PENSOFT
SENCKENBERG world of biodiversity
Leibniz Association

A new species of stream-living toad (Anura: Bufonidae: *Bufo*) from Guangdong, China

Shuo Qi[1], Zhi-Tong Lyu[1], Han-Ming Song[1], Shi-Chao Wei[2], Qi-Feng Zhong[3], Ying-Yong Wang[1]

1 *State Key Laboratory of Biocontrol/ The Museum of Biology, School of Life Sciences/ School of Ecology, Sun Yat-sen University, Guangzhou 510275, Guangdong, China*

2 *Guangdong Academy of Forestry, Guangzhou 510520, Guangdong, China*

3 *Management Bureau of Guangdong Nankunshan Nature Reserve, Huizhou 516876, Guangdong, China*

https://zoobank.org/0773AD34-C135-4D66-B0EF-C3255FE25693

Corresponding author: Ying-Yong Wang (wangyy@mail.sysu.edu.cn)

Academic editor Raffael Ernst | **Received** 7 December 2022 | **Accepted** 5 June 2023 | **Published** 21 July 2023

Citation: Qi S, Lyu Z-T, Song H-M, Wei S-C, Zhong Q-F, Wang Y-Y (2023) A new species of stream-living toad (Anura: Bufonidae: *Bufo*) from Guangdong, China. Vertebrate Zoology 73 677–689. https://doi.org/10.3897/vz.73.e98558

Abstract

In this work, we describe a new species of genus *Bufo*, *Bufo exiguus* **sp. nov.** from Mt. Nankun, Guangdong Province, China. This new species can be distinguished from all congeners by significant divergences in the mitochondrial 16S rRNA and CO1 genes and by a combination of morphological characters: small body size, tympanum absent, parotoid glands small and olive-shaped, tarsal fold absent, dorsal body with a fine vertebral line and white nuptial spinules present on dorsal and inner surfaces of fingers I and II in males. At present, *Bufo exiguus* **sp. nov.** is only known from the slow-flowing montane streams from its type locality and its conservation status should be carefully addressed.

Keywords

Bufo cryptotympanicus, *Bufo exiguus* **sp. nov.**, niche differentiation, taxonomy, true toad

Introduction

The genus *Bufo* Garsault, 1764, as a typical representative of the Old World, is widespread from temperate Eurasia and adjacent islands extending to North Africa and the Middle East, eastwards to Japan and through mainland China and southwards to Myanmar and northern Vietnam (Frost 2023). Previous systematic studies support the genus *Bufo* as a monophyletic group, but the phylogenetic relationships within the genus have been a controversial topic for many years and have still not been resolved (Macey et al. 1998; Liu et al. 2000; Fu et al. 2005; Igawa et al. 2006; Zhan and Fu 2011; Recuero et al. 2012; Che et al. 2020; Othman et al. 2022; Dufresnes and Litvinchuk 2022). Despite the controversy, the monophyletic *Bufo* is recognised to contain 24 species at present and about three-quarters (18 species) of this genus are known to occur in China (Frost 2023).

Liu and Hu (1962) described *Bufo cryptotympanicus*, based on a series of specimens from Huaping Nature Reserve, Longsheng, Guangxi, China. Later studies reported two more distribution sites in Guangxi (Xing'an and Ziyuan) expanding its distribution range to Guangdong (Longmen, Heyuan and Ruyuan), Yunnan (Lyuchun) and northern Vietnam (Lao Cai and Da Nang) (Yang 1991; Fei et al. 2009; Nguyen et al. 2009 and references there-

in; Li et al. 2011). Yang (2008) re-identified the Lyuchun (previously transliterated as Luchun) population as a new species and transferred it to the genus *Torrentophryne* Yang, 1996, namely *T. luchunnica* Yang & Rao, 2008. Therefore, the distribution range of *B. cryptotympanicus* is recorded from northern Guangxi and northern Guangdong in China and northern Vietnam.

During our field surveys in Mt. Nankun, Longmen County, Guangdong Province, China (Fig. 1), we collected a series of specimens of small-sized *Bufo* species near mountain streams, which were previously reported as *B. cryptotympanicus* (Fei et al. 2009, 2010, 2012; Li et al. 2011; Fei and Ye 2016). However, these specimens displayed significant morphological differences from other congeners, including small body size, tympanum absent, parotoid glands small and olive-shaped, tarsal fold absent, dorsal body with a fine vertebral line and white nuptial spinules present on dorsal and inner surfaces of fingers I and II in males. Further molecular data revealed that these specimens belong to a distinct evolutionary lineage separated from all other recognised *Bufo* species and, hence, it should be considered as a new species and described herein.

Materials and Methods

Specimens and morphology

Five specimens of the genus *Bufo* were collected from Mt. Nankun, Guangdong, China from 2015 to 2017. All specimens were fixed in 10% buffered formalin and later transferred to 70% ethanol and deposited in the Museum of Biology, Sun Yat-sen University (**SYS**) and Chengdu Institute of Biology, Chinese Academy of Sciences (**CIB**), China.

All measurements were taken with digital callipers (Neiko 01407A Stainless Steel 6-Inch Digital Caliper, USA) to the nearest 0.1 mm. Morphological characters used and their measurement methods followed Fei et al. (2009), the webbing formula followed Savage and Heyer (1997) and the computational method for sexual size dimorphism index (SSDi) followed Nali et al. (2014).

The following measurements and abbreviations are used: **SVL** snout-vent length (from tip of snout to vent); **HDL** head length (from the tip of snout to the rear of jaws); **HDW** head width (head width at commissure of jaws); **SNT** snout length (from the tip of snout to the anterior corner of eye); **IND** internarial distance (distance between nares); **IOD** interorbital distance (minimum distance between middle upper eyelids); **ED** eye diameter (diameter of exposed portion of eye ball); **HND** hand length (distance from distal end of radioulna to tip of phalanx of finger III); **RAD** radioulna length; **FTL** foot length (distance from distal end of tibia to tip of distal phalanx of toe IV); **TIB** tibia length (distance from knee to heel, flexed at 90°); **PL**, maximum parotoid gland length; **PW**, maximum parotoid gland width. The sex of specimens was determined by dissection or observation of secondary sexual characters (i.e., the presence of nuptial pads in males). Enlarged gonads and mature eggs were considered evidence of adulthood.

In addition, the specimens were compared with other congeners of genus *Bufo* on the basis of descriptions in literature (Matsui 1976; Kou 1984; Yang et al. 1996; Litvinchuk et al. 2008; Yang and Rao 2008; Zhao 2008; Kuo, 2009; Fei and Ye 2016; Rao 2022) and specimens stored in SYS.

Molecules

For phylogenetic analysis, 20 tissue samples (liver or muscle) were used. The samples were obtained from euthanised specimens and then preserved in 95% ethanol and stored at –20°C. In addition, 44 sequences of genus *Bufo* were obtained from GenBank for phylogenetic analyses. GenBank accession numbers of all sequences are shown in Table 1.

Genomic DNA was extracted from muscle tissue samples, using a DNA extraction kit from Tiangen Biotech (Beijing) Co., Ltd. Two fragments of the mitochondrial genes that encode partial 16S ribosomal RNA gene (16S) and partial cytochrome oxidase subunit I gene (CO1) were amplified. Primers used for 16S were L3975 (5'-CGCCTGTTTACCAAAAACAT-3') and H4551 (5'-CCGGTCTGAACTCAGATCACGT-3') following Simon et al. (1994) and primers used for CO1 were dgLCO (5'-GGTCAACAAATCATAAAGAYATYGG-3') and dgHCO (5'-AAACTTCAGGGTGACCAAARAAYCA-3') following Meyer et al. (2005). PCR amplifications were processed with the cycling conditions of the initial denaturing step at 95°C for 5 min; 35 cycles of denaturing at 95°C for 40 s, annealing at 53°C for 40 s and extending at 72°C for 1 min; and a final extending step of 72°C for 10 min. PCR products were purified with spin columns and then sequenced with both forward and reverse primers using BigDye Terminator Cycle Sequencing Kit following the guidelines, on an ABI Prism 3730 automated DNA sequencer by Wuhan Tianyi Huiyuan Bioscience & Technology Inc.

