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Revalidated after having been described more than a century ago: *Calamaria berezowskii* Günther, 1896 (Squamata, Colubridae) from Sichuan, Southwestern China

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Abstract

The reed snakes of the genus *Calamaria* Boie, 1827 are one of the largest groups of Asian snakes, distributed from northeast India to the Maluku Islands of east Indonesia. Recent research on the genus in China has revealed that the species diversity of the group was underestimated. In this study, morphological comparisons and mitochondrial DNA analysis revealed that a junior synonym of *C. pavimentata* Duméril, Bibron & Duméril, 1854 — *Calamaria berezowskii* Günther, 1896 is valid, hence we redescribed and recovered the validity of *C. berezowskii*. This species can be distinguished from other congeners by the combination of the following characters: four supralabials; one preocular; rostral shield width larger than height; mental not touching anterior chin shields; eye diameter less than the distance from eye to mouth edge; less than 1/2 of the posterior chin shield meets in the midline; dorsal scales reduced to six rows at tail; indistinct light ring present in the nuchal region or a more or less distinct yellowish collar. Phylogenetically, this species is sister to *C. pavimentata*, with significant genetic differences (0.190) on mitochondrial gene Cyt *b*.

Key Words

Calamaria berezowskii, Colubridae, morphology, phylogenetics, taxonomy

Introduction

Calamaria Boie, 1827 is the largest group of the colubrid subfamily Calamariinae (Reed Snakes), containing more than 68 species (Uetz et al. 2023). In mainland Southeast Asia and China, 20 species of *Calamaria* are known at present, including *C. lumbricoidea* Boie, 1827; *C. albiventer* (Gray, 1834); *C. schlegeli* Duméril, Bibron & Duméril, 1854; *C. pavimentata* Duméril, Bibron & Duméril, 1854; *C. lovii* Boulenger, 1887; *C. septentrionalis* Boulenger, 1890; *C. prakkei* Lidth De Jeude, 1893; *C. buchi* Marx & Inger, 1955; *C. yunnanensis* Chernov, 1962 (Yeung et al. 2022; Uetz et al. 2023); *C. thanhi* Ziegler & Quyet, 2005; *C. gialaiensis* Ziegler, Nguyen & Nguyen, 2009; *C. sangi* Nguyen, Koch et Ziegler, 2009; *C. abramovi* Orlov, 2009; *C. concolor* Orlov, Nguyen, Nguyen, Ananjeva &

Ho, 2010; *C. andersoni* Yang & Zheng, 2018; *C. dominici* Ziegler, Tran & Nguyen, 2019; *C. strigiventris* Poyarkov, Nguyen, Orlov & Vogel, 2019; *C. nebulosa* Lee, 2021; *C. arcana* Yeung, Lau & Yang, 2022; *C. jinggangensis* Cai, Jiang, Wu, Huang, Fei & Ding, 2023.

There were only three species of genus *Calamaria* recorded in China 26 years ago: *C. pavimentata*, *C. septentrionalis* and *C. yunnanensis* (Zhao et al. 1998). Recently, three more species were described: *C. andersoni*, *C. arcana*, *C. jinggangensis* (Yang and Zheng 2018; Yeung et al. 2022; Cai et al. 2023; Uetz et al. 2023). The species diversity of the genus in China is suggested to be underestimated, and those widely distributed species should be re-evaluated. For example, the widely recorded species *C. pavimentata* has a synonym, *C. berezowskii* Günther 1896, which was described based on two specimens from Lun-ngan-fu (龙安府 Long'an Fu, now 龙安镇 Long'an Town of 平武县 Pingwu County) of Sze-chuen (Sichuan Province of China) (Günther 1896).

During the scientific expeditions in the Gongga Mountains, Luding County, Sichuan Province, between 2017 and 2022, we collected three *Calamaria* specimens. Morphological and molecular phylogenetic analyses showed that these specimens represent a species that differs from all currently recognized congeners of the genus. However, the morphology of these specimens matches the original description of *C. berezowskii*. Besides, Luding County and Pingwu County both are located on the eastern slope of Qinghai-Tibet Plateau bordering the Sichuan Basin. Therefore, we identify the specimens as *C. berezowskii*, and the species is revalidated and redescribed here.

