

A new species of *Boulenophrys* from central Hunan Province, China (Anura: Megophryidae)

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<https://zoobank.org/73D439BE-596C-4A69-BCA3-7E4B35440C11>

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Academic editors Raffael Ernst | **Received** 22 January 2023 | **Accepted** 18 September 2023 | **Published** 3 October 2023

Citation: Qian T, Hu K, Mo X, Gao Z, Zhang N, Yang D (2023) A new species of *Boulenophrys* from central Hunan Province, China (Anura, Megophryidae). Vertebrate Zoology 73 915–930. <https://doi.org/10.3897/vz.73.e100889>

Abstract

We re-examined the megophryinid population from Mt. Hengshan, Hunan Province, China previously identified as *Boulenophrys brachykolos* (under the name *Megophrys brachykolos*). Based on newly obtained molecular data, this population appears to be an independent lineage with a relatively distant phylogenetic relationship to *B. brachykolos* sensu stricto. Furthermore, this population exhibits distinct morphological characteristics that distinguish it from all its congeners. Therefore, we propose to recognize the Hengshan population of *B. brachykolos* as a new species, *Boulenophrys hengshanensis* sp. nov. described herein.

Keywords

Frog diversity, morphology, phylogeny, taxonomy

Introduction

The genus *Boulenophrys* Fei, Ye and Jiang, 2016 is the largest branch of the Asian horned frog subfamily Megophryinae Bonaparte, 1850, contains 65 recognized species, distributed throughout southern China, and south to the Indochina Peninsula (Frost 2023; Lyu et al. 2023). Due to the conserved morphological characters, a large number of cryptic species from southern China were unrecognized in the past, and have been revealed based on large-scale molecular sampling efforts (i.e. Chen et al. 2017; Liu et al. 2018). This also resulted in taxonomic uncertainty in many species that were generally considered widespread. For example, *Boulenophrys brachykolos* (Inger and Romer, 1961) was previously regarded as widespread throughout southern China and northern Vietnam (Fei et

al. 2009; Orlov et al. 2002). However, only specimens from its type locality Hong Kong and adjacent Shenzhen, Guangdong Province were confirmed by molecular and morphological data (Lyu et al. 2023). The record of *B. brachykolos* from Mt. Dayaoshan, Guangxi Province was recently revised and identified as an independent species *B. yaoshanensis* Qi et al., 2021. Additionally, the record from Mt. Mangshan, Hunan Province was cast into doubt following random tadpole sampling (Qian et al. 2023). As a result, Lyu et al. (2023) concluded that *B. brachykolos* is restricted to Hong Kong and Shenzhen and suggested to remove all records from other localities.

Mo et al. (2009) first reported the presence of *B. brachykolos* on Mt. Hengshan, Hunan Province, providing

measurements of five specimens (sex unmentioned). Shen et al. (2014) subsequently provided a detailed description and identified four vouchered female specimens from Hunan Province as *B. brachykolos*, two of which were collected from Mt. Hengshan. All specimens examined by Shen et al. (2014) displayed a series of morphological characteristics, including relatively smooth dorsum skin, a small horn-like tubercle on the upper eyelid, short hindlimbs with heels not touching each other when hindlimbs held at right angles to body, the absence of vomerine teeth, toes with rudimentary webbing and lacking lateral fringes, and an interorbital triangle dorsum pattern. These characteristics were consistent with the original description of *B. brachykolos* by Inger and Romer (1961), as well as the revised description based on the examination of topotype specimens by Fei et al. (2009). However, the absence of molecular data for this population called for a re-examination of its identification as *B. brachykolos*.

To clarify the taxonomic status of the *Boulenophrys* population from Mt. Hengshan, a field survey was carried out in June 2021, during which a series of specimens was collected. To determine the phylogenetic position of this population, DNA sequences were extracted from the newly collected specimens and compared to those of its congeners. The Hengshan population and both the topo-typical *B. brachykolos* specimens and all known *Boulenophrys* congeners were also morphologically examined and compared. Our results revealed that the specimens from Mt. Hengshan represent an independent lineage and can be separated from all known congeners by a series of morphological characteristics. Thus, we propose to recognize the *B. brachykolos* population from Mt. Hengshan as a new species, which we describe below.

Materials and Methods

A total of 20 newly collected specimens from Mt. Hengshan, Nanyue District, Hengyang Prefecture, Hunan Province, China were preserved in 75% alcohol and deposited in the Institute of Wildlife Conservation, Central South University of Forestry and Technology (CSUFT). For DNA extraction, tissue samples (liver) were preserved in 95% ethanol. Two museum specimens of the Hengshan population were preserved in formalin and deposited in the College of Life Sciences, Hunan Normal University (HNNU). Comparative specimens were examined from CSUFT and the Museum of Biology, Sun Yat-sen University (SYS) as listed in Appendix I.

Molecular data

We selected six newly collected specimens that exhibited several variable morphological features (see Variation) for DNA extraction. Two mitochondrial genes, namely the genes for 16S ribosomal RNA (16S) and cytochrome C oxidase subunit I (CO1) were selected for molecular

analysis following Qi et al. (2021). Genomic DNA was extracted from 95%-ethanol-preserved tissues using a Tsingke TSP201-200 DNA extraction kit. Primer sequences were retrieved from the literature: L3975 and H4551 for 16S following Simon et al. (1994) and dgLCO and dgH-CO for CO1 following Meyer et al. (2005). PCR amplification was carried out in 50 μ l reaction volumes under the following conditions: 98°C for 2 min; 30 cycles of 98°C for 10 sec, 55°C for 10 sec, 72°C for 10 sec, with a final extension step at 72°C for 5 min. PCR purification and sequencing were performed by Biomarker Technologies Co. (Beijing, China).

For reconstructing the phylogenetic tree, the corresponding sequences of 64 known *Boulenophrys* species and an undescribed species from Hunan Province (“*Megophrys*” sp. 27 of Liu et al. (2018)) were downloaded from GenBank. We selected *Xenophrys glandulosa* and *X. mangshanensis* as outgroups, because these taxa performed well in several *Boulenophrys* studies (e.g. Lyu et al. 2020, 2021; Qi et al. 2021; Wang et al. 2022). All samples used in the molecular analysis are listed in Table 1. Sequences of two genes were aligned respectively in MEGA 6 (Tamura et al. 2013) by the MUSCLE algorithm (Edgar 2004) with default parameters. Finally, the alignment of 545 base pairs (bp) of 16S and 627 bp of CO1, were concatenated into an 1172-bp-long dataset. Phylogenetic trees were constructed using Bayesian inference (BI) implemented in MrBayes 3.2.5 (Ronquist et al. 2012). The best-fitting models and the best partitioning scheme were selected using the corrected Akaike information criterion (AICc) in PartitionFinder 2 (Lanfear et al. 2017). The selected partitions and models were GTR + I + G for 16S, SYM + I for CO1 first codon position, HKY for CO1 second codon position, and GTR + G for CO1 third codon position. Two independent runs were conducted during the BI analyses with 10,000,000 generations each, and they were sampled every 1000 generations with the first 25% of samples discarded as burn-in, resulting in a potential scale reduction factor (PSRF) of <0.005.

Morphological data

Morphological measurements from preserved specimens were conducted as described by Lyu et al. (2021) using a digital caliper (to the nearest 0.1 mm). Definitions were as follows: Snout-vent length from tip of snout to vent (**SVL**); head length from the point behind the angle of the jaw to tip of snout (**HDL**); head width at the widest point (**HDW**); snout length from the tip of snout to the anterior corner of the eye (**SNT**); internasal distance (**IND**); interorbital distance (**IOD**), the minimum distance between upper eyelids; eye diameter (**ED**); tympanum diameter (**TD**); tympanum-eye distance from the anterior edge of tympanum to posterior corner of the eye (**TED**); hand length from the proximal border of the outer palmar tubercle to the tip of finger III (**HND**); length of lower arm and hand, from the flexed elbow to the tip of finger III (**LAH**); tibia length from the outer surface of the flexed knee to the heel (**TIB**); foot-tarsus length from the distal

Table 1. Specimens, localities, and GenBank accession numbers of all samples used in the molecular analysis.