DNA sequences were aligned in MEGA 6 (Tamura et al. 2013) by the Clustal W algorithm with default parameters (Thompson et al. 1997). Two gene segments, 987 base pairs (bp) of 16S and 635 bp of CO1, were concatenated seriatim into a 1322 bp single sequence. The dataset was partitioned according to genes and for codons for CO1 and tested respectively in jModelTest v.2.1.2 with Akaike and Bayesian Information Criteria, all resulting in the best-fitting nucleotide substitution models of GTR + I + G. Sequenced data were analysed using Bayesian Inference (BI) in MrBayes 3.2.4 (Ronquist et al. 2012) and Maximum Likelihood (ML) in RaxmlGUI 1.3 (Silvestro and Michalak 2012). Two independent runs were conducted in the BI analysis with 2,000,000 generations each and 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. In the ML anal-

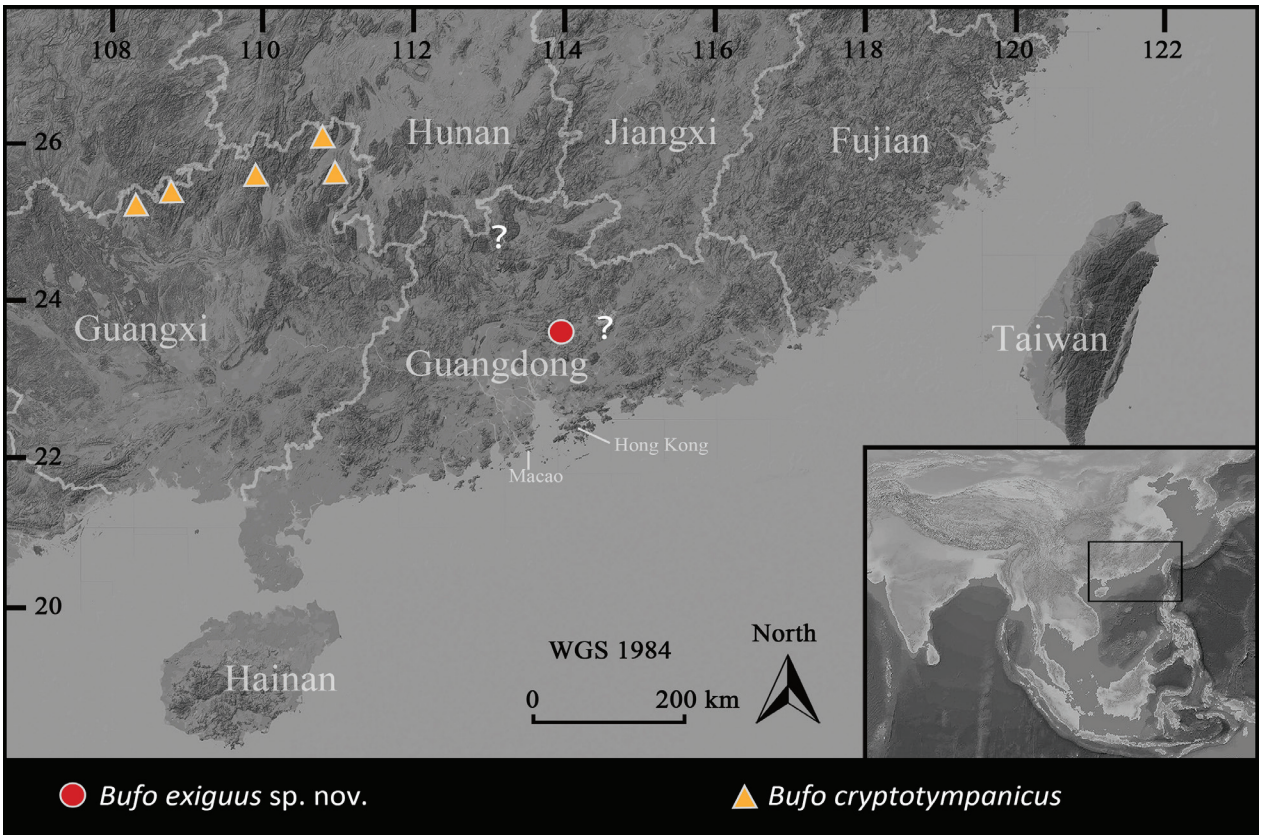

Figure 1. Geographic distribution of *Bufo exiguus* **sp. nov.** and *B. cryptotympanicus*. "?" indicates the unconfirmed historic record of *B. cryptotympanicus* in Guangdong Province, China.

Table 1. Localities, voucher information and GenBank accession numbers for all samples used in this study.

ID	Species	Localities	Voucher ID	GenBank accession numbers		References
				16S	CO1	
1	*Bufo exiguus* **sp. nov.**	China: Mt. Nankun, Longmen County, Guangdong	SYS a003605	OR096253	OR096112	This study
2	*Bufo exiguus* **sp. nov.**	China: Mt. Nankun, Longmen County, Guangdong	SYS a005715	OR096254	OR096113	This study
3	*Bufo exiguus* **sp. nov.**	China: Mt. Nankun, Longmen County, Guangdong	SYS a005716	OR096255	OR096114	This study
4	*Bufo exiguus* **sp. nov.**	China: Mt. Nankun, Longmen County, Guangdong	SYS a005790	OR096256	OR096115	This study
5	*Bufo exiguus* **sp. nov.**	China: Mt. Nankun, Longmen County, Guangdong	SYS a005791	OR096257	OR096116	This study
6	*Bufo andrewsi*	China: Mt. Gaoligong, Tengchong City, Yunnan	SYS a003828	OR096258	OR096117	This study
7	*Bufo andrewsi*	China: Lijiang City, Yunnan	KIZ YPX14110	MW023953	/	Che et al. (2020)
8	*Bufo andrewsi*	China: Anzhou District, Mianyang City, Sichuan	SYS a005394	OR096259	OR096118	This study
9	*Bufo aspinia*	China: Yangbi Yi Autonomous County, Yunnan	KIZ 93A011	AF160787	/	Liu et al. (2000)
10	*Bufo bankorensis*	China: Kuantzuling, Tainan City, Taiwan	n/a	AB159589	/	Igawa et al. (2006)
11	*Bufo bankorensis*	China: Fuyang Nature Park, Taipei City, Taiwan	BB098	JN653290	/	Recuero et al. (2012)
12	*Bufo bankorensis*	China: Fuyang Nature Park, Taipei City, Taiwan	BB099	JN653291	/	Recuero et al. (2012)
13	*Bufo bufo*	France: Le Boujon	BB143	JN647131	/	Recuero et al. (2012)
14	*Bufo bufo*	Sweden: Göteborg,Slätta damm, Vastra Götaland	BB169	JN647146	/	Recuero et al. (2012)

