in this survey as *B. changyangensis*, representing a new provincial record for Hunan Province, China. With this discovery, the number of *Boulenophrys* species known from Hunan has increased from 19 to 20 (AmbhibiaChina 2026), providing foundational data for future conservation listings and local biodiversity resource assessments in the province. Additionally, the morphometric data provided in this study expand the known morphological variation of *B. changyangensis* and supplement the morphological dataset for this species (Table 3).

Table 3. Measurements of the males of *Boulenophrys changyangensis* (mm). Morphometric measurements of the type series were obtained from Li et al. (2025).

Characters	JSU HJ2026073	JSU HJ2026074	MT CY20220806006	MT CY20220806002	MT CY20220806003	MT CY20220806004	MT CY20220806005	Range (mean ± SD)
SVL	38.7	36.0	39.4	43.3	41.5	42.0	41.3	36.00–43.30 (40.31 ± 2.46)
HDL	13.3	12.4	10.9	11.4	12.3	11.4	11.9	10.90–13.30 (11.94 ± 0.80)
HDW	12.9	12.1	12.9	12.7	13.1	12.9	13.6	12.10–13.60 (12.89 ± 0.45)
SL	4.3	4.2	4.6	4.6	6.0	5.2	5.6	4.20–6.00 (4.93 ± 0.68)
ED	3.7	3.6	4.6	4.0	3.6	4.4	4.2	3.60–4.60 (4.01 ± 0.40)
IND	3.4	3.2	5.1	4.4	5.3	4.8	4.5	3.20–5.30 (4.39 ± 0.81)
IOD	2.3	2.8	4.1	4.0	4.2	4.3	4.6	2.30–4.60 (3.76 ± 0.86)
UEW	3.3	3.0	3.1	3.7	3.4	3.8	3.7	3.00–3.80 (3.43 ± 0.31)
TYD	2.2	2.1	3.0	2.6	3.0	3.1	3.3	2.10–3.30 (2.76 ± 0.46)
LAL	18.6	17.6	20.1	19.2	20.9	19.7	19.8	17.60–20.90 (19.41 ± 1.07)
LW	2.6	2.4	2.6	3.0	2.8	3.0	3.0	2.40–3.00 (2.77 ± 0.24)
ML	10.0	8.4	11.0	10.4	10.5	10.8	10.9	8.40–11.00 (10.29 ± 0.90)
HLL	65.8	57.7	62.7	62.7	63.6	58.2	64.7	57.70–65.80 (62.20 ± 3.11)
THL	19.8	18.0	17.9	17.6	18.5	15.9	17.5	15.90–19.80 (17.89 ± 1.17)
TL	19.5	18.0	19.9	19.7	20.2	18.4	20.4	18.00–20.40 (19.44 ± 0.91)
TW	4.6	3.9	3.5	4.0	4.5	4.5	4.1	3.50–4.60 (4.16 ± 0.40)
TFL	27.5	25.7	29.8	28.4	28.7	27.2	30.0	25.70–30.00 (28.19 ± 1.52)
FL	18.0	15.6	18.4	17.6	17.5	16.7	18.7	15.60–18.70 (17.50 ± 1.06)

Table 4. Intraspecific uncorrected *p*-distances of *Boulenophrys changyangensis* based on the 525-bp of the mitochondrial 16S gene.

No.	Species	1	2	3	4	5	6
1	*B. changyangensis* JSU HJ2026073						
2	*B. changyangensis* JSU HJ2026074	0.000					
3	*B. changyangensis* MT CY20220806005	0.012	0.012				
4	*B. changyangensis* MT CY20220806004	0.012	0.012	0.000			
5	*B. changyangensis* MT CY20220806006	0.012	0.012	0.000	0.000		
6	*B. changyangensis* MT CY20220806002	0.014	0.014	0.002	0.002	0.002	
7	*B. changyangensis* MT CY20220806003	0.012	0.012	0.000	0.000	0.000	0.002

Table 5. Intraspecific uncorrected *p*-distances of *Boulenophrys changyangensis* based on the 557-bp of the mitochondrial COI gene.