ID	Species	Voucher	Locality	16S	CO1	Reference
1	<i>Boulenophrys hengshanensis</i> sp. nov.	CSUFT HS210603	Mt. Hengshan, Nanyue Dist., Hengyang, Hunan, China	ON209287	---	This study
2	<i>B. hengshanensis</i> sp. nov.	CSUFT HS210606	Mt. Hengshan, Nanyue Dist., Hengyang, Hunan, China	ON209292	---	This study
3	<i>B. hengshanensis</i> sp. nov.	CSUFT HS210608	Mt. Hengshan, Nanyue Dist., Hengyang, Hunan, China	ON209289	OQ910104	This study
4	<i>B. hengshanensis</i> sp. nov.	CSUFT HS210610	Mt. Hengshan, Nanyue Dist., Hengyang, Hunan, China	ON209293	OQ910105	This study
5	<i>B. hengshanensis</i> sp. nov.	CSUFT HS210612	Mt. Hengshan, Nanyue Dist., Hengyang, Hunan, China	ON209288	OQ910106	This study
6	<i>B. hengshanensis</i> sp. nov.	CSUFT HS210614	Mt. Hengshan, Nanyue Dist., Hengyang, Hunan, China	ON209291	OQ910107	This study
7	<i>B. hengshanensis</i> sp. nov.	CSUFT HS210617	Mt. Hengshan, Nanyue Dist., Hengyang, Hunan, China	ON209294	OQ910108	This study
8	<i>B. hengshanensis</i> sp. nov.	CSUFT HS210621	Mt. Hengshan, Nanyue Dist., Hengyang, Hunan, China	ON209290	OQ910109	This study
9	<i>B. xuefengmontis</i>	SYS a004364	Wugang County, Hunan, China	MH406813	MH406275	Lyu et al. (2023)
10	<i>B. xuefengmontis</i>	SYS a004365	Wugang County, Hunan, China	MH406814	MH406276	Lyu et al. (2023)
11	“ <i>Megophrys</i> ” sp. 27	SYS a004279	Taoyuan County, Hunan, China	MH406793	MH406255	Liu et al. (2018)
12	“ <i>Megophrys</i> ” sp. 27	SYS a004280	Taoyuan County, Hunan, China	MH406794	MH406256	Liu et al. (2018)
13	<i>B. acuta</i>	SYS a002159	Heishiding Nature Reserve, Guangdong, China	MF667869	MH406099	Liu et al. (2018)
14	<i>B. angka</i>	KIZ 040592	Kiew Mae Pan Nature Trail, Doi Inthanon, Chiang Mai, Thailand	MN508048	---	Wu et al. (2019)
15	<i>B. anlongensis</i>	CIB AL21090531017	Anlong County, Guizhou, China	MT823185	MT823262	Li et al. (2020)
16	<i>B. baishanzuensis</i>	CIB QY20200719001	Baishanzu National Park, Qingyuan, Zhejiang, China	MW001150	MT998291	Wu et al. (2020)
17	<i>B. baolongensis</i>	KIZ 019216	Baolong Town, Chongqing, China	KX811813	KX812093	Chen et al. (2017)
18	<i>B. binchuanensis</i>	KIZ 019442	Mt. Jizu, Yunnan, China	KX811850	KX812113	Chen et al. (2017)
19	<i>B. binglingensis</i>	SYS a005313	Mt. Wawu, Sichuan, China	MH406892	MH406354	Liu et al. (2018)
20	<i>B. boettgeri</i>	SYS a004149	Mt. Wuyi, Fujian, China	MF667878	MH406247	Liu et al. (2018)
21	<i>B. brachykolos</i>	SYS a002258	Hong Kong, China	KJ560403	MH406120	Liu et al. (2018)
22	<i>B. caobangensis</i>	IBER 4385	Nguyen Binh, Cao Bang, Vietnam	LC483945	---	Nguyen et al. (2020)
23	<i>B. caudoprocta</i>	SYS a004281	Zhangjiajie, Hunan, China	MH406795	MH406257	Liu et al. (2018)
24	<i>B. cheni</i>	SYS a002142	Taoyuandong, Hunan, China	KJ560398	MH406098	Liu et al. (2018)
25	<i>B. chishuiensis</i>	CIB CS20190518019	Chishui County, Guizhou, China	MN954708	MN928959	Xu et al. (2020)
26	<i>B. congjiangensis</i>	GZNU 20200706003	Yueliangshan Nature Reserve, Congjiang, Guizhou, China	MW959773	MW959761	Luo et al. (2021)
27	<i>B. daiyunensis</i>	SYS a001733	Mt. Daiyun, Fujian, China	MH406643	MH406079	Liu et al. (2018)
28	<i>B. daoji</i>	SYS a004088	Fenghua, Zhejiang, China	MH406782	MH406242	Liu et al. (2018)
29	<i>B. daweimontis</i>	KIZ 048938	Mt. Dawei, Yunnan, China	KX811870	KX812126	Chen et al. (2017)
30	<i>B. dongguanensis</i>	SYS a001973	Mt. Yiping, Guangdong, China	MH406647	MH406083	Liu et al. (2018)
31	<i>B. fansipanensis</i>	AMS R186115	Sa Pa, Lao Cai, Vietnam	MH514887	MW086548	Tapley et al. (2021)
32	<i>B. fengshunensis</i>	SYS a004724	Fengshun County, Guangdong, China	MH406848	MH406310	Liu et al. (2018)
33	<i>B. frigida</i>	AMS R186131	Mount Ky Quan San, Bat Xat District, Lao Cai, Vietnam	MT364279	MW086550	Tapley et al. (2021)
34	<i>B. hoanglienensis</i>	VNMN 2018.02	Sa Pa, Lao Cai, Vietnam	MH514889	MW086551	Tapley et al. (2021)
35	<i>B. boettgeri</i>	SYS a002703	Mt. Huangshan, Anhui, China	MF667883	MH406161	Lyu et al. (2023)
36	<i>B. hungtai</i>	SYS a007575	Jiexi County, Guangdong, China	OL635592	OL634859	Wang et al. (2022)
37	<i>B. insularis</i>	SYS a002168	Nan’ao Island, Guangdong, China	MF667886	MF667923	Liu et al. (2018)
38	<i>B. jiangi</i>	CIB KKS20180426001	Kuankuoshui Nature Reserve, Guizhou, China	MN107744	MN107749	Liu et al. (2020)
39	<i>B. jingdongensis</i>	SYS a003928	Mt. Wuliang, Yunnan, China	MH406773	MH406232	Liu et al. (2018)
40	<i>B. jinggangensis</i>	SYS a004028	Mt. Jinggang, Jiangxi, China	MH406780	MH406239	Liu et al. (2018)
41	<i>B. jiulianensis</i>	SYS a004219	Mt. Jiulian, Jiangxi, China	MH406791	MH406253	Liu et al. (2018)
42	<i>B. kuatunensis</i>	SYS a003449	Guadun, Fujian, China	MF667881	MH406206	Liu et al. (2018)
43	<i>B. leishanensis</i>	SYS a002213	Mt. Leigong, Guizhou, China	MH406673	MH406113	Liu et al. (2018)
44	<i>B. liboensis</i>	GNUG 20150813001	Libo, Guizhou, China	MF285253	---	Zhang et al. (2017)
45	<i>B. lini</i>	SYS a002381	Mt. Jinggang, Jiangxi, China	MF667874	MH406135	Liu et al. (2018)

ID	Species	Voucher	Locality	16S	CO1	Reference
46	<i>B. lishuiensis</i>	CIB WYF00169	Lishui, Zhejiang, China	KY021418	---	Wang et al. (2017b)
47	<i>B. lushuiensis</i>	CIB YN201909288	Lushui County, Yunnan, China	MW001225	MW000912	Shi et al. (2021)
48	<i>B. minor</i>	SYS a003209	Dujiangyan, Sichuan, China	MF667862	MH406194	Liu et al. (2018)
49	<i>B. mirabilis</i>	SYS a002192	Huaping Nature Reserve, Guangxi, China	MH406669	MH406109	Liu et al. (2018)
50	<i>B. mufumontana</i>	SYS a006391	Mt. Mufu, Pingjiang County, Hunan, China	MK524105	MK524136	Wang et al. (2019a)
51	<i>B. nankunensis</i>	SYS a004501	Mt. Nankun, Guangdong, China	MH406822	MH406284	Liu et al. (2018)
52	<i>B. nanlingensis</i>	SYS a001964	Nanling Nature Reserve, Guangdong, China	MH406646	MH406082	Liu et al. (2018)
53	<i>B. obesa</i>	SYS a002272	Heishiding Nature Reserve, Guangdong, China	KJ579122	MH406124	Liu et al. (2018)
54	<i>B. omeimontis</i>	SYS a005301	Mt. Le, Sichuan, China	MH406887	MH406349	Liu et al. (2018)
55	<i>B. palpebralespinosa</i>	KIZ 011650	Pu Hu Nature Reserve, Thanh Hoa, Vietnam	KX811889	KX812138	Chen et al. (2017)
56	<i>B. puningensis</i>	SYS a005770	Puning City, Guangdong, China	OL635585	OL634853	Wang et al. (2022)
57	<i>B. qianbeiensis</i>	CIB TZ20190608017	Huanglian Nature Reserve, Guizhou, China	MT651554	MT654521	Su et al. (2020)
58	<i>B. rubrimera</i>	AMS R177676	Sa Pa, Lao Cai, Vietnam	MF536419	MW086542	Tapley et al. (2021)
59	<i>B. sangzhiensis</i>	SYS a004306	Zhangjiajie, Hunan, China	MH406797	MH406259	Liu et al. (2018)
60	<i>B. sanmingensis</i>	SYS a002486	Mt. Emeifeng, Fujian, China	MH406697	MH406145	Liu et al. (2018)
61	<i>B. shimentaina</i>	SYS a002077	Shimentai Nature Reserve, Guangdong, China	MH406655	MH406092	Liu et al. (2018)
62	<i>B. shunhuangensis</i>	HNNU 18NS01	Nanshan Forest Park, Hunan, China	MK836023	MK977594	Wang et al. (2019b)
63	<i>B. spinata</i>	SYS a002226	Mt. Leigong, Guizhou, China	MH406675	MH406115	Liu et al. (2018)
64	<i>B. tongboensis</i>	SYS a002415	Mt. Tongbo, Jiangxi, China	MH406690	MH406138	Liu et al. (2018)
65	<i>B. tuberogranulata</i>	SYS a004310	Badagongshan Nature Reserve, Hunan, China	MH406801	MH406263	Liu et al. (2018)
66	<i>B. wugongensis</i>	SYS a004800	Mt. Wugong, Jiangxi, China	MH406853	MH406315	Liu et al. (2018)
67	<i>B. wuliangshanensis</i>	SYS a003924	Mt. Wuliang, Yunnan, China	MH406771	MH406230	Liu et al. (2018)
68	<i>B. wushanensis</i>	SYS a003008	Mt. Wu, Hubei, China	MH406732	MH406184	Liu et al. (2018)
69	<i>B. xiangnanensis</i>	SYS a002874	Mt. Yangming, Hunan, China	MH406713	MH406165	Liu et al. (2018)
70	<i>B. xianjuensis</i>	CIB XJ190505	Xianju County, Zhejiang, China	MN563753	MN563769	Wang et al. (2020)
71	<i>B. yangmingensis</i>	SYS a002877	Mt. Yangming, Hunan, China	MH406716	MH406168	Liu et al. (2018)
72	<i>B. yaoshanensis</i>	SYS a002189	Dayaoshan Nature Reserve, Guangxi, China	MH406667	MH406107	Liu et al. (2018)
73	<i>B. yingdeensis</i>	SYS a002100	Shimentai Nature Reserve, Guangdong, China	MH406658	MH406095	Liu et al. (2018)
74	<i>B. yunkaiensis</i>	SYS a004637	Yukaishan Nature Reserve, Guangdong, China	MH406843	MH406305	Liu et al. (2018)
75	<i>Xenophrys glandulosa</i>	SYS a003758	Mt. Gaoligong, Yunnan, China	MH406755	MH406214	Liu et al. (2018)
76	<i>X. mangshanensis</i>	SYS a002177	Mt. Sanyue, Guangdong, China	MH406666	MH406106	Liu et al. (2018)