ID	Species	Localities	Voucher ID	GenBank accession numbers		References
				16S	CO1	
15	*Bufo bufo*	Poland: Zgorzelec	BB174	JN647149	/	Recuero et al. (2012)
16	*Bufo cryptotympanicus*	China: Mt. Mao'er, Xing'an County, Guangxi	SYS a006558	OR096260	OR096119	This study
17	*Bufo cryptotympanicus*	China: Guangxi	KIZ 07606	MW023951	/	Che et al. (2020)
18	*Bufo* cf. *cryptotympanicus*	Vietnam: Fan-Si-Pan; Lao Cai	AMNH 13198	AF160789	/	Liu et al. (2000)
19	*Bufo eichwaldi*	Azerbaijan: Agoshapeshta, Lerik	BB062	JN647240	/	Recuero et al. (2012)
20	*Bufo eichwaldi*	Azerbaijan: Mt. Talysh, Kizhaba, Astara	BB084	JN647239	/	Recuero et al. (2012)
21	*Bufo eichwaldi*	Azerbaijan: Agoshapeshta, Lerik	BB091	JN647238	/	Recuero et al. (2012)
22	*Bufo formosus*	Japan: Hakodate, Hokkaido	n/a	AB159561	/	Igawa et al. (2006)
23	*Bufo formosus*	Japan: Hirosaki, Aomori	n/a	AB159562	/	Igawa et al. (2006)
24	*Bufo formosus*	Japan: Wakuya, Miyagi	n/a	AB159564	/	Igawa et al. (2006)
25	*Bufo gargarizans*	China: Hejiang County, Sichuan	SYS a004929	OR096261	OR096120	This study
26	*Bufo gargarizans*	China: Fenghua District, Ningbo City, Zhejiang	SYS a006788	OR096262	OR096121	This study
27	*Bufo gargarizans*	China: Mt. Mogan, Deqing County, Zhejiang	SYS a007985	OR096263	OR096122	This study
28	*Bufo gargarizans miyakonis*	Japan: Miyako Islands	n/a	AB159588	/	Igawa et al. (2006)
29	"*Bufo pageoti*"	Myanmar: Laiva village, Falam Township, Chin	CAS 233251	KF665335	/	Liedtke et al. (2016)
30	*Bufo praetextatus*	Japan: Arashiyama, Kyoto	n/a	AB159579	/	Igawa et al. (2006)
31	*Bufo praetextatus*	Japan: Kodera, Hyogo	n/a	AB159580	/	Igawa et al. (2006)
32	*Bufo praetextatus*	Japan: Nichinan, Tottori	n/a	AB159581	/	Igawa et al. (2006)
33	*Bufo sachalinensis*	Republic of Korea: Donghaemyeon, Goseong, Goseong	NIBRAM 0000100413	JQ815292	JQ844500	Jeong et al. (2013)
34	*Bufo sachalinensis*	Republic of Korea: Siheung, Gyeonggi	NIBRAM 0000100345	JQ815293	JQ844501	Jeong et al. (2013)
35	*Bufo sachalinensis*	Republic of Korea: Hanam, Hwacheon, Gangwon	NIBRAM 0000100330	JQ815294	JQ844502	Jeong et al. (2013)
36	*Bufo spinosus*	France: Saint Bonnet en Champsaur	OBYX19	JN647181	/	Recuero et al. (2012)
37	*Bufo spinosus*	Spain: Sadernes	BB012	JN647222	/	Recuero et al. (2012)
38	*Bufo spinosus*	Morocco: Ifrane	BB119	JN647215	/	Recuero et al. (2012)
39	*Bufo stejnegeri*	China: Benxi City, Liaoning	SYS a007664	OR096264	OR096124	This study
40	*Bufo stejnegeri*	Republic of Korea: Seo, Yangyang, Gangwon	NIBRAM 0000000192	JQ815295	JQ844503	Jeong et al. (2013)
41	*Bufo stejnegeri*	Republic of Korea: Toji, Gurye, Jeollanam	NIBRAM 0000100334	JQ815296	JQ844504	Jeong et al. (2013)
42	*Bufo tibetanus*	China: Biru County, Tibet	KIZ 08276	MW133326	/	Che et al. (2020)
43	*Bufo tibetanus*	China: Bomê County, Tibet	SYS a006632	OR096265	OR096123	This study
44	*Bufo torrenticola*	Japan: Tenkawa, Nara	BB100	JN653292	/	Recuero et al. (2012)
45	*Bufo torrenticola*	Japan: Neo, Gifu	n/a	AB159577	/	Igawa et al. (2006)
46	*Bufo torrenticola*	Japan: Neo, Gifu	n/a	AB159578	/	Igawa et al. (2006)
47	*Bufo tuberculatus*	China: Batang County, Sichuan	KIZ 019288	MW133327	/	Che et al. (2020)
48	*Bufo tuberculatus*	China: Batang County, Sichuan	KIZ 019290	MW133329	/	Che et al. (2020)
49	*Bufo tuberospinius*	China: Mt. Gaoligong, Tengchong City, Yunnan	SYS a003773	OR096266	OR096125	This study
50	*Bufo tuberospinius*	China: Mt. Gaoligong, Tengchong City, Yunnan	SYS a003811	OR096267	OR096126	This study
51	*Bufo tuberospinius*	China: Tengchong City, Yunnan	KIZ 91A089	AF160788	/	Liu et al. (2000)
52	*Bufo verrucosissimus*	Georgia: Malaya Ritza Lake, Bzypi Gorge, Abkhazia	BB059	JN647227	/	Recuero et al. (2012)

ID	Species	Localities	Voucher ID	GenBank accession numbers		References
				16S	CO1	
53	*Bufo verrucosissimus*	Russia: Aderbiyevka, Gelenjik District, Krasnodar	BB083	JN647233	/	Recuero et al. (2012)
54	*Bufo verrucosissimus*	Russia: Novoyekaterinovskaya, Shpakovskiy District, Stavropol	BB085	JN647234	/	Recuero et al. (2012)
55	*Duttaphrynus melanostictus*	China: Dongguan City, Guangdong	SYS a001988	OR096268	OR096127	This study
56	*Duttaphrynus melanostictus*	China: Dongguan City, Guangdong	SYS a001989	OR096269	OR096128	This study
57	*Duttaphrynus stuarti*	China: Gongshan County, Yunnan	SYS a007708	OR096270	OR096129	This study
58	*Strauchbufo raddei*	China: Laoting County, Hebei	SYS a002919	OR096271	OR096130	This study
59	*Strauchbufo raddei*	China: Laoting County, Hebei	SYS a002920	OR096272	OR096131	This study

ysis, a bootstrap consensus tree inferred from 1000 replicates was generated. Nodes with Bayesian posterior probabilities (BPP) ≥ 0.95 and the bootstrap supports (BS) for Maximum Likelihood analysis ≥ 70 were considered as strongly supported (Huelsenbeck et al. 2001; Wilcox et al. 2002). Pairwise distances (p distances) were calculated in MEGA6 using the uncorrected model. Gaps/Missing Data Treatment used the complete-deletion option, substitutions to include the d: Transitions + Transversions option.

Results

The ML and BI analyses resulted in essentially identical topologies (Fig. 2), but there were discordant topologies at some nodes within the *Bufo gargarizans* complex. The mean uncorrected p distances, based on 16S amongst all in-group species used in this study, are given in Table 2.

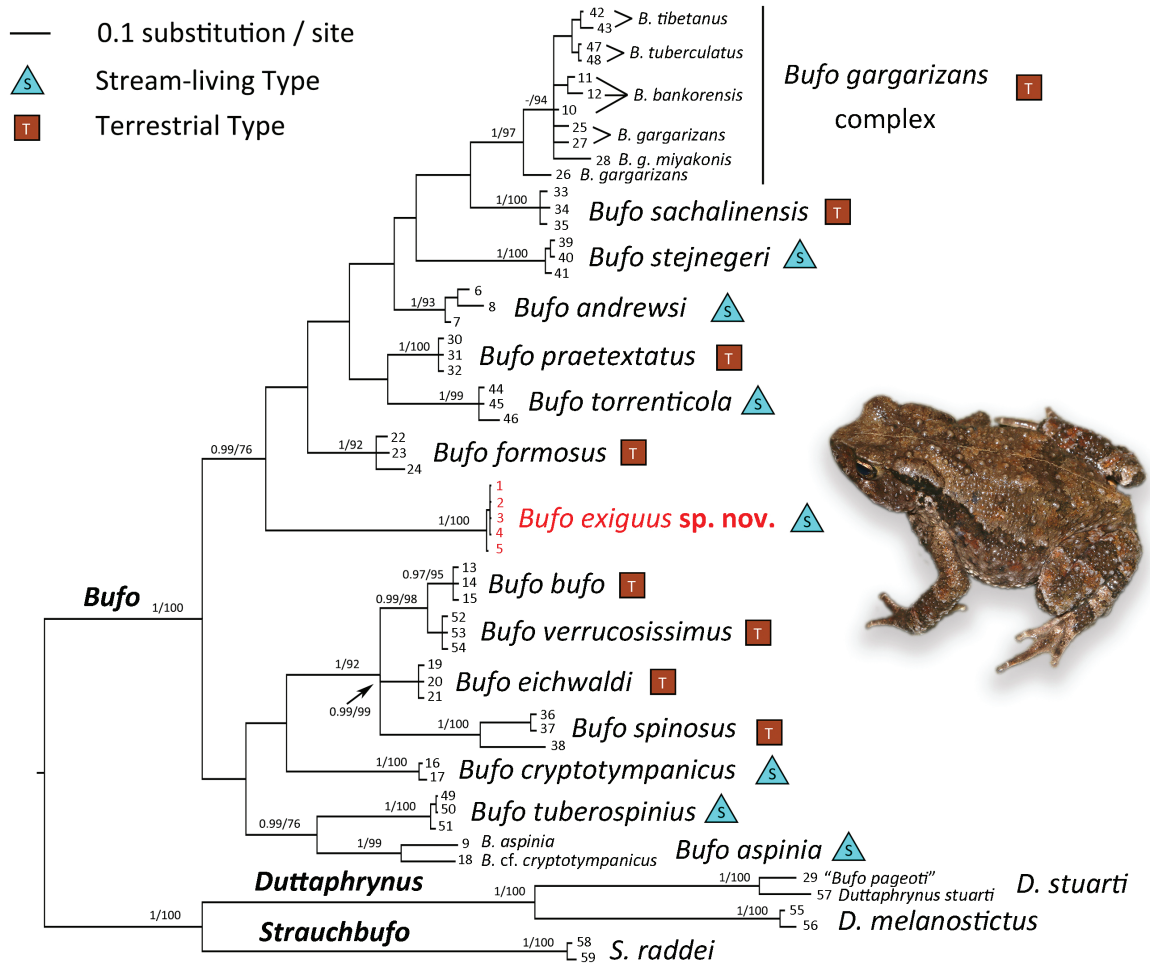

Figure 2. Bayesian inference tree inferred from 16S rRNA and CO1 genes. The Bayesian posterior probabilities (BPP) < 0.95 or the bootstrap supports (BS) < 70 are not given in the tree.