Materials and methods

Sampling

Three specimens of *Calamaria* were collected from eastern slope of Mt. Gongga, Moxi Town, Luding County, Ganzi Tibetan Autonomous Prefecture, Sichuan Province, China during 2017 to 2022 (Fig. 1). One adult female GXNU DLR195 (29.645105°N, 102.111076°E, 1736 m a.s.l.), one adult male GXNU DLR194 (29.615872°N, 102.109208°E, 1680 m a.s.l.), one juvenile female GXNU 20221215002 (29.641749°N, 102.110340°E, 1827 m a.s.l.), collected by Xu Zhang on 31 August 2018, Li Ding on 21 August 2017 and Congcong Du on 15 December 2022. The three specimens were fixed and stored in 80% ethanol and deposited at the School of Life Sciences, Guangxi Normal University.

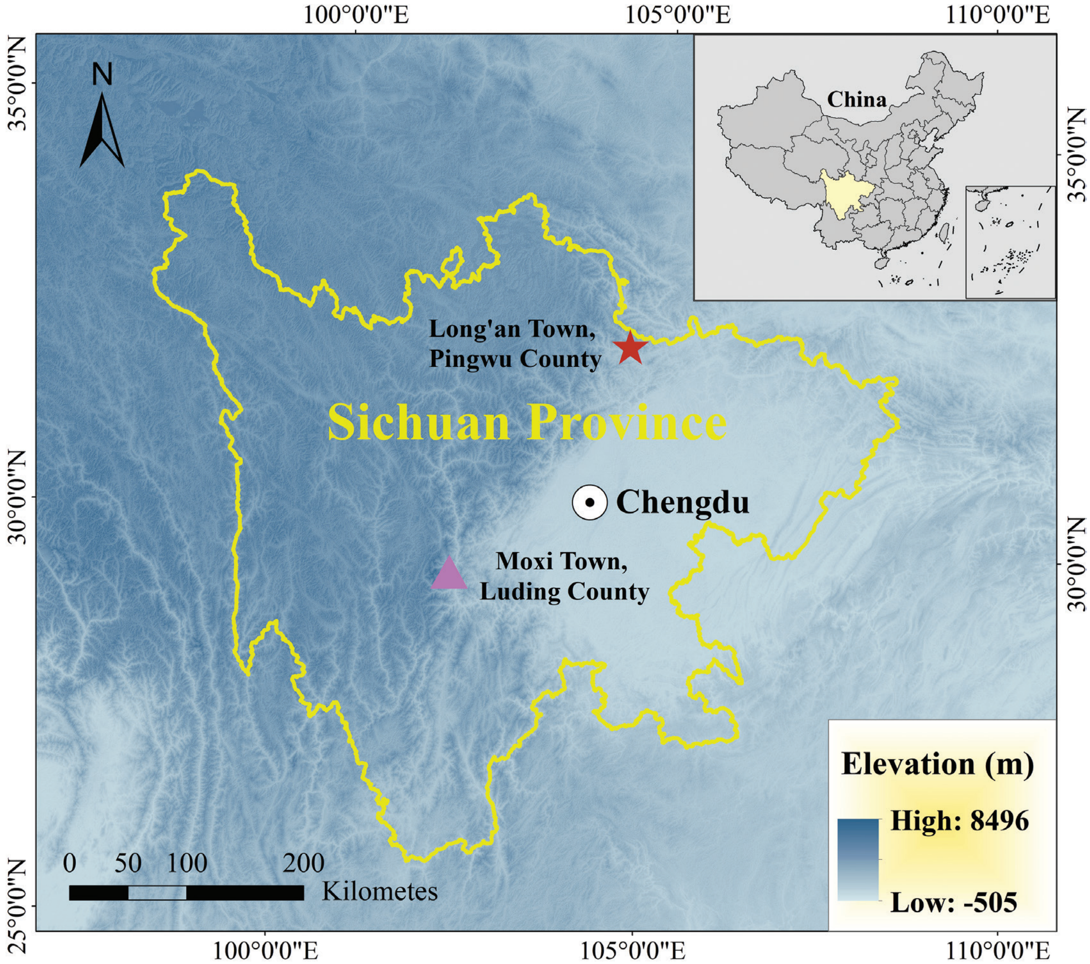


Figure 1. Distribution map of *Calamaria berezowskii.* Red star indicates the type locality, purple triangle indicates the distribution found in this study.