end of the shank to the tip of digit IV (**FTL**). Sex was determined by the presence of nuptial pads/spines in males and/or gonadal inspection.

Morphological data for genera delimitation was based on information that summarized by Lyu et al. (2023). Comparative data of *Boulenophrys* species were based on the examination of museum specimens listed in Appendix I and on information available in the literature (Inger and Romer 1961; Tian et al. 2000; Ye et al. 2007; Jiang et al. 2008; Mo et al. 2010; Wang et al. 2012, 2014, 2017a, 2017b, 2019a, 2019b, 2020, 2022; Liao et al. 2013; Li et al. 2014, 2018, 2020; Shen et al. 2014; Fei and Ye 2016; Tapley et al. 2017, 2018, 2021; Zhang et al. 2017; Messenger et al. 2019; Wu et al. 2019, 2020; Liu et al. 2020; Lyu et al. 2020, 2021, 2023; Nguyen et al. 2020; Su et al. 2020; Xu et al. 2020; Luo et al. 2021; Shi et al. 2021; Qi et al. 2021).

Acoustic data

Advertisement calls were recorded using a TASCAM DR05X digital recorder (96 kHz, 24-bit) with a built-in microphone from a male paratype specimen on 4 June 2021 at about 20:48 h. Calls were recorded at approximately 0.2 m with the frog. The ambient temperature was not recorded. Calls were analyzed with Raven Pro 1.6 software (Cornell Lab of Ornithology). Spectrograms were produced applying a Hann window with 512 samples window size, and overlap of 50%, and a hop size of 256 samples. Call parameters were measured following Tapley et al. (2021): Call duration (ms), inter-call interval (ms), number of calls per call groups, call repetition rate (calls/s), number of pulses per call, and dominant frequency (kHz).

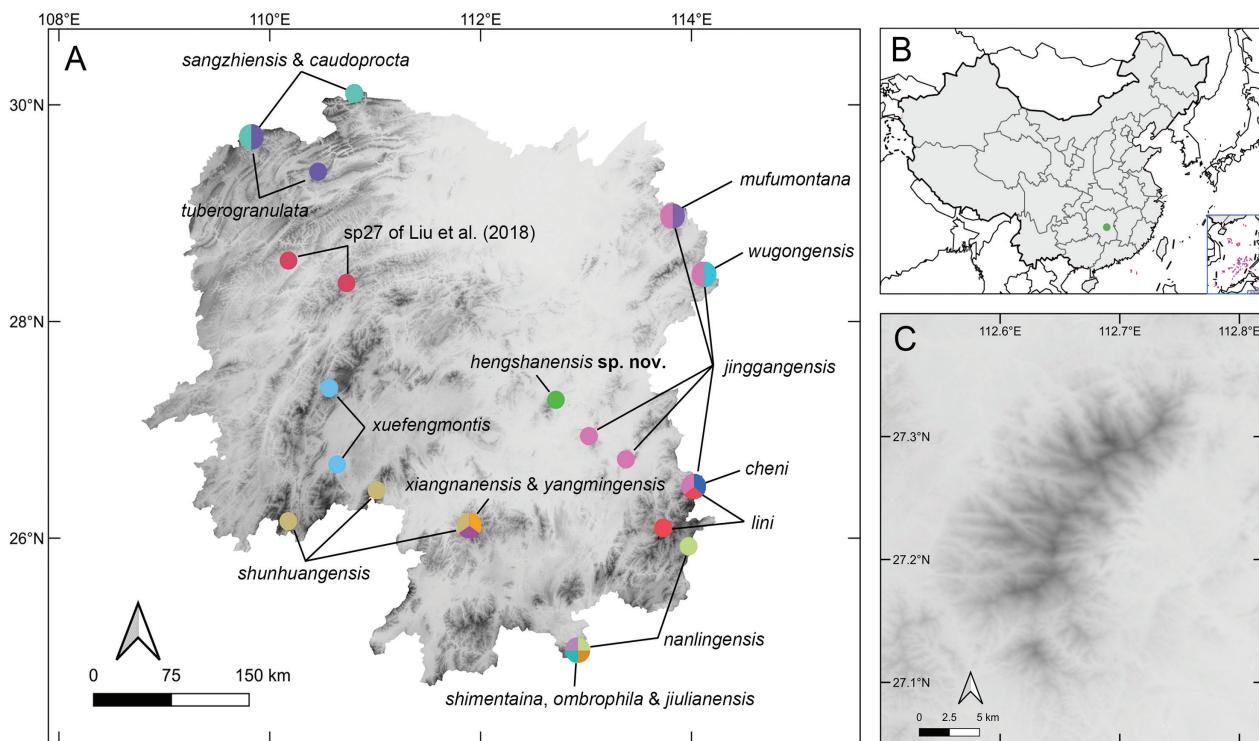


Figure 1. Distribution map of *Boulenophrys* species in Hunan Province (A). The green spot in (B) indicates the collection site of *Boulenophrys hengshanensis* sp. nov. in China. The map in (C) represents a zoomed-in topographic map of Mt. Hengshan.

Mapping

The software Quantum GIS (QGIS v.3.16, Hannover) was used to make a topographic map (Fig. 1) using the 30 m spatial resolution Advanced Spaceborne Thermal Emission and Reflection (ASTER) Global Digital Elevation Model (GDEM) layer available from Geospatial Data Cloud (<http://gscloud.cn>). GPS coordinates were retrieved from the literature (Wang et al. 2019a; Lyu et al. 2020, 2023; Shang et al. 2023; Qian et al. 2023) or from information recorded with museum specimens. Localities reported in literature without coordinates (Shen et al. 2014; Liu et al. 2018; Gao et al. 2022) were estimated using TIANDITU (<https://maps.tianditu.gov.cn>, accessed on 23 December 2022).

Results

The reconstructed Bayesian topology is shown in Fig. 2. The samples from Mt. Hengshan clustered into a poorly supported clade (Bayesian Posterior Probability [BPP] value 0.68) with *B. shunhuangensis*, *B. mirabilis*, *B. xuefengmontis* and “*Megophrys*” sp. 27 in Liu et al. (2018). Within this clade, each lineage is strongly supported with a BPP value of 1.00, except for *B. shunhuangensis* and *B. mirabilis*. The Hengshan population is sister to “*Megophrys*” sp. 27 from Taoyuan County, about 200 km northwest of Mt. Hengshan (see Fig. 1). In addition, the undescribed species shows a relatively distant phylogenetic relationship to *B. brachykolos*.

Based on examination of topotype specimens of *B. brachykolos* and the revised diagnosis provided by Lyu et al. (2023), our undescribed species from Mt. Hengshan is distinguished from *B. brachykolos* by the tongue not notched posteriorly (vs. notched posteriorly), the absence of vomerine teeth and vomerine ridges (vs. presence of vomerine ridges and weakly developed vomerine teeth), and a relative finger length of III>I>II>IV (vs. III>I>IV>II). Furthermore, this undescribed species is separated from all known congeners by a series of diagnostic morphological traits (see Comparisons) and described herein as a new species.

Family: Megophryidae

Subfamily: Megophryinae

Boulenophrys hengshanensis sp. nov.