Table 2. Uncorrected p distances (%) of the 16S gene amongst species of genus *Bufo*. A "/" indicates not available.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	*Bufo exiguus* **sp. nov.**	0.0																			
2	*Bufo andrewsi*	6.4	0.3																		
3	*Bufo aspinia*	8.6	5.7	/																	
4	*Bufo bankorensis*	5.3	3.2	7.3	0.3																
5	*Bufo bufo*	7.7	6.5	8.5	5.9	0.0															
6	*Bufo cryptotympanicus*	7.6	5.4	6.2	5.1	5.4	0.2														
7	*Bufo* cf. *cryptotympanicus*	9.1	6.5	2.2	7.8	7.9	6.5	/													
8	*Bufo eichwaldi*	7.7	6.2	7.9	6.2	2.4	4.9	7.9	0.0												
9	*Bufo formosus*	5.3	4.1	7.9	3.6	7.6	6.5	8.5	7.4	0.8											
10	*Bufo gargarizans*	5.4	3.1	7.3	0.6	5.8	5.3	7.9	6.1	3.6	0.6										
11	*Bufo g. miyakonis*	6.1	4.0	8.3	1.2	6.9	6.2	8.8	7.2	4.5	1.3	/									
12	*Bufo praetextatus*	7.2	3.4	7.7	5.0	6.6	6.7	8.5	7.4	4.2	4.7	5.0	0.0								
13	*Bufo sachalinensis*	7.4	3.4	6.9	2.8	6.3	6.4	7.4	6.6	5.3	2.9	3.7	5.5	0.0							
14	*Bufo spinosus*	9.7	7.5	9.0	7.3	4.4	7.0	9.1	4.1	8.0	7.3	8.6	7.8	7.9	2.0						
15	*Bufo stejnegeri*	6.3	2.6	7.1	4.5	6.6	7.0	7.9	7.4	4.6	4.2	5.2	3.2	4.2	8.6	0.0					
16	*Bufo tibetanus*	5.2	3.6	7.6	0.8	6.0	5.8	7.9	6.2	4.1	0.9	1.6	5.4	3.3	7.1	4.9	0.2				
17	*Bufo torrenticola*	7.1	4.8	8.6	6.1	7.3	8.6	8.3	8.1	4.7	6.1	6.7	3.6	6.4	8.0	4.9	6.6	0.3			
18	*Bufo tuberculatus*	5.3	3.7	7.2	1.0	6.1	5.9	8.0	6.4	4.2	1.0	1.7	5.5	3.5	7.2	5.0	0.4	6.7	0.0		
19	*Bufo tuberospinius*	8.6	6.8	4.0	7.6	7.6	5.1	4.5	7.1	8.0	7.8	8.8	8.5	7.7	8.6	7.6	7.8	8.8	8.0	0.0	
20	*Bufo verrucosissimus*	7.7	6.5	8.2	5.9	0.7	5.6	7.7	2.2	7.6	5.8	6.9	6.6	6.3	4.2	6.0	6.0	6.8	6.1	7.4	0.0

The phylogenetic analyses recovered a strongly-supported monophyletic lineage containing all *Bufo* samples used in this study (BPP = 1.00; BS = 100), except a misidentified sequence from GenBank (KF665335). Two branches within genus *Bufo* are shown in our phylogenetic tree, but with low intrageneric nodal supports, so intrageneric phylogenetic relationships are not clear. Our phylogenetic analyses showed that *B. bankorensis*, *B. gargarizans*, *B. tibetanus* and *B. tuberculatus* were clustered into a species complex with low intrapopulation genetic differentiation, the systematic relationships amongst them requiring more evidence to determine. Besides, the three samples, identified as *B. cryptotympanicus*, were not clustered in a monophyletic group. Two samples from Guangxi, China were clustered into one lineage with low genetic differentiation (0.2%), representing the true *B. cryptotympanicus*. However, another *B. cryptotympanicus* sample from Vietnam was clustered with a topotypic sample of *B. aspinia* into one clade with significant genetic differentiation (2.2%).

The results recovered an unnamed independent evolutionary lineage from Mt. Nankun, Guangdong, China with strong nodal support (BPP = 1.00; BS = 100) and low intrapopulational genetic differentiation (0%), which is significantly different from other congeners in phylogeny. Therefore, this unnamed population from Mt. Nankun is considered as a separately evolving lineage and an undescribed species.

The diagnostic characters separating all species of *Bufo* are given in Table 4. The morphological examination on the specimens from Mt. Nankun also revealed a combination of morphological characters that distinctly differed from all other known species in the genus *Bufo*, including small body size, tympanum absent, parotoid glands small and olive-shaped, tarsal fold absent, dorsal body with a fine vertebral line and white nuptial spinules present on dorsal and inner surfaces of fingers I and II in males. Based on the molecular and morphological evidence, we hereby describe these specimens from Mt. Nankun as a new species, *Bufo exiguus* **sp. nov.**

Taxonomic account

Bufo exiguus sp. nov.

https://zoobank.org/4CCAB971-D935-4E34-B16F-447BD54AC947

Figures 3 and 4

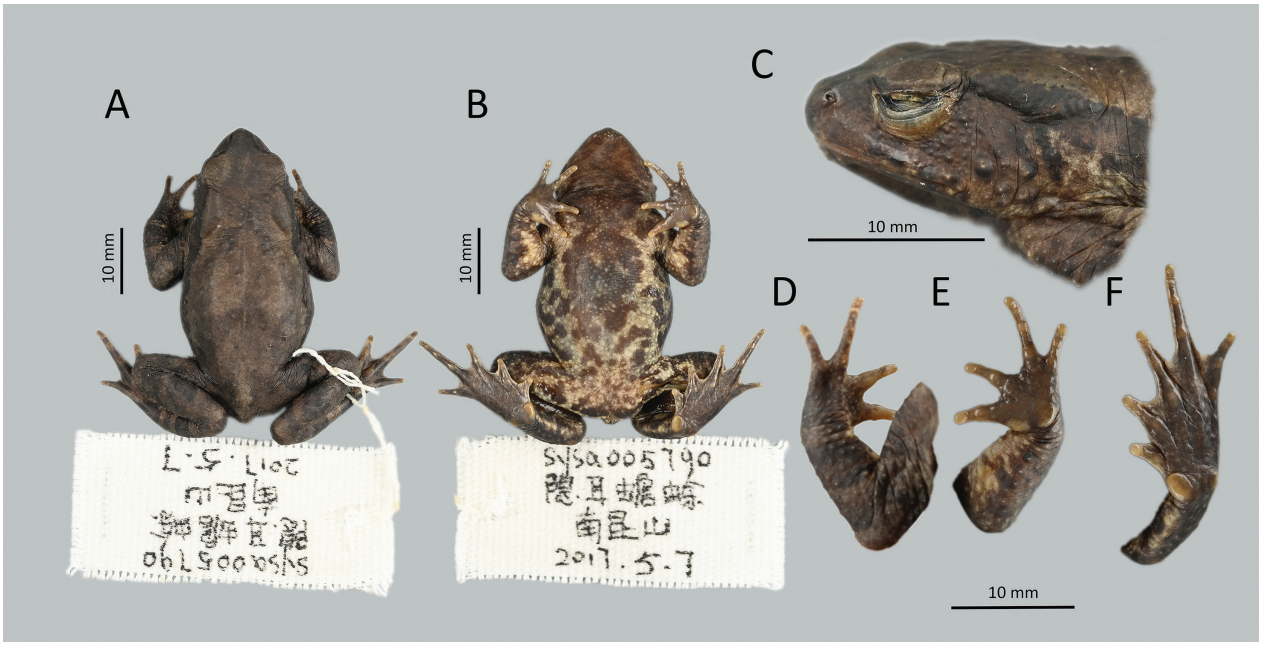

Figure 3. Morphological features of the adult male holotype SYS a005790 of *Bufo exiguus* **sp. nov.** in preservative. **A** dorsal; **B** ventral; **C** close-up of head; **D** dorsal view of right hand; **E** ventral view of left hand; **F** ventral view of left foot. Photos by Shuo Qi.