No.	Species	1	2	3	4	5	6
1	*B. changyangensis* JSU HJ2026073						
2	*B. changyangensis* JSU HJ2026074	0.005					
3	*B. changyangensis* MT CY20220806005	0.025	0.023				
4	*B. changyangensis* MT CY20220806004	0.023	0.022	0.002			
5	*B. changyangensis* MT CY20220806006	0.025	0.023	0.000	0.002		
6	*B. changyangensis* MT CY20220806002	0.025	0.023	0.000	0.002	0.000	
7	*B. changyangensis* MT CY20220806003	0.025	0.023	0.000	0.002	0.000	0.000

During specimen collection, both male specimens were found on exposed rocks beside a stream, where they were emitting advertisement calls (Figure 1). The collection took place at approximately 00:00 on June 6, at a temperature of 23 °C and an elevation of 824 m a.s.l. In addition, an individual of *Lycodon rufozonatus* Cantor, 1842 was observed nearby, which may represent a potential predator during the breeding season of this species. In the same area, within the bushes along the stream, a closely related species, *B. xuefengmontis* Lyu & Wang, 2023 was also heard producing advertisement calls, indicating that the two species share an overlapping breeding season. However, males of the two species appear to differ in their choice of calling microhabitats.

The new record was discovered in the Hunan Xiaoxi National Nature Reserve, which is within the southeastern interior of the Wuling Mountains, in north-western Hunan. This region has recently been recognised as one of the biodiversity hotspots for amphibians in China (Xu et al. 2024). Lately, several new amphibian species have been described from this area, including *Tylototriton gaowangjienensis* Huang, Xiang, Zhang, Wu & Jiang, 2024 and *Leptobrachella yongshunensis* Huang, Wu, Jiang & Zhang, 2025 (Huang et al. 2024, 2025). Most of these taxa are characterised by narrow distributions and high endemism, collectively contributing to the remarkable amphibian diversity of this hotspot. The continuous discovery of new records and new species indicates that the amphibian diversity in this region remains incompletely documented and warrants further investigation.

Genetic distances and phylogenetic analyses based on mitochondrial 16S and COI genes revealed moderate genetic differentiation between the Hunan population discovered in this study and the type specimens in the Hubei population (Figure 3, Tables 4, 5). As a small-bodied frog species that relies on mountain stream habitats and exhibits strong site fidelity, this genetic divergence is likely driven by geographical barriers, such as the large rivers (e.g. Lishui and Qingjiang rivers) and intervening plains that separate the two regions (Figure 1). A similar pattern has been observed in sibling species pairs distributed on opposite sides of the Lishui River, such as *L. wulingensis* and *L. yongshunensis* (Huang et al. 2025). In this biodiversity hotspot, the isolation among mountain habitats caused by large rivers and surrounding lowland plains may be one of the key factors shaping local diversity, a phenomenon that can be explained by the sky-island effect (MacArthur and Wilson 1967). Indeed, many amphibian species that belong to the same genus but represent distinct evolutionary clades are also distributed in this region (Li et al. 2024; Xiang et al. 2024), collectively highlighting the complexity underlying the formation of this biodiversity hotspot.

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Additional information

Conflict of interest

The authors declare that no competing interests exist.

Ethical statement

No ethical statement is reported.

Use of artificial intelligence (AI)

The authors accept full responsibility for the content of the manuscript, including the disclosure of any use of AI. No AI tools were used in the preparation of this manuscript.

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Author contributions

Conceptualization: J. Huang, W. Jiang. Data curation: J. Huang, Y. Xiao, Y. Liu, J. Song. Formal analysis: J. Huang, Y. Liu, Y. Tian, T. Wu. Funding acquisition: J. Huang, T. Wu, W. Jiang. Investigation: J. Huang, Y. Xiao, J. Song, J. Zhou, Y. Tian. Methodology: J. Huang, Y. Liu, T. Wu, W. Jiang. Resources: W. Xu, T. Wu, W. Jiang. Supervision: T. Wu, W. Jiang. Visualization: J. Huang, Y. Xiao. Project administration: J. Huang, W. Jiang. Validation: W. Jiang, T. Wu., W. Xu. Writing – original draft: J. Huang, W. Jiang. Writing – review and editing: J. Huang, T. Wu, W. Jiang.

Author ORCID iDs

Jie Huang https://orcid.org/0009-0005-2565-3109
Yang Xiao https://orcid.org/0009-0009-9306-5172
Yongxiang Tian https://orcid.org/0000-0001-9249-8114
Tao Wu https://orcid.org/0009-0009-4991-005X
Wansheng Jiang https://orcid.org/0000-0002-6498-944X

Data availability

All data that support the findings of this study are available in the main text and Supplementary materials:

Table S1. Uncorrected *p*-distances within the genus *Boulenophrys* based on the 525-bp mitochondrial 16S gene. [https://doi.org/10.15560/22.5.801.suppl1] **Table S2.** Uncorrected *p*-distances within the genus *Boulenophrys* based on the 557-bp mitochondrial COI gene. https://doi.org/10.15560/22.5.801.suppl2]

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