<https://zoobank.org/A03130D7-3C1E-4DAB-8AFC-A0F-ABD61BB28>

Figures 3–5, Table 2

Chresonymy. *Megophrys brachykolos* – Mo et al. (2009), Shen et al. (2014), Gao et al. (2022)

Holotype. HNNU 0706000A, adult female, collected by Youhui Shen from Nanyue Hengshan, Hengyang, Hunan Province, China in June 2007.

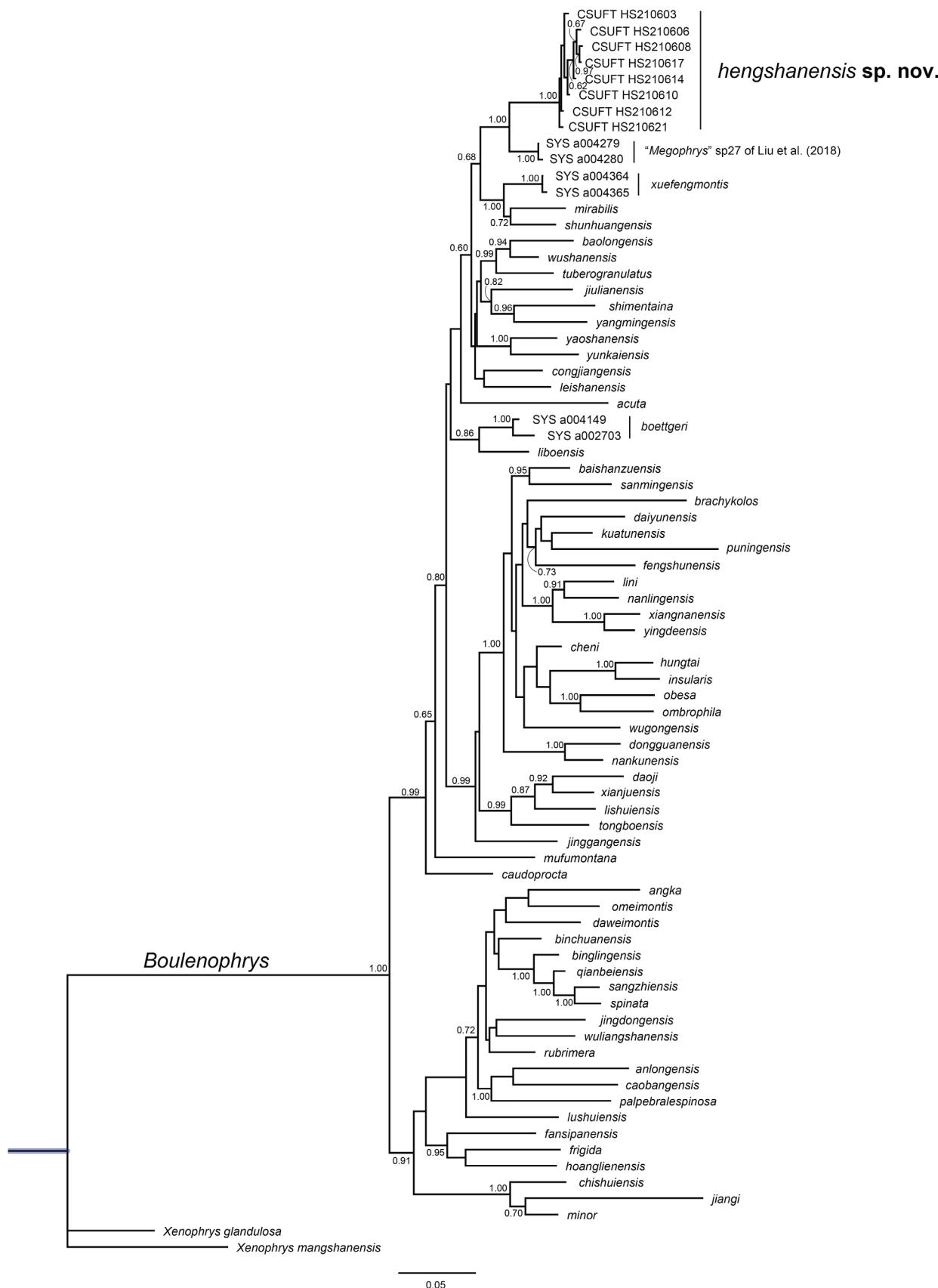


Figure 2. Bayesian topology of *Boulenophrys* based on the 16S and CO1 genes. Numbers on nodes are Bayesian posterior probabilities (BPP) greater than or equal to 0.60.

Paratypes. HNNU 1997:6-17, adult female, collected by Youhui Shen from Laotage, Nanyue Hengshan, Hengyang, Hunan Province, China on 17 June 1997; CSUFT

HS210602–603, 605–610, 612–616, and 618–620, sixteen adult males; CSUFT HS210604, 611, 617, and 621, four adult females, collected by KH and ZG from Nanyue



Figure 3. Holotype of *Boulenophrys hengshanensis* sp. nov. (HNNU 0706000A, adult female). **A** Dorsal, **B** ventral views of body; **C** lateral view of head; **D** volar view of hand; **E** plantar view of foot. Scale bars equal to 10 mm.

Hengshan Scenic Area (112.714N, 27.277E, ca. 450 m a.s.l.), Nanyue District, Hengyang Prefecture, Hunan Province, China on 4 June 2021.

Etymology. The specific epithet “*hengshanensis*” refers to Mt. Hengshan, the type locality of the new species.

Diagnosis. Among the ten genera of the subfamily Megophryinae, the new species is assigned to *Boulenophrys* based on its slender body (vs. thickset and stocky in *Brachytarsophrys*), a distinct tympanum visible (vs. concealed and invisible in *Brachytarsophrys*), a narrower and non-depressed head (vs. enlarged and depressed in *Brachytarsophrys*, *Grillitschia*, *Pelobatrachus*, and *Megophrys*), head without a transverse groove behind (vs. present in *Brachytarsophrys*, *Pelobatrachus*, and *Megophrys*), the short temporal region being relatively clos-

er to the posterior corner of eye (vs. elongated and far from posterior corner of eye in *Grillitschia*, *Megophrys*, and *Pelobatrachus*), maxillary teeth present (vs. absent in *Ophryophryne*), a small horn-like tubercle on upper eyelid present (vs. margin of eyelid smooth in *Atympantanophrys*), supratympanic fold distinctly curved over tympanum (vs. slightly curved or not curved in *Atympantanophrys*), asperities on lower jaw absent (vs. present in *Xenophrys*), subarticular tubercles on fingers present (vs. absent in *Jingophrys*, *Xenophrys*, and *Sarawakiphrys*), nuptial pad present on fingers I and II in breeding males (vs. absent in *Sarawakiphrys*, and absent or only present on finger I in *Jingophrys*), and the absence of ventrolateral tubercles (vs. present in *Jingophrys*).

Boulenophrys hengshanensis sp. nov. can be further distinguished from its congeners by the combination of: (1) body of moderate size of SVL 35.7–41.2 mm (n =

16) in males, and 37.5–50.2 mm ($n = 6$) in females; (2) hindlimbs short, heels not meeting each other when hindlimbs held at right angles to body, TIB/SVL 0.38–0.46; (3) tympanum rounded, upper margin concealed by supratympanic fold, TD/ED 0.48–0.63; (4) absence of vomerine teeth and vomerine ridges; (5) posterior margin of tongue not notched; (6) dorsum skin rough with several granules and not continuous X-shaped skin ridges in males, relatively smooth in females; (7) ventral surface smooth with rounded tubercles; (8) two metacarpal tubercles present; (9) distinct subarticular tubercles on bases of each finger; (10) fingers lacking webs and lateral fringes; (11) relative finger length III > I > II ≥ IV; (12) rudimentary toe webs, absent lateral fringes; (13) the presence of dense tiny spines on surface of temporal region, upper and lower lips, and from the loreal region to the tip of snout in males; and (14) in breeding males, nuptial pads with small dense spines on fingers I–II.

Description of the holotype. Adult female, moderate body size, SVL 37.5 mm; head wider than long, HDL/HDW 0.97; snout short, with a bluntly rounded tip, SNT/HDL 0.33; eye large, with vertical pupils, ED/HDL 0.32; tympanum distinct and round, with upper border concealed by supratympanum fold, TD/ED 0.53; oval nostrils, orientated laterally, narial rim slightly prominent; absence of vomerine teeth and vomerine ridge; maxillary teeth present; rounded tongue with no notch at the posterior margin.

Fingers without webbing and lateral fringes; relative finger length III > I > II > IV; distinct subarticular tubercles present at the bases of each finger; large inner metacarpal tubercle, relatively small outer metacarpal tubercle, both distinctly visible.

Hindlimbs short, TIB/SVL 0.45; heels not touching each other when hindlimbs held at right angles to the body; toes with rudimentary webs and lacking lateral fringes; relative toe length IV > III > V > II > I; subarticular tubercles distinct at bases of toes I, II, and III, absent at base of toe IV, and very small and flat at base of toe V; inner metatarsal tubercle elongated and large, outer metatarsal tubercle absent.