Chresonymy. *Bufo cryptotympanicus* — Fei et al. (2009, 2010): Longmen County; Li et al. (2011): Mt. Nankun, Longmen County. *Torrentophryne cryptotympanicus* — Fei et al. (2012): Longmen County; Fei and Ye (2016): Longmen County.

Holotype. SYS a005790 (Fig. 3), adult male, collected on 7 May 2017 by Zhi-Tong Lyu (ZTL), Jian Wang (JW) and Hai-Long He (HLH) from Mt. Nankun (23˚38′31″ N, 113˚50′53″ E; 564 m a.s.l.), Longmen County, Huizhou City, Guangdong Province, China.

Paratypes. Four adult and one subadult specimens from the same locality as the holotype (500–600 m a.s.l.): adult male CIB 19028 (field number: SYS a003605) collected on 14 April 2015 by ZTL, JW and Run-Lin Li; adult females SYS a005715 (Fig. 4A) and SYS a005716 (Fig. 4C) collected on 9 April 2017 by ZTL and JW; adult female SYS a005791 collected on 7 May 2017 by ZTL, JW and HLH; subadult female SYS a005584 (Fig. 4B) collected on 11 November 2016 by ZTL, JW and Ying-Yong Wang.

Diagnosis. *Bufo exiguus* **sp. nov.** is distinguished from other species in the genus *Bufo* by a combination of the following morphological characters: (1) small body size (adult males with SVL 43.2–43.3 mm, adult females with SVL 48.5–52.4 mm); (2) tympanum absent; (3) parotoid glands small, olive-shaped; (4) tarsal fold absent; (5) dorsal body with a fine vertebral line; (6) numerous white granular nuptial spinules present on dorsal and inner surfaces of fingers I and II in males.

Etymology. The specific name *exiguus* means small in Latin and refers to its small body size. According to their distribution and ecological habit, we suggest the common name as "Guangdong stream toad" and Chinese formal name as "guǎng dōng xī chán" (广东溪蟾).

Description of the holotype. Adult male (Fig. 3), body size small and stubby (SVL 43.3 mm); head width slightly larger than head length (HL/HW = 0.98); snout obtusely acuminate in dorsal review, tip of snout vertical in lateral profile; nostrils closer to tip of snout than to eye; canthus rostralis distinct, internarial distance less than interorbital distance (IND/IOD = 0.79); loreal region slightly concave; eye moderate, diameter larger than interorbital distance (ED/IOD = 1.05), pupil horizontal elliptic; tympanum absent; parotoid glands olive-shaped, parallel in position (PW/PL = 0.51); maxillary and vomerine teeth absent; vocal sacs and splits absent.

Radioulnar length almost equal to hand length (RAD/HND = 1.01); hand without webbing, fingers without lateral fringes, relative finger length I = II < IV < III; tips of fingers round, not enlarged, without toe pads; subarticular tubercles prominent, rounded and not divided, present in each phalangeal joint; inner metacarpal tubercle long oval and prominent, lying at the base of finger I; outer metatarsal tubercle rounded, indistinct, boundary unclear; nuptial pad on the medial dorsal surface of the first and second fingers, bearing numerous white granular nuptial spinules.

Hind-limbs short and stout, tibio-tarsal articulation reaching middle point of parotoid gland when hind-limb stretched alongside the body; heels not meeting when flexed hind-limbs held at right angles to body axis; tibia length 0.40 of SVL and foot length 0.60 of SVL; relative toe length I < II < V < III < IV; tips of toes round, not dilated; toes with lateral fringes and with one third webbing, webbing formula I0-1^{+}-II0^{-} 1^{-}III1–2^{-}IV2^{-}-½V; subarticular tubercles rounded, not divided; inner and outer metatarsal tubercle ovoid, the former being much larger than the latter; tarsal fold absent.

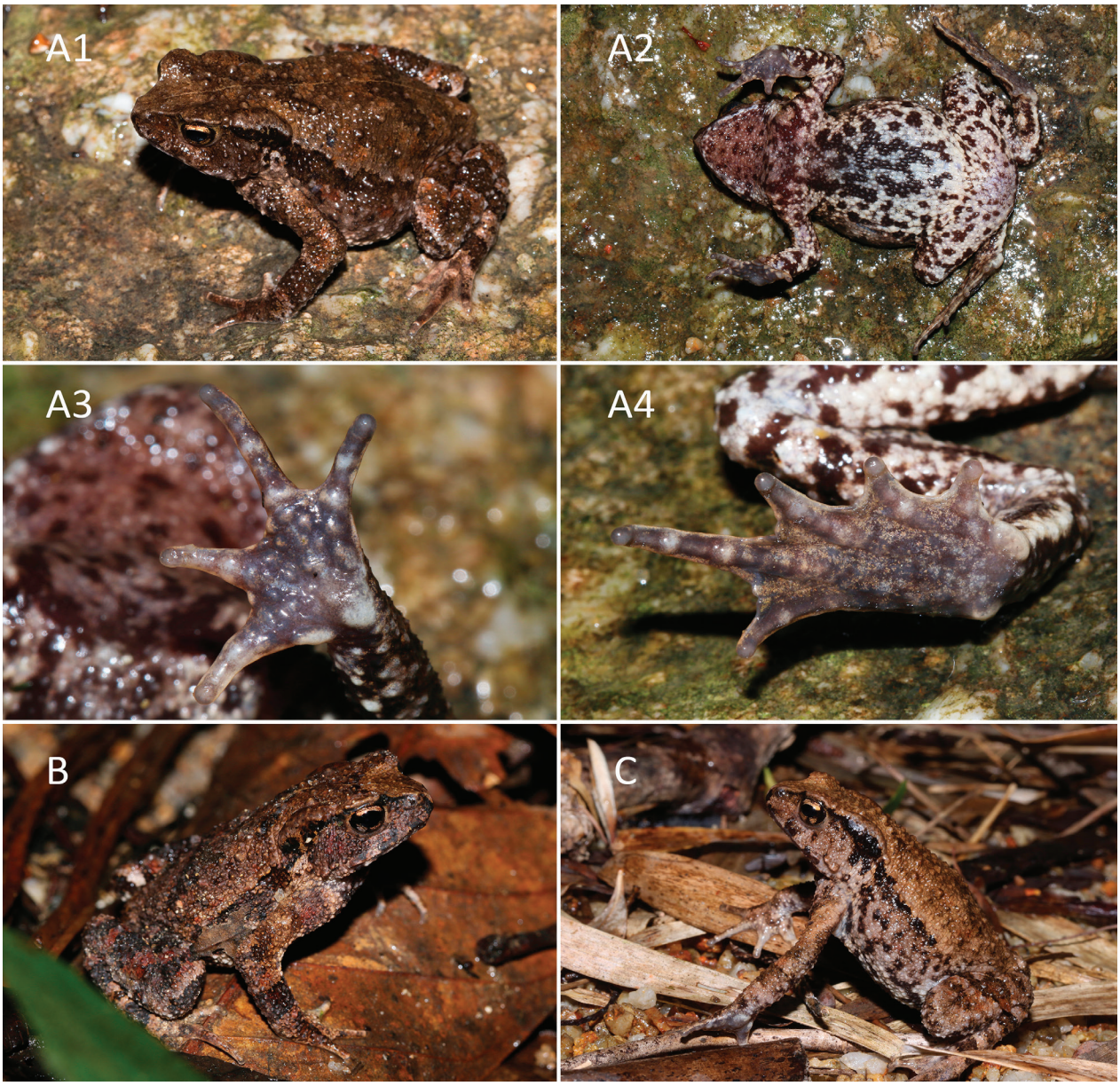

Figure 4. Paratypes of *Bufo exiguus* **sp. nov.** in life. **A1** adult female SYS a005715, dorsolateral view; **A2** adult female SYS a005715, ventral view; **A3** adult female SYS a005715, ventral view of left hand; **A4** adult female SYS a005715, ventral view of right foot; **B** subadult female SYS a005584; **C** adult female SYS a005716. Photos by Lyu Zhi-Tong (A, B) and Jian Wang (C).

Dorsal surface of head and body relatively smooth, all the tubercles and granules without horny spinules; a few rounded tubercles and granules scattered on dorsum, especially in the region between parotoid glands, without large warts. Loreal region relatively smooth, only with a few flat granules; some small tubercles and granules present in temporal region, two larger tubercles at the mandibular articulation; flanks relatively smooth, only with a few granules; dorsal surfaces of limbs relatively smooth, fore-limbs only with small granules, hind-limbs with small granules, some large flat tubercles scattered on dorsal surfaces of tibia; ventral surfaces of head and body wrinkled, with scattered small granules; ventral surfaces of hands and feet with a few flat tubercles.