Molecular phylogenetic analysis

Genomic DNA was extracted from muscle or liver tissue taken from the collected specimens using the TIANamp Marine Animals DNA Kit (TIANGEN Biotech). We sequenced a fragment of the mitochondrial gene cytochrome *b* (Cyt *b*) using the primer pair L14910/H16064 (Burbrink et al. 2000). PCR amplification was performed in 25 μl reactions containing 12.5 μl 2× SanTaq PCR Master Mix (with Blue Dye), 10 μl ddH$_2$O, 1 μl F-primers, 1 μl R-primers and 0.5 μl DNA template. The PCR conditions were an initial denaturing step at 94 °C for 5 min, followed by 35 cycles: denaturing at 94 °C for 30 s, annealing at 48.5 °C for 45 s, an extension step at 72 °C for 35 s; and a final extension of 72 °C for 10 min. Finally, the PCR products were sent to Sangon Biotechnologies Co., Ltd. (Shanghai, China), where the purified PCR products were sequenced using the same forward and reverse primers. Sequences for comparison of available species were downloaded from GenBank (Table 1).

We evaluated and manually corrected the sequencing peak maps of the three sequences obtained, downloaded 29 Cyt *b* sequences of 12 species of the genus *Calamaria* that have been reported in Southeast Asia and China in GenBank, and selected three species *Elaphe quatuorlineata*, *Lycodon rufozonatus* and *Orientocoluber spinalis* as outgroups (Yang and Zheng 2018; Yeung et al. 2022), for a total of 35 sequences to be used for multiple sequence comparisons. All sequences were aligned with other retrieved sequences in the same gene loci by using software MEGA 11 (Kumar et al. 2018). Phylogenetic trees were constructed based on mitochondrial gene Cyt *b* using maximum likelihood (ML) and Bayesian inference (BI). Maximum likelihood analysis was conducted in RAxML v8.2.4 (Stamatakis 2014). Confidence intervals were determined with 1000 bootstrap replicates utilizing the rapid bootstrap option under the GTR+GAMMA substitution model. Bayesian inference analysis was conducted in MrBayes 3.2 (Ronquist et al. 2012). PartitionFinder 2.1.1 software was used to select the most suitable nucleotide substitution models for Cyt *b* sequence data based on the Bayesian information criterion (BIC): GTR+G, HKY+G and GTR+G (Lanfear et al. 2012; Lanfear et al. 2017). We ran our analyses for 20 million generations with the chains, sampling every 1000 generations using the Markov Chain Monte Carlo (MCMC). After removing outgroup taxa, MEGA11 (Kumar et al. 2018) was used to calculate uncorrected pairwise sequence divergence between the *Calamaria* species.

Table 1. DNA sequences used in this study.