Dorsal skin rough, with several posterior enlarged tubercles on dorsum and flanks; weakly X-shaped skin ridge and dorsolateral skin ridges on dorsum; small horn-like tubercle at the edge of each upper eyelid; supratympanic fold distinct and narrow, extending from posterior eye borders, curves down broadly through upper tympanum and gradually widen above insertion of arm; flanks with sparse tubercles; ventral surface of head and body granular; thigh smooth anteriorly, with dense tubercles posteriorly; pectoral and femoral glands prominent, the latter closer to knee than to vent.

Coloration of holotype. In preservation, dorsally light brown without distinct markings; several brown narrow stripes present on lower arms, hands, thighs and shanks; dark brown pigmentation present on hindlimbs posteriorly where meets the venter. Ventrally, skin beige with light brown pigmentation; several dark brown blotches present

on lower lip; a distinct, short, dark brown stripe longitudinally on throat; a dark brown, wide band present on ventrolateral margin of trunk; several brown spots scattered on belly; inner thigh almost clear proximally, with several brown spots on distal half; shanks and foot scattered with brown spots; pectoral and femoral glands clear, unpigmented; palm and sole brown with pale tubercles.

Coloration of paratype in life. A color photograph of the holotype in life is available in Shen et al. (2014), but with a low resolution. The detailed description of the coloration in life was based on newly collected paratype specimens. — **MALE.** The following description is based on a male paratype CSUFT HS210605 (Fig. 4A–B). The dorsal surface is light brown with dark brown markings; reddish small tubercles cover the dorsal surface; a wide dark brown band is present on each upper eyelid, and extends posteriorly where the two bands meet each other; the dorsal pattern shows several transverse bars; a distinct large X-shaped pattern is present on the middle of the dorsum; the intersection of the X-shape is elongated longitudinally; wide dark brown bars are present on dorsal surface of lower arms and hindlimbs, and form large blotches on elbows. The background coloration on ventral surface is dark purple; the skin on chin and chest is rather dark, with several white blotches and stripes that are embellished with red pigmentation; four distinct longitudinal stripes on throat; on the chest, the background skin color is gradually faded; dense red spots are distinctly visible, and extend laterally to flanks; two white pectoral glands are distinctly prominent; white blotches cover the most of the belly, which is scattered with grey and dark brown spots, and sparse red spots; the ventral surface of the thigh is purple, with white tubercles proximally and white blotches distally; two white femoral glands are distinctly prominent; the palm and sole are dark purple; the digital discs, palm tubercles are pale whitish-purple; the groin is pigmented red. Laterally, the enlarged portion of supratympanic fold above insertion of arm is beige; the iris is reddish-white; the sclera is golden; the tympanum is golden with dark brown pigmentation, with a distinct dark spot in the center. — **FEMALE.** A female paratype CSUFT HS210604 (Fig. 4C–D) has a similar dorsal pattern to the description above; the ventral surface is paler, and with fewer reddish pigmentation; the inner aspect of thigh lacks white blotches, but with pale gray pigmentation and several black dots; the tip of toe is pink, with pale orange pigmentation.

Sexual secondary characteristics. In male specimens, tubercles with tiny central spines are present on the surface of the temporal region, on the upper and lower lip, and from the loreal region to the tip of snout; similar spines are also present on the tubercles around the cloacal region, the inner aspects of thigh, shank, and tarsus. Several individuals show these spines on the outer aspect of the hindlimbs and lower arms, and alongside the ridge of supratympanic fold (CSUFT HS210602–603, 613, 616, 618–620). Nuptial pads are present on fingers I and II. Additionally, male specimens have strong arms during

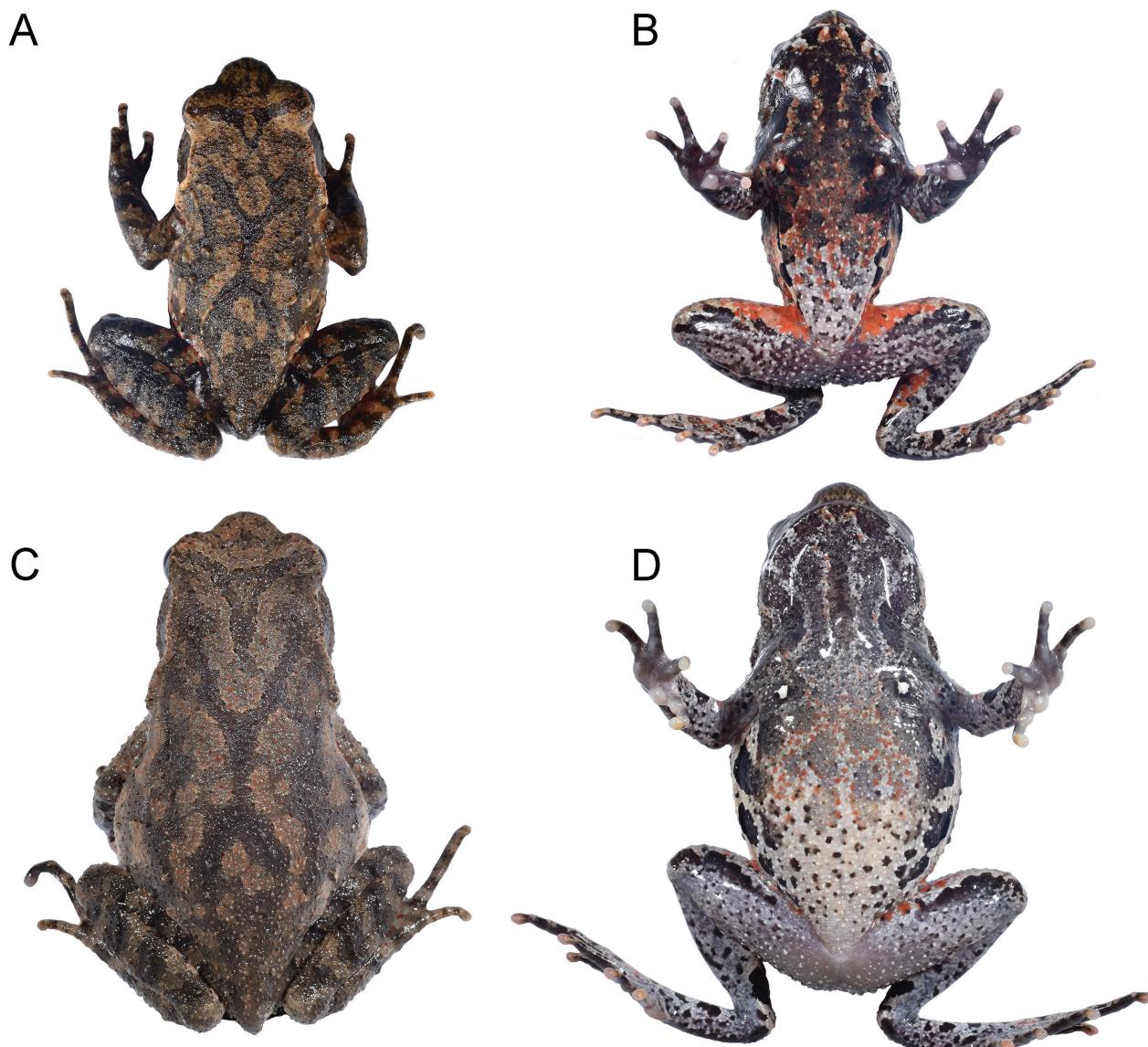


Figure 4. Paratypes of *Boulenophrys hengshanensis* sp. nov. in life. **A, B** Male paratype CSUFT HS210605; **C, D** Female paratype CSUFT HS210604.

the breeding season, and show a rough dorsum with granules, while the dorsum of female specimens is relatively smooth.

Variation. Measurements of all type specimens are shown in Table 2. The relative finger length is III>I>II=IV in several specimens (CSUFT HS210601–603, 609, 611, 613–616). The extension of supratympanic fold above insertion of arm forms a depressed supraaxillary gland in two specimens (CSUFT HS210604, 620), and is slightly swollen in five specimens (CSUFT HS210606, 611–14), and is very swollen in several specimens (CSUFT HS210602–603, 605, 607, 609–610, 615–619, 621). In life, several individuals (CSUFT HS210608, 15, 18, 21) show an extremely dark skin on the venter and an absence of red pigmentation, sometimes rendering the longitudinal stripes on throat indistinguishable (CSUFT HS210615).

Advertisement call. Call descriptions are based on the calls of male paratype CSUFT HS210614 (SVL 39.1

mm). Spectrograms and waveforms are shown in Fig. 5. Six call groups with a total of 135 calls were analyzed. Each call group contains an average of 22.5 ± 2.9 (20–28) calls. Calls were an average of 100 ± 6.8 (87–123) ms in duration, with an average inter-call interval of 168 ± 48.7 (95–492) ms. Calls were repeated at a rate of 3.7 ± 0.1 (3.6–3.9) calls/s per call group. Each call consisted of 18 ± 1.6 (14–24) pulses. The dominant frequency was 2.7 ± 0.1 (2.5–2.8) kHz, no evident harmonics.