Colouration of holotype in preservative. Dorsal ground colour of head and body faded to greyish-brown; a dark brown "V"-shaped marking between eyes, two "inverted V"-shaped markings on the back of body; an indistinct thin vertebral line running from the snout to the upper part of the vent; dorsal surface of limbs greyish-brown, a dark brown band on forearm, some blurry blotches in upper arm, several distinct dark brown bands on femur, tibia and tarsus. Some dark brown blotches on the edge of the lips and mandibular articulation; a dark brown stripe from the nare across lower part of parotoid gland, extending to the groin. Ventral surface of throat and chest brown with a few blurry spots and worm-like markings; ventral surface of body pale yellow with dark brown worm-like markings, many markings agglomerating into a large blotch in the centre of the belly; ventral surfaces of limbs pale yellow, with dark brown spots and worm-like markings; ventral surface of hands and feet dark brown, tips of digits, subarticular tubercles and metatarsal tubercles fading to pale white.

Table 3. Measurements (in mm) and ratios of the type series of *Bufo exiguus* **sp. nov.**, * indicates the holotype.

	SYS a005790*	CIB 19028	SYS a005584	SYS a005715	SYS a005716	SYS a005791
Sex	Male	Male	Subadult female	Female	Female	Female
SVL	43.3	43.2	36.8	52.4	49.7	48.5
HDL	12.8	13.2	12.1	15.3	14.9	13.8
HDW	13.1	13.9	13.7	16.2	16.1	14.8
SNT	5.5	5.4	5.2	6.5	6.0	6.0
IND	3.1	3.3	2.7	3.6	3.8	3.4
IOD	3.9	4.0	4.0	5.1	5.0	4.8
ED	4.1	4.0	3.6	4.8	4.5	4.3
HND	10.7	9.6	9.8	13.1	11.9	12.3
RAD	10.8	10.2	9.6	12.8	12.1	11.4
FTL	29.7	28.0	24.1	32.9	31.5	30.0
TIB	17.2	16.5	14.6	19.2	18.8	18.0
PL	8.5	8.7	6.6	9.6	9.0	8.9
PW	4.3	3.9	3.5	4.9	4.6	4.7
HDL/SVL	0.30	0.31	0.33	0.29	0.30	0.28
HDW/SVL	0.30	0.32	0.37	0.31	0.32	0.31
HDL/HDW	0.98	0.95	0.88	0.94	0.92	0.93
FTL/SVL	0.69	0.65	0.65	0.63	0.63	0.62

Morphological variation. Measurements of all individuals are given in Table 3 and the aspects of three female paratypes are given in Figure 4.

Adult females are larger than males, SSDi = 0.84. Both males have nuptial pads on the dorsal surfaces of the first and second fingers, bearing granular white nuptial spinules. All the paratypes share similar aspects with the holotype in general, apart from the following differences. The relative finger length I < II < IV < III in four paratypes (CIB 19028, SYS a005584, 5715 and 5716); tibio-tarsal articulation reaching to the posterior edge of the parotoid gland when the hind-limb is stretched alongside the body in SYS a005791. Besides, based on existing specimens, the females have rougher skin texture than males, dorsal surfaces of head and body with many rounded tubercles and granules, but all the tubercles and granules are without horny spinules; in the loreal region and temporal region, small tubercles and granules, two or three larger tubercles at the mandibular articulation are present; dorsal surfaces of limbs rough, fore-limbs with many small granules, hind-limbs with small granules and tubercles, some large tubercles scattered on dorsal surfaces of tibia; ventral surfaces of hands and feet with multiple flat tubercles.

As the general aspect of colouration of the holotype in life is not available, we describe the colouration of three female paratypes in life below.

All female paratypes share similar colouration in life. The dorsal surface of the head and body brown or yellowish-brown with a few separated dark brown and rusty red blotches; a dark brown "V"-shaped or inverted hollow triangularly-shaped marking between eyes, two "inverted V"-shaped markings on the back of body; a thin vertebral line running from the snout to the upper part of the vent. Dorsal surface of limbs have the same colour as the dorsal surface of the body, a distinct dark brown band on forearm, some indistinct blotches on the upper arm, several distinct dark brown bands on the femur, tibia and tarsus (rusty red blotches scattered on the limbs in SYS a005584). Some dark brown blotches on the edge of the lips and mandibular articulation; a dark brown stripe from the nares across the lower part of parotoid glands and flanks, extending to the groin (stripe discontinuous in the flanks in SYS a005716). Ventral surface of throat and chest light brown with a few darker spots and worm-like markings; ventral surface of body white with many dark brown worm-like markings, mostly in the centre of the belly; ventral surfaces of limbs showing the same colour as the ventral surface of the body, with dark brown spots and worm-like markings; ventral surface of hands and feet greyish-brown, tips of digits, subarticular tubercles and metatarsal tubercles faintly white. Pupil black; iris golden with black reticular markings, black blotches in four directions.

Comparisons. *Bufo exiguus* **sp. nov.** are distinctly different from all other recognised congeners by having a relatively small body size, SVL < 50 mm in adult males and < 60 mm in adult females (vs. SVL > 50 mm in adult males in *B. andrewsi*, *B. aspinius*, *B. bankorensis*, *B. bufo*, *B. cryptotympanicus*, *B. eichwaldi*, *B. formosus*, *B. gargarizans*, *B. luchunnicus*, *B. menglianus*, *B. minshanicus*, *B. pageoti*, *B. praetextatus*, *B. spinosus*, *B. stejnegeri*, *B. tibetanus*, *B. torrenticola*, *B. tuberculatus*, *B. tuberospinius*, *B. verrucosissimus*, *B. yongdeensis* and *B. yunlingensis*; SVL > 60 mm in adult females in *B. andrewsi*, *B. aspinius*, *B. bankorensis*, *B. bufo*, *B. eichwaldi*, *B. formosus*, *B. gargarizans*, *B. menglianus*, *B. minshanicus*, *B. pageoti*, *B. praetextatus*, *B. spinosus*, *B. tibetanus*, *B. torrenticola*, *B. tuberospinius*, *B. verrucosissimus*, *B. yongdeensis* and *B. yunlingensis*), tympanum absent (vs. present in *B. andrewsi*, *B. bankorensis*, *B. bufo*, *B. eichwaldi*, *B. formosus*, *B. gargarizans*, *B. menglianus*, *B. minshanicus*, *B. praetextatus*, *B. sachalinensis*, *B. spi-*

Table 4. Diagnostic characters separating 25 species of the genus *Bufo*.

	SVL in males (mm)	SVL in females (mm)	Tympanum absent (0) or present (1)	Tarsal fold absent (0) or present (1)	Vertebral line absent (0) or present (1)	Number of adult specimens examined	References
Bufo exiguus **sp. nov.**	43.2–43.3	48.5–52.4	0	0	1	2 males, 3 females	This study
Bufo ailaoanus	38.5–41.1	52.0–55.0	0	0	0	2 males, 4 females	Fei and Ye (2016)
Bufo andrewsi	63.0–89.5	85.0–116.0	1	1	0 or 1	114 males, 80 females	Fei and Ye (2016); this study
Bufo aspinius	65.0–80.0	81.0–103.0	0	0 or 1	1	80 males, 60 females	Fei and Ye (2016)
Bufo bankorensis	64.0–100.0	72.8–130.0	1	0	0 or 1	87 males, 24 females	Fei and Ye (2016); Kuo (2009); this study
Bufo bufo	61.0–72.4	74.9–94.1	1	0	0	13 males, 8 females	Litvinchuk et al. (2008)
Bufo cryptotympanicus	65.0–70.0	60.0–77.0	0	0	1	6 males, 10 females	Fei and Ye (2016)
Bufo eichwaldi	67.8–71.2	78.2	1	0	0	2 males, 1 female	Litvinchuk et al. (2008)
Bufo formosus	110.0–158.0	142.5–159.2	1	0	0	19 males, 4 females	Matsui (1976)
Bufo gargarizans	79.2–106.1	97.6–120.5	1	0 or 1	0 or 1	28 males, 27 females	Fei and Ye (2016); this study
Bufo luchunnicus	58	55.0–79.0	0	1	1	1 male, 6 females	Fei and Ye (2016)
Bufo menglianus	/	80.0–98.0	1	0	1	2 females	Fei and Ye (2016)
Bufo minshanicus	53.0–64.8	72.0–90.0	1	0	0	72 males, 50 females	Fei and Ye (2016)
Bufo pageoti	52.0–66.0	83.0	0	1	0 or 1	6 males, 4 females	Fei and Ye (2016)
Bufo praetextatus	87.0–116.5	94.0–134.8	1	0	0	23 males, 20 females	Matsui (1976)
Bufo sachalinensis	47.0–87.0	42–97.0	1	1	0	27 males, 75 females	Zhao (2008)
Bufo spinosus	94.8	76.8–134.0	1	0	0	1 male, 2 females	Litvinchuk et al. (2008)
Bufo stejnegeri	53.0–58.0	50.0–58.2	0	1	1	9 males, 11 females	Fei and Ye (2016); this study
Bufo tibetanus	61.5–64.0	70.0–77.0	1	1	1	80 males, 51 females	Fei and Ye (2016); this study
Bufo torrenticola	70.5–108.2	91.0–118.2	1	0	0	14 males, 33 females	Matsui (1976)
Bufo tuberculatus	61.0–76.3	58.0–89.0	1	1	0	46 males, 29 females	Fei and Ye (2016)
Bufo tuberospinius	52.0–66.0	63.6–85.0	0	1	1	8 males, 10 females	Yang and Rao (2008); this study
Bufo verrucosissimus	58.4–75.4	89.5–119.2	1	0	0	5 males, 16 females	Litvinchuk et al. (2008)
Bufo yongdeensis	50.0–65.0	59.0–80.0	0	0	0 or 1*	2 males, 2 females	Rao (2022)
Bufo yunlingensis	67.0	75.0	1	1	0 or 1**	2 females***	Rao (2022)

*Rao (2022) recorded *Bufo yongdeensis* "with a distinct light-coloured vertebral line" in main text (p. 219), but a contradictory diagnosis "without light-coloured vertebral line" was given in supplemental description (p. 432, line 7) and then mentioned "with a distinct light-coloured vertebral line in general" (p. 432, line 11).

**Rao (2022) recorded *Bufo yunlingensis* "without light-coloured vertebral line in general" (p. 431, line 14) and then used "without light-coloured vertebral line" to compare with *B. gargarizans* (p. 431, line 23). However, we can clearly see that the photos of type specimen have a light-coloured vertebral line in preservative (p. 431).

***Rao (2022) designated two female specimens as the type specimen of *Bufo yunlingensis*, but sharing same voucher number. The snout-vent length of the male was given in the morphological description, but no more additional specimens mentioned (p. 431, line 11).

nosus, *B. tibetanus*, *B. torrenticola*, *B. tuberculatus*, *B. verrucosissimus* and *B. yunlingensis*), tarsal fold absent (vs. present in *B. andrewsi*, *B. luchunnicus*, *B. pageoti*, *B. sachalinensis*, *B. stejnegeri*, *B. tibetanus*, *B. tuberculatus*, *B. tuberospinius* and *B. yunlingensis*) and presence of a thin vertebral line (vs. absent in *B. ailaoanus*, *B. bufo*, *B. eichwaldi*, *B. formosus*, *B. minshanicus*, *B. sachalinensis*, *B. spinosus*, *B. torrenticola*, *B. tuberculatus* and *B. verrucosissimus*). Comparative data of *Bufo exiguus* **sp. nov.** with other recognised members of the genus *Bufo* are listed in Table 4.

Distribution and natural history. The new species is currently only known from the type locality, Mt. Nankun, Longmen County, Huizhou City, Guangdong Province, China. However, another two historic records (Heyuan City and Shaoguan City), designated as *B. cryptotympanicus* from Guangdong Province, are worthy of careful re-examination (Fig. 1).

This species inhabits slow-flowing montane streams, generally surrounded by evergreen and deciduous broad-leaved mixed forest, at elevations of 500–600 m a.s.l. All mature individuals were observed at night after rain in

April and May. Both males possessed nuptial spinules, but no eggs were observed in the adult female's oviduct by dissection, suggesting their breeding season should be earlier than April. Nevertheless, the tadpoles and most of the other ecology and types of behaviour of this toad remain unknown.

Discussion

The genus *Bufo* has a long history and its concept has been changed many times (Frost 2023). Similar to previous molecular phylogenetic studies, our results support the current recognition of genus *Bufo* as a monophyletic group with high diversity, but the intrageneric phylogenetic relationships are still not resolved. Our phylogenetic results showed that *B. bankorensis*, *B. gargarizans*, *B. tibetanus* and *B. tuberculatus* were clustered into the *B. gargarizans* complex with low intrapopulation genetic differentiation, but due to the lack of samples of topotype and low support at some nodes, the taxonomic placements of these four species require more evidence to determine. In addition, we suggest the sample (AMNH 13198), reported as *B. cryptotympanicus* from Vietnam, is probably a misidentification of *B. aspinia* and, therefore, the record of this species from Vietnam requires further classification. It can be seen that the lack of available sequences and misidentified sequences for some species has affected the understanding of the systematics of the genus *Bufo* and the link to voucher specimens is needed to assist the identification. Besides that, frequent taxonomic changes indicate that previous knowledge on the diversity and taxonomy of genus *Bufo* is inadequate and more work is needed in the future.

There are generally two different ecological niches for the congeners of genus *Bufo*: stream-living type and terrestrial type (Fong et al. 2020). Our phylogenetic analysis shows that these two niche differentiations have occurred multiple times in the genus *Bufo*, but the phylogenetic relationships have not been resolved between each other. The stream-living species usually have small to medium body size, no tympanum, abdominal sucker in the tadpole stage, prefer wetter environments and even inhabit montane streams and spawn in streams rather than lentic habitats (Matsui 1976; Yang et al. 1996; Fong et al. 2020; this study). On the other hand, the terrestrial congeners usually have a medium to large body size, tympana are present and are mainly terrestrial beyond the breeding seasons. In addition, stream-living species are more vulnerable to habitat loss, human activities and other threats than terrestrial congeners (Jiang and Xie 2021a, b). The majority of these species are restricted to relatively small distribution areas and several species (*Bufo ailaoanus*, *B. aspinius*, *B. luchunnicus*, *B. menglianus*, *B. exiguus* and *B. yongdeensis*) are currently only known from their type localities, which are more vulnerable and careful attention should be paid to their conservation.

Acknowledgements

We are grateful to Jian Wang for his help in the field and the preparation of the manuscript; to Si-Yu Zhang for her help in molecular analysis; to Annemarie Ohler for her constructive comments on this manuscript. This work was supported by DFGP Project of Fauna of Guangdong-202115 and the National Animal Collection Resource Center, China.

References

Che J, Jiang K, Yan F, Zhang YP (2020) Amphibians and Reptiles in Tibet: Diversity and Evolution. Science Press, Beijing [in Chinese].

Dufresnes C, Litvinchuk SN (2022) Diversity, distribution and molecular species delimitation in frogs and toads from the Eastern Palaearctic. Zoological Journal of the Linnean Society 195: 695–760. https://doi.org/10.1093/zoolinnean/zlab083

Fei L, Hu SQ, Ye CY, Huang YZ (2009) Fauna Sinica. Amphibia Vol. 2 Anura. Science Press, Beijing [in Chinese].

Fei L, Ye CY, Jiang JP (2010) Colored Atlas of Chinese Amphibians. Sichuan Publishing House of Science & Technology, Chengdu [in Chinese].

Fei L, Ye CY, Jiang JP (2012) Colored Atlas of Chinese Amphibians and their Distributions. Sichuan Publishing House of Science & Technology, Chengdu [in Chinese].

Fei L, Ye CY (2016) Amphibians of China. Vol. 1. Science Press, Beijing [in Chinese].

Fong JJ, Yang BT, Li PP, Waldman B, Min MS (2020) Phylogenetic systematics of the water toad (*Bufo stejnegeri*) elucidates the evolution of semi-aquatic toad ecology and Pleistocene glacial refugia. Frontiers in Ecology and Evolution 7: 523. https://doi.org/10.3389/fevo.2019.00523

Frost DR (2023) Amphibian Species of the World: An Online Reference. Version 6.1. American Museum of Natural History, New York, USA. Available from: https://amphibiansoftheworld.amnh.org/index.php [accessed 16 May 2023].