Comparisons. By having a moderate body size of SVL 35.7–41.2 mm ($n = 17$) in males, and 37.5–50.2 mm ($n = 5$) in females, *B. hengshanensis* sp. nov. differs from the larger species *B. binlingensis* (vs. 45.1–51.0 mm [$n = 3$] in males), *B. caudoprocta* (vs. 70.8–81.3 mm [$n = 3$] in males, and 77.8 mm [$n = 1$] in female), *B. chishuiensis* (vs. 43.1–44.1 mm [$n = 3$] in males), *B. jingdongensis* (vs. 53.0–56.5 mm [$n = 3$] in males, and 63.5 mm [$n = 1$] in female), *B. liboensis* (vs. 60.8–70.6 mm [$n = 8$] in females), *B. mirabilis* (vs. 55.8–61.4 mm [$n = 2$] in males,

Table 2. Measurements of the type series of *Boulenophrys hengshanensis* sp. nov. The voucher number of the holotype is highlighted by an asterisk. For abbreviations, see Materials and Methods.

Voucher	Sex	SVL	HDL	HDW	SNT	IND	IOD	ED	TD	TED	HND	RAH	TIB	FTL	FL
CSUFT HS210602	M	36.5	13.6	14.1	4.3	4.0	3.8	5.0	2.5	1.7	9.8	16.9	16.4	24.2	14.9
CSUFT HS210603	M	36.0	13.4	14.0	3.7	4.3	3.4	5.4	2.7	1.5	9.1	16.9	16.3	24.1	14.6
CSUFT HS210605	M	36.8	13.3	14.0	3.9	4.5	3.5	5.2	2.5	1.7	10.0	17.7	17.1	24.9	15.6
CSUFT HS210606	M	38.0	13.8	14.1	4.0	3.9	3.5	5.0	2.6	1.6	9.5	17.2	16.1	23.1	14.2
CSUFT HS210607	M	39.1	14.2	14.7	4.5	5.1	4.1	6.3	3.0	1.6	9.6	17.2	16.9	24.6	14.9
CSUFT HS210608	M	39.0	13.5	13.9	4.1	4.3	3.5	5.2	2.8	1.5	9.4	16.3	16.3	23.2	14.5
CSUFT HS210609	M	39.4	14.9	15.1	4.5	4.7	4.2	5.5	3.1	1.7	10.8	18.1	18.1	25.8	16.2
CSUFT HS210610	M	41.2	15.1	16.3	4.5	4.7	4.0	5.6	2.8	1.8	10.4	18.7	18.2	26.5	16.8
CSUFT HS210612	M	39.7	14.4	15.0	4.2	4.6	3.8	5.4	2.7	2.1	9.4	17.3	16.7	23.9	14.6
CSUFT HS210613	M	39.6	13.7	13.8	4.0	4.6	3.7	5.5	3.2	1.5	9.9	17.4	16.5	23.5	14.6
CSUFT HS210614	M	39.1	13.9	14.2	4.2	5.0	3.8	5.4	2.8	1.8	10.2	17.6	17.5	24.6	15.4
CSUFT HS210615	M	35.8	13.0	13.0	4.1	4.4	3.8	5.1	2.6	1.6	9.4	16.5	16.0	21.8	13.9
CSUFT HS210616	M	38.3	13.6	13.9	4.1	4.2	3.5	5.4	2.8	1.7	9.8	17.8	17.7	24.9	15.4
CSUFT HS210618	M	40.4	13.4	13.8	4.2	4.1	4.0	5.2	2.7	1.8	9.9	17.9	18.2	25.0	15.3
CSUFT HS210619	M	40.3	13.6	14.1	4.3	4.6	3.6	5.5	3.1	1.9	10.0	17.4	15.9	24.2	15.4
CSUFT HS210620	M	35.7	13.2	13.5	4.1	4.4	3.7	5.2	2.7	1.7	9.6	17.5	16.3	24.0	15.4
HNNU 0706000A*	F	37.5	14.1	14.5	4.7	4.3	3.6	4.5	2.4	2.8	10.1	17.4	16.7	23.8	16.4
HNNU 1997:6-17	F	47.9	16.4	17.1	5.4	4.7	4.0	5.7	3.6	3.4	11.9	20.8	18.2	27.4	18.6
CSUFT HS210604	F	50.2	16.4	17.3	5.2	4.8	4.6	6.2	3.2	2.6	12.2	22.7	21.4	29.0	19.1
CSUFT HS210611	F	41.8	14.4	15.0	4.1	4.1	3.5	5.3	2.7	1.9	10.1	19.2	18.8	26.2	16.3
CSUFT HS210617	F	47.6	16.4	17.3	5.1	5.1	4.0	6.2	3.7	2.1	11.0	19.9	20.0	28.8	18.9
CSUFT HS210621	F	43.8	15.1	15.3	4.1	5.0	4.8	5.9	3.3	2.1	11.0	20.5	19.3	27.8	17.6

and 68.5–74.8 mm [n = 2] in females), *B. omeimontis* (vs. 56.0–59.5 mm [n = 10] in males, and 58.0–72.5 mm [n = 3] in females), *B. qianbeiensis* (vs. 49.3–58.2 mm [n = 6] in males), *B. sangzhiensis* (vs. 53.0–60.8 mm [n = 7] in males, and 73.0 mm [n = 1] in female), *B. shuichengensis* (vs. 102.0–118.3 mm [n = 8] in males, and 99.8–115.6 mm [n = 7] in females), *B. spinata* (vs. 47.2–54.4 mm [n = 18] in males, and 54.0–55.0 mm [n = 2] in females); and the smaller species *B. acuta* (vs. 27.1–33.0 mm [n = 10] in males, and 34.4 mm [n = 1] in female), *B. angka* (vs. 31.2–32.1 mm [n = 2] in males), *B. baishanzensis* (vs. 28.4–32.4 mm [n = 6] in males), *B. cheni* (vs. 26.2–29.5 mm [n = 15] in males, and 31.8–34.1 [n = 3] in females), *B. congjiangensis* (vs. 28.6–33.4 mm [n = 15] in males), *B. daiyunensis* (vs. 27.6–28.7 mm [n = 4] in males, and 33.7–35.6 mm [n = 3] in females), *B. daoji* (vs. 32.6–33.6 mm [n = 4] in males), *B. frigida* (vs. 30.3–31.8 mm [n = 4] in males), *B. jiangi* (vs. 39.5–40.4 mm [n = 2] in females), *B. jiulianensis* (vs. 30.4–33.9 mm [n = 9] in males), *B. kuatunensis* (vs. 26.2–31.4 mm [n = 24] in males), *B. lishuiensis* (vs. 31.0–34.8 mm [n = 2] in males), *B. myfumontana* (vs. 30.1–30.8 mm [n = 2] in males, and 36.3 mm [n = 2] in females), *B. nankunensis* (vs. 29.9–34.9 mm [n = 11] in males), *B. ombrophila* (vs. 27.4–34.5 mm [n = 5] in males, and 32.8–35.0 mm [n = 4] in females), *B. rubrimera* (vs. 26.7–30.5 mm [n = 8] in males), *B. sanmingensis* (vs. 28.0–30.6 mm [n = 12] in males, and 32.9 mm [n = 1] in female), *B. shimentaina* (vs. 28.0–30.6 mm [n = 12] in males), *B. shunhuangensis* (vs. 30.3–33.7 mm [n = 10] in males), *B. tongboensis* (vs. 26.5–31.5 mm [n = 5] in males), *B. wugongensis* (vs. 31.0–34.1 mm [n = 4] in males), *B. wuliangshanensis*

(vs. 27.3–31.6 mm [n = 10] in males), *B. wushanensis* (vs. 30.4–35.5 mm [n = 10] in males), *B. yingdeensis* (vs. 33.2–35.3 mm [n = 4] in males), *B. puningensis* (vs. 31.7–34.6 mm [n = 4] in males), and *B. hungtai* (vs. 25.8–33.3 mm [n = 12] in males).

Boulenophrys hengshanensis sp. nov. differs from its phylogenetically closest congeners *B. xuefengmontis*, *B. shunhuangensis*, and *B. mirabilis* by heels not meeting each other when hindlimbs are held at right angles to the body (vs. just meeting in *B. xuefengmontis*, and overlapping in *B. shunhuangensis* and *B. mirabilis*), the presence of dense tiny spines on the surface of the tympanic region, upper and lower lip, and from the loreal region to the tip of snout in males (vs. absent in *B. xuefengmontis*), a relative finger length of III>I>II>IV (vs. III>IV>II>I in *B. shunhuangensis*, and III>IV>I>II in *B. xuefengmontis* and *B. mirabilis*), the upper margin of the tympanum concealed by a supratympanic fold (vs. tympanum with clear boundary in *B. mirabilis*), a small horn-like tubercle on the upper eyelid (vs. large in *B. mirabilis*), the absence of lateral fringes on the fingers (vs. presence in *B. mirabilis*), an internasal distance larger than the interorbital distance (vs. smaller in *B. mirabilis*), and the presence of distinct subarticular tubercles on bases of each finger (vs. indistinct in *B. mirabilis*).

Compared to other congeners occurring in Hunan Province, *B. hengshanensis* sp. nov. differs by the absence of vomerine teeth and vomerine ridges (vs. presence of vomerine teeth in *B. caudoprocta*, *B. jiulianensis*, *B. nanlingensis*, and *B. shimentaina*; *B. xiangnanensis* and *B. yangmingensis* lack vomerine teeth, but have weak or strong vomerine ridges), the unnotched posteri-

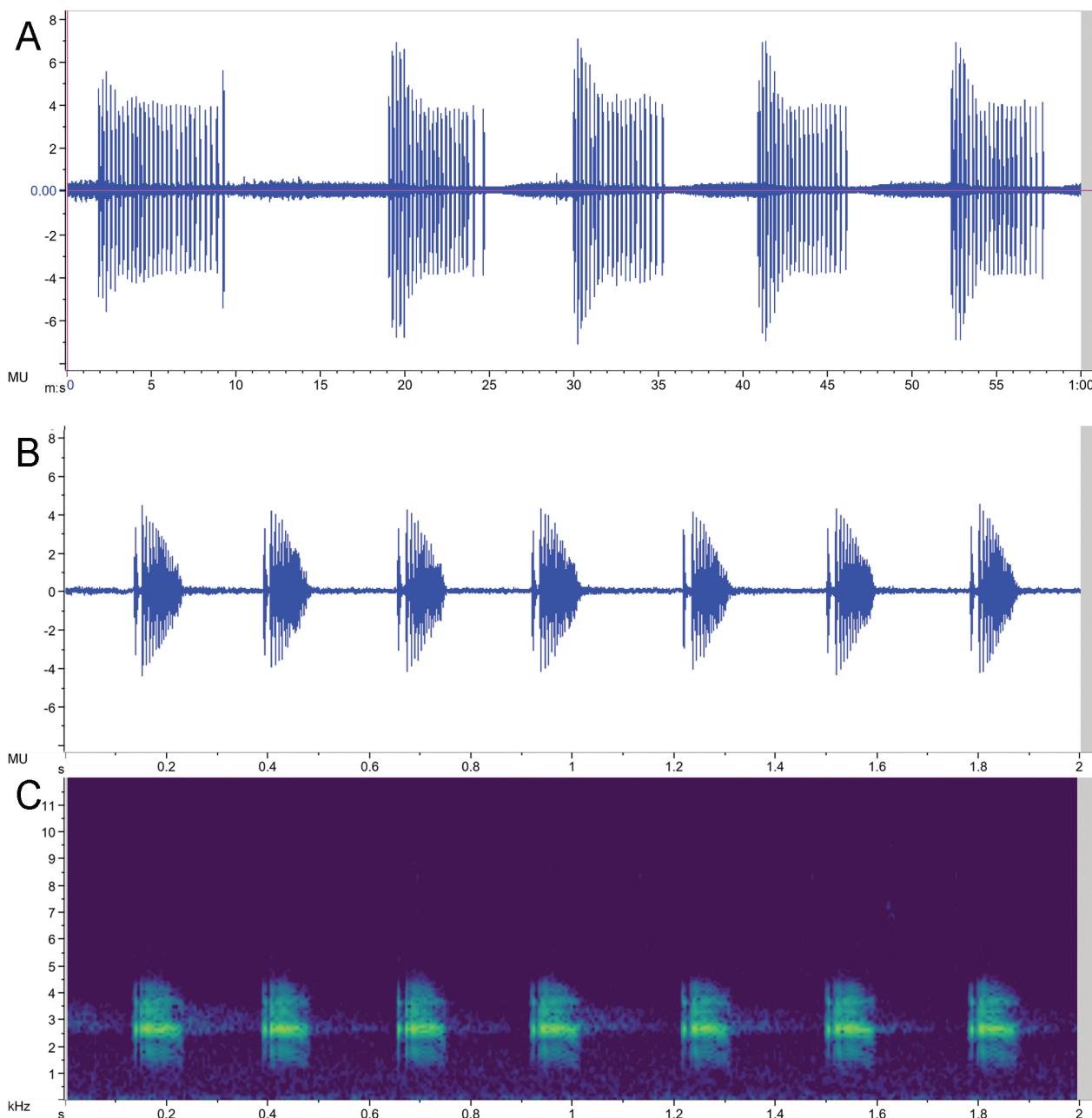


Figure 5. Advertisement call of *Boulenophrys hengshanensis* sp. nov. **A** 60 s waveform showing five call groups; **B** 2 s waveform; **C** respective spectrogram showing seven calls from male paratype CSUFT HS210614.

or margin of tongue (vs. notched in *B. cheni*, *B. nanlingensis*, *B. juiyanensis*, and *B. sangzhiensis*), by a small horn-like tubercle on the upper eyelid (vs. a large tubercle in *B. caudoprocta*, and *B. jinggangensis*). *Boulenophrys hengshanensis* sp. nov. differs from *B. cheni*, *B. lini*, *B. sangzhiensis* [male only], and *B. xiangnanensis* by lateral fringes on toes absent (vs. moderate or wide fringes), by having only rudimentary webbing on toes (vs. 1/4 on each side of toe IV in *B. sangzhiensis*), the absence of lateral fringes on fingers (vs. present in *B. shimentaina*), the presence of distinct subarticular tubercles on bases of each finger (vs. absent or indistinct in *B. shimentaina*, and *B. juiyanensis*), heels not meeting each other when hindlimbs are held at right angles to the body (vs. just touching or overlapping in *B. cheni*, *B. jinggangensis*, *B. juiyanensis*, *B. lini*, *B. mufumontana*, *B. nanlingensis*, *B.*

sangzhiensis, *B. shimentaina*, *B. tuberogranulata*, and *B. yangmingensis*), the upper margin of tympanum concealed by a supratympanic fold (vs. tympanum with clear boundary in *B. jinggangensis*, *B. juiyanensis*, *B. lini*, *B. ombrophila*, *B. shimentaina*, *B. wugongensis*, *B. xiangnanensis*, and *B. yangmingensis*), the presence of dense tiny spines on the surface of the tympanic region, upper and lower lip, and from the loreal region to the tip of snout in males (vs. absent in *B. tuberogranulata*), nuptial pads with small, dense spines present on fingers I-II in breeding males (vs. absence of nuptial pads in breeding males in *B. nanlingensis*, and nuptial pads with large spines in *B. sangzhiensis*), a relative finger length of III>I>II>IV (vs. II>IV>I>II in *B. jinggangensis*, II>IV>I>II in *B. lini*, III>IV>II>I in *B. cheni*, II>IV=I>II in *B. dongguanensis*, III>IV=I>II in *B. wugongensis*, and III>I>IV=II in *B. mu-*



Figure 6. Unvouchedered calling male frog of *Boulenophrys hengshanensis* sp. nov. under a bush.

fumontana), and the presence of vocal sacs in males (vs. absent in *B. caudoprocta*).

Furthermore, *B. hengshanensis* sp. nov. differs by the absence of vomerine teeth and vomerine ridges from *B. daiyunensis*, *B. daweimontis*, *B. dongguanensis*, *B. fansipanensis*, *B. fengshunensis*, *B. frigida*, *B. hoanglienensis*, *B. insularis*, *B. jingdongensis*, *B. liboensis*, *B. lushuiensis*, *B. nankunensis*, *B. palpebralespinosa*, *B. puningensis*, *B. qianbeiensis*, *B. rubrimera*, *B. tongboensis*, and *B. yingdeensis*, all of which have vomerine teeth, and it further differs from *B. obesa*, *B. yaoshanensis*, and *B. xianjuensis*, all of which lack vomerine teeth but have weak or strong vomerine ridges. *Boulenophrys hengshanensis* sp. nov. differs by the posterior margin of the tongue being not notche, from *B. binlingensis*, *B. boettgeri*, *B. jingdongensis*, *B. kuatunensis*, *B. liboensis*, *B. minor*, *B. omeimontis*, *B. sanmingensis*, *B. spinata*, and *B. tongboensis*, all of which have a notch on the posterior tongue. Furthermore, *B. hengshanensis* sp. nov. differs from *B. baolongensis*, *B. hoanglienensis*, *B. insularis*, *B. lushuiensis*, *B. qianbeiensis*, and *B. shuichengensis*, all of which have a weakly or feebly notched tongue on posterior margin, by having a small horn-like tubercle on upper eyelid. *Boulenophrys hengshanensis* sp. nov. differs from *B. acuta*, *B. liboensis*, *B. palpebralespinosa*, and *B. shuichengensis*, all of which have a large horn-like tubercle on upper eyelid, by lateral fringes on toes being absent. *Boulenophrys hengshanensis* sp. nov. differs from *B. binchuanensis*, *B. boettgeri*, *B. jingdongensis*, *B. liboensis*, *B. qianbeiensis*, *B. sanmingensis*, *B. shuichengensis*, *B. spinata*, and *B. wushanensis* (male only), all of which have moderate or wide lateral fringes on toes, by having only rudimentary webbing on toes. *B. hengshanensis* sp. nov. further differs