Fu J, Weadick CJ, Zeng X, Wang Y, Liu Z, Zheng Y, Li C, Hu Y (2005) Phylogeographic analysis of the *Bufo gargarizans* species complex: A revisit. Molecular Phylogenetics and Evolution 37: 202–213. https://doi.org/10.1016/j.ympev.2005.03.023

Garsault FAP de (1764) Les figures des plantes et animaux d'usage en médecine, décrits dans la *Matière Médicale* de Mr. Geoffroy Médecin. Mrs. Defehrt, Prevost, Duflos, Martinet & Co., Paris, [i–v] + 1–20, pl. 644–729.

Huelsenbeck JP, Ronquist F, Nielsen R, Bollback JP (2001) Bayesian inference of phylogeny and its impact on evolutionary biology. Science 294: 2310–2314. https://doi.org/10.1126/science.1065889

Igawa T, Kurabayashi A, Nishioka M, Sumida M (2006) Molecular phylogenetic relationship of toads distributed in the Far East and Europe inferred from the nucleotide sequences of mitochondrial DNA genes. Molecular Phylogenetics and Evolution 38: 250–260. https://doi.org/10.1016/j.ympev.2005.09.003

Jeong TJ, Jun J, Han S, Kim HT, Oh K, Kwak M (2013) DNA barcode reference data for the Korean herpetofauna and their applications. Molecular Ecology Resources 13: 1019–1032. https://doi.org/10.1111/1755-0998.12055

Jiang JP, Xie F (2021a) China's Red List of Biodiversity: Vertebrates. Volume IV, Amphibians (I). In: Jing ZG (Ed.) China's Red List of Biodiversity: Vertebrates. Science Press, Beijing, 1–413.

Jiang JP, Xie F (2021b) China's Red List of Biodiversity: Vertebrates. Volume IV, Amphibians (II). In: Jing ZG (Ed.) China's Red List of Biodiversity: Vertebrates. Science Press, Beijing, 414–1052.

Kou Z (1984) Preliminary reports on the herpetofauna of Shuitang and Zhelong districts of the eastern slope of Mt. Ailao, with description of a new species. Acta Herpetologica Sinica 3: 39–45 [in Chinese].

Kuo CF (2009) Altitude and breeding site effects on morphology of *Bufo bankorensis*. Master Thesis, Graduate Institute of Forestry and Natural Resources, College of Agriculture, National Chiayi University Chiayi City [in Chinese].

Li ZC, Xiao Z, Liu SR (2011) Amphibians and Reptiles of Guangdong. Guangdong Science and Technology Press, Guangzhou [in Chinese].

Liedtke HC, Müller H, Rödel MO, Menegon M, Gonwouo LN, Barej MF, Gvoždík V, Schmitz A, Channing A, Nagel P, Loader SP (2016) No ecological opportunity signal on a continental scale? Diversification and life-history evolution of African true toads (Anura: Bufonidae). Evolution 70: 1717–1733. https://doi.org/10.1111/evo.12985

Litvinchuk SN, Borkin LJ, Skorinov LJ, Rosanov JM (2008) A new species of common toads from the Talysh Mountains of south-eastern Caucasus: Genome size, allozyme, and morphological evidences. Russian Journal of Herpetology 15: 19–43.

Liu CC, Hu SQ (1962) A herpetological report of Kwangsi. Acta Zoologica Sinica 14 (Supplement): 73–104.

Liu W, Lathrop A, Fu J, Yang D, Murphy RW (2000) Phylogeny of East Asian bufonids inferred from mitochondrial DNA sequences (Anura: Amphibia). Molecular Phylogenetics and Evolution 14: 423–435. https://doi.org/10.1006/mpev.1999.0716

Macey JR, Schulte II JA, Larson A, Fang Z, Wang Y, Tuniyev BS, Papenfuss TJ (1998) Phylogenetic relationships of toads in the *Bufo bufo* species group from the eastern escarpment of the Tibetan Plateau: A case of vicariance and dispersal. Molecular Phylogenetics and Evolution 9: 80–87. https://doi.org/10.1006/mpev.1997.0440

Matsui M (1976) A new toad from Japan. Contributions from the Biological Laboratory Kyoto University 25: 1–9.

Meyer CP, Geller JB, Paulay G (2005) Fine scale endemism on coral reefs: Archipelagic differentiation in turbinid gastropods. Evolution 59: 113–125. https://doi.org/10.1111/j.0014-3820.2005.tb00899.x

Nali RC, Zamudio KR, Haddad CF, Prado CP (2014) Size-dependent selective mechanisms on males and females and the evolution of sexual size dimorphism in frogs. The American Naturalist 184: 727–740. https://doi.org/10.1086/678455

Othman SN, Litvinchuk SN, Maslova I, Dahn H, Messenger KR, Andersen D, Jowers MJ, Kojima Y, Skorinov DV, Yasmiba K, Chuang MF, Chen YH, Bae Y, Hoti, Jang Y, Borzee A (2022) From Gondwana to the Yellow Sea, evolutionary diversifications of true toads *Bufo* sp. in the Eastern Palearctic and a revisit of species boundaries for Asian lineages. eLife 11: e70494. https://doi.org/10.7554/eLife.70494

Rao D (2022 "2020"). Atlas of Wildlife in Southwest China: Amphibians. In: Zhu JG (Ed.) Atlas of Wildlife in Southwest China. Beijing Press, Beijing, 1–448 [in Chinese].

Recuero E, Canestrelli D, Vörös J, Szabó K, Poyarkov NA, Arntzen JW, Crnobrnja-Isailovic J, Kidov AA, Cogălniceanu D, Caputo FP, Nascetti G, Martínez-Solano I (2012) Multilocus species tree analyses resolve the radiation of the widespread *Bufo bufo* species group (Anura, Bufonidae). Molecular Phylogenetics and Evolution 62: 71–86. https://doi.org/10.1016/j.ympev.2011.09.008

Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542. https://doi.org/10.1093/sysbio/sys029

Savage JM, Heyer WR (1997) Digital webbing formulae for anurans: A refinement. Herpetological Review 28: 131.

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P (1994) Evolution, weighting and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Annals of the Entomological Society of America 87: 651–701. https://doi.org/10.1093/aesa/87.6.651

Silvestro D, Michalak I (2011) RaxmlGUI: A graphical front-end for RAxML. Organisms Diversity & Evolution 12: 335–337. https://doi.org/10.1007/s13127-011-0056-0

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution 30: 2725–2729. https://doi.org/10.1093/molbev/mst197

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 25: 4876–4882.

Wilcox TP, Zwickl DJ, Heath TA., Hillis DM (2002) Phylogenetic relationships of the dwarf boas and a comparison of Bayesian and bootstrap measures of phylogenetic support. Molecular Phylogenetics and Evolution 25: 361–371. https://doi.org/10.1016/S1055-7903(02)00244-0

Yang DT, Liu WZ, Rao DQ (1996) A new toad genus of Bufonidae – *Torrentophryne* from Transhimalaya mountain of Yunnan of China with its biology. Zoological Research 17: 353–359.

Yang DT, Rao DQ (2008) Amphibia and Reptilia of Yunnan. Yunnan Science & Technology Press, Kunming [in Chinese].

Zhan A, Fu J (2011) Past and present: Phylogeography of the *Bufo gargarizans* species complex inferred from multi-loci allele sequence and frequency data. Molecular Phylogenetics and Evolution 61: 136–148. https://doi.org/10.1016/j.ympev.2011.06.009

Zhao WG (2008) The Amphibia and Reptilia Fauna of Heilongjiang. Science Press, Beijing [in Chinese].

Appendix

Specimens of genus *Bufo* examined in this study.

B. andrewsi (2): **China: Sichuan:** Mianyang: SYS a005394; **Yunnan:** Tengchong: Mt. Gaoligong: SYS a003828

B. bankorensis (2): **China: Taiwan:** Miaoli: Sanyi: SYS a003429; Natou: Sanxi: SYS a003430

B. cryptotympanicus (1): **China: Guangxi:** Xing'an, Mt. Maoer: SYS a006558 (juvenal)

B. gargarizans (12): **China: Guangdong:** Shixing: Chebaling NR: SYS a006728; Nanxiong: Mt. Qingzhang: SYS a008922, 8951; Lianzhou: Mt. Dadong: SYS a009075; **Hunan:** Guidong: Mt. Bamian: SYS a006192, 6193, 6249; **Zhejiang:** Fenghua: SYS a006785–6789

B. stejnegeri (1): **China: Liaoning:** Benxi: SYS a007664

B. tibetanus (5): **China: Tibet:** Bomê: SYS a006632, 8527–8530

B. tuberospinius (1): **China: Yunnan:** Tengchong, Mt. Gaoligong: SYS a003773, 3811