from *B. jingdongensis* (vs. half-webbed), *B. qianbeiensis* (vs. one-third webbing), *B. shuichengensis* (vs. 1/3 one each side of toe IV), and *B. spinata* (vs. half-webbed). By the absence of lateral fringes on fingers, *B. hengshanensis* sp. nov. differs from *B. daiyunensis*, *B. daoji*, and *B. tongboensis*, all of which have lateral fringes on fingers. By the presence of distinct subarticular tubercles on bases of each finger, *B. hengshanensis* sp. nov. differs from *B. wuliangshanensis*, *B. nankunensis*, *B. fansipanensis*, *B. hoanglienensis*, *B. frigida*, and *B. rubrimera*, in all of which subarticular tubercles are absent or indistinct; it also differs in this trait from *B. angka* (subarticular tubercles only present on the base of fingers I-II). *Boulenophrys hengshanensis* sp. nov. differs by heels not meeting each other when the hindlimbs are held at right angles to the body from *B. acuta*, *B. anlongensis*, *B. baishanzuensis*, *B. baolongensis*, *B. binlingensis*, *B. boettgeri*, *B. conjiangensis*, *B. chishuiensis*, *B. daoji*, *B. jiangi*, *B. jingdongensis*, *B. leishanensis*, *B. liboensis*, *B. lishuiensis*, *B. jiangi*, *B. obesa*, *B. omeimontis*, *B. palpebralespinosa*, *B. qianbeiensis*, *B. sanmingensis*, *B. spinata*, *B. tongboensis*, *B. wuliangshanensis*, *B. xianjuensis*, *B. yunkaiensis*, and *B. yaoshanensis*, in all of which the heels are touching or overlapping. Furthermore, *B. hengshanensis* sp. nov. differs by the upper margin of the tympanum concealed by a supratympanic fold from *B. acuta*, *B. boettgeri*, *B. dongguanensis*, *B. fengshunensis*, *B. hungtai*, *B. insularis*, *B. kuatunensis*, *B. leishanensis*, *B. lishuiensis*, *B. nankunensis*, *B. obesa*, *B. puningensis*, *B. rubrimera*, *B. xianjuensis*, *B. yingdeensis*, *B. yaoshanensis*, and *B. yunkaiensis*, all of which have a tympanum with clear boundary. *Boulenophrys hengshanensis* sp. nov. differs by the presence of dense tiny spines on the surface of the

tympanic region, the upper and lower lip, and from the loreal region to the tip of the snout in males from *B. hungtai*, *B. puningensis*, *B. yaoshanensis*, and *B. yingdeensis*, all of which are lacking these spines. *Boulenophrys hengshanensis* sp. nov. differs by nuptial pads with small, dense spines present on fingers I–II in breeding males from *B. daiyunensis* having no nuptial pads in breeding males; it further differs from *B. daoji*, *B. sanmingensis*, and *B. angka*, all of which nuptial pad only present on finger I, and from *B. qianbeiensis*, and *B. spinata*, both have large spines on nuptial pads. By having a relative finger length of III > I > II ≥ IV, *B. hengshanensis* sp. nov. differs from *B. daiyunensis* (vs. III>IV>II=I), *B. sanmingensis* (vs. III>IV>II>I), *B. tongboensis* (vs. III>IV>I>II), *B. dongguanensis* (III>IV≥I>II), *B. nankunensis* (vs. III>IV>I>II), *B. xianjuensis* (vs. III>IV>I>II), *B. puningensis* (vs. III>IV>II=I), *B. hungtai* (vs. III>IV>II>I), and *B. baishanzuensis* (vs. III>IV>II>I). By the presence of outer metacarpal tubercle, *B. hengshanensis* sp. nov. differs from *B. fansipanensis* and *B. hoanglienensis*, in which the outer metacarpal tubercle is absent.

Distribution and Habitat. The new species is currently only known from Mt. Hengshan, Nanyue District, Hengyang Prefecture, Hunan Province, China. The amphibian fauna in this area was first reported by Shen (1965), with a total of 14 amphibians recorded: *Bufo gargarizans* Cantor, 1842, *Fejervarya multistriata* (Hallowell, 1860), *Hoplobatrachus chinensis* (Osbeck, 1765), *Quasipaa boulengeri* (Günther, 1889), *Rana hanluica* Shen, Jiang & Yang, 2007 (reported as *Rana japonica japonica* Boulenger, 1879), *Pelophylax hubeiensis* (Fei & Ye, 1982), *Pelophylax nigromaculatus* (Hallowell, 1860), *Hylarana latouchii* (Boulenger, 1899), *Hylarana guentheri* (Boulenger, 1882), *Polypedates megacephalus* Hallowell, 1861, *Microhyla heymonsi* Vogt, 1911, *M. pulchra* (Hallowell, 1860), and *M. fissipes* Boulenger, 1884. Due to the following checklist reported by Shen (1983), *Andrias davidianus* (Blanchard, 1871), *Amolops sinensis* Lyu, Wang & Wang, 2019 (reported as *Amolops ricketti* [Boulenger, 1899]), *Zhangixalus dennysi* (Blanford, 1881) were also recorded in Mt. Hengshan. A relatively comprehensive amphibian survey in Mt. Hengshan was conducted from 2006 to 2007 by Mo et al. (2009). These authors reported the new species (identified as *B. brachykolos*) from the forest road between 744–900 m elevation during night surveys from 19:00 to 23: 00 h. They also recorded *H. guentheri*, *F. multistriata*, and *M. heymonsi* occurred between 200–300 m elevation; *P. megacephalus* and *M. heymonsi* between 600–700 m elevation; and *Bu. gargarizans*, *R. hanluica* between 1000–1100 m elevation. During our rapid survey conducted in June 2021, we observed *Bu. gargarizans*, *Quasipaa exilispinosa* (Liu & Hu, 1975), *P. megacephalus*, and *A. sinensis* to co-exist with *B. hengshanensis* sp. nov. at 450 m elevation. The calling activities of male *B. hengshanensis* sp. nov. started at about 18:00 h and did not decline until 23:00 h. Calling male frogs were found sitting in a roadside ditch, with shallow flowing water attaining the feet, or hide in the bush, or perched on low plants (Fig. 6).

Conservation status. Mt. Hengshan is a relatively isolated mountain in Hunan Province (Fig. 1). Unsuitable habitat (e.g. farmland, country, rivers, and roads) and large regions with low altitude areas around Hengshan could be barriers to the dispersal of the new species. As noticed by Mo et al. (2009), Mt. Hengshan is protected by the Hunan Nanyuehengshan National Nature Reserve, but it is also a famous tourist destination (known as Nanyuehengshan Scenic Area). The potential threat to all amphibians in Mt. Hengshan is mainly human disturbance from tourist areas (Mo et al. 2009).

Discussion

The discovery of the new species sheds light on the previously underestimated diversity within central Hunan Province, where mountains are isolated and sporadic. The related species (*B. shunhuangensis*, *B. xuefengmontis*, and “*Megophrys*” sp. 27) were all found in hilly areas in western Hunan. Thus, the herpetofauna of the isolated Mt. Hengshan in central Hunan is similar to the herpetofauna of the western hills (Xuefeng Mountains), but not the herpetofauna of the southern hills (Nanling Mountains) or eastern hills (Luoxiao Mountains).

Before the description of *B. hengshanensis* sp. nov., 16 species had been reported from Hunan Province either as the type locality or with confirmed species identity using molecular data, which are: *B. caudoprocta*, *B. cheni*, *B. jinggangensis*, *B. jiulianensis*, *B. lini*, *B. musumontana*, *B. nanlingensis*, *B. ombrephila*, *B. sangzhiensis*, *B. shimentaina*, *B. shunhuangensis*, *B. tuberogranulata*, *B. wugongensis*, *B. xiangnanensis*, *B. xufengmontis*, and *B. yangmingensis* (Lyu et al. 2023; Shang et al. 2023). The description of *B. hengshanensis* sp. nov. increased the total number of species of *Boulenophrys* from Hunan Province to 17, highlighting the high species richness of *Boulenophrys* in this region. However, some records of *Boulenophrys* species in Hunan Province, such as *B. kuatunensis* and *B. boettgeri*, have been denied by Lyu et al. (2023) due to taxonomic uncertainties. Further investigation of *Boulenophrys* species in Hunan Province is warranted to better understand the diversity and evolutionary history of this genus in this biologically important region.

Acknowledgements

We thank Jian Wang and an anonymous reviewer for their helpful comments on the previous version of this manuscript. We thank Yingyong Wang and Shuo Qi for access to museum specimen collections and associated support. We also thank Yuan Li for the help in molecular data analysis, Dejia Hou for generating DEM files, Leqiang Zhu for the assistance during specimen examination. TQ thanks the Cornell Lab of Ornithology for providing license support for Raven Pro software. This work was supported by the National Natural Science Foundation of China (Grant No. 31472021), the Project for Wildlife Conservation and

Management of the National Forestry and Grassland Administration of China (Grant No. 2022-HN-001), and the Wildlife Conservation Project of Hunan Province (Grant No. HNYB2022-001).

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Appendix 1

Specimens examined

- B. brachykolos* (13): China: Hong Kong (type locality): SYS a001502–1503; Guangdong: Shenzhen: Mt. Yangtaishan: SYS a002051–2056, 2453–2454; Dapeng Peninsula: SYS a002406–2408.
- B. tuberogranulata* (12): China: Hunan: Zhangjiajie: Mt. Tianzishan: CSUFT 091–099, 206–208.
- B. sangzhiensis* (9): China: Hunan: Sangzhi: Mt. Tianpingshan: CSUFT 2006006–2006014.