No.	Taxa	Voucher	Locality	Cyt *b*	Reference
1	*C. septentrionalis*	HSR19100	Mt. Huangshan, Anhui, China	OQ354842	Cai et al. 2023
2	*C. septentrionalis*	HS11145	Mt. Nanling, Guangdong, China	OQ354840	Cai et al. 2023
3	*C. septentrionalis*	DL2021610-1	Huangsha, Guangxi, China	OQ354838	Cai et al. 2023
4	*C. septentrionalis*	KFBG14506	Hainan Island, China	MH445956	Yang and Zheng 2018
5	*C. septentrionalis*	ROM35605	Nguyên Bình, CaoBang, Vietnam	AF471081	Lawson et al. 2005
6	*C. septentrionalis*	ROM35597	Cao Bang, Vietnam	KX694890	Alencar et al. 2016
7	*C. pavimentata*	KFBG14507	Ningming, Guangxi, China	MH445957	Yang and Zheng 2018
8	*C. andersoni*	HSR20101	Dehong, Yunnan, China	OQ354844	Cai et al. 2023
9	*C. andersoni*	HSR20181	Tengchong, Yunnan, China	OQ354845	Cai et al. 2023
10	*C. andersoni*	SYSr001699	Yingjiang, Yunnan, China	MH445955	Yang and Zheng 2018
11	*C. yunnanensis*	ROM41547	Simao, Yunnan, China	KX694891	Zaher et al. 2009
12	*C. yunnanensis*	YPx503	Unknown	JQ598922	Grazziotin et al. 2012
13	*C. arcana*	HS17082	Mt. Dawu, Guangdong, China	OQ354835	Cai et al. 2023
14	*C. arcana*	KFBG14611	Mt. Dadongshan, Guangdong, China	ON482335	Yeung et al. 2022
15	*C. arcana*	GP9975	Yongxing, Hunan, China	OP980549	Cai et al. 2023
16	*C. arcana*	DLR199	Mt. Wuyi, Fujian, China	OQ354834	Cai et al. 2023
17	*C. jinggangensis*	DL20200725	Mt. Jinggangshan, Jiangxi, China	OQ354830	Cai et al. 2023
18	*C. jinggangensis*	DL20200625-2	Mt. Jinggangshan, Jiangxi, China	OQ354831	Cai et al. 2023
19	*C. jinggangensis*	DL20200625-3	Mt. Jinggangshan, Jiangxi, China	OQ354832	Cai et al. 2023
20	*C. jinggangensis*	DL20200625-4	Mt. Jinggangshan, Jiangxi, China	OQ354833	Cai et al. 2023
21	*C. muelleri*	RMB1283	Gowa, South Sulawesi, Indonesia	MT819391	Weinell et al. 2021
22	*C. muelleri*	TNHC58955	Gowa, South Sulawesi, Indonesia	MT819390	Weinell et al. 2021
23	*C. lumbricoidea*	USMHC1560	Penang, Peninsular, Malaysia	MN338526	Quah et al. 2019
24	*C. palavanensis*	KU311411	Mt. Mantalingahan, Palawan, Philippine	MT819387	Weinell et al. 2021
25	*C. palavanensis*	KU309445	Barangay Irawan, Palawan, Philippine	MT819386	Weinell et al. 2021
26	*C. gervaisii*	KU334485	Municipality, Ilocos Sur, Philippines	MT819385	Weinell et al. 2021
27	*C. gervaisii*	KU324661	Puguis, Benguet, Philippines	MT819384	Weinell et al. 2021
28	*C. schlegeli*	LSUHC10278	Perak, Peninsular, Malaysia	MN338525	Quah et al. 2019
29	*C. nebulosa*	FMNH258666	Phongsaly, Laos	MN338524	Quah et al. 2019
30	*C. berezowskii*	GXNU DLR194	Mt. Gongga, Sichuan, China	PP747047	This study
31	*C. berezowskii*	GXNU DLR195	Mt. Gongga, Sichuan, China	PP747048	This study
32	*C. berezowskii*	GXNU 20221215002	Mt. Gongga, Sichuan, China	PP747049	This study
33	*Elaphe quatuorlineata*	LSUMZ40626	Turkey, European Turkey	AY486931	Nagy et al. 2004
34	*Lycodon rufozonatus*	LSUMZ44977	Unknown	AF471063	Lawson et al. 2005
35	*Orientocoluber spinalis*	MVZ211019	Yinnan, Ningxia, China	AY486924	Nagy et al. 2004

Morphological analysis

Terminology and descriptions follow the views of Inger and Marx (1965) and Ziegler et al. (2008). Body and tail length were measured with a tape ruler to the nearest 1 mm: total length (**TL**), from the tip of snout to the tip of tail; snout-vent length (**SVL**), from the tip of snout to posterior margin of cloaca; tail length (**TaL**), from posterior margin of cloaca to the tip of tail. Other measurements were conducted with a digital caliper to the nearest 0.1 mm: head length (**HL**), from the snout tip to the posterior margin of the mandible; head width (**HW**), measured at the widest part of the head on posterior side; head height (**HH**), at the maximal highest part of the head; the eye horizontal diameter (**EyeD**); and eye-mouth distance (**Eye–Mouth D**), measured from the anterior point of the eye to the mouth gap. Ventral scales (**VEN**) were counted according to Dowling (1951). The enlarged shield(s) anterior to the first ventral were regarded as preventral(s). The number of dorsal scale rows (**DSR**) are given at one head length behind head, at midbody, and at one head length before vent, respectively. The tail tip was not included in the number of subcaudal scales (**SC**). Sex was determined by examining the presence or absence of hemipenis.

Type specimen (lectotype ZISP 8823) was examined for morphological comparisons. Relevant morphological data of other *Calamaria* species were obtained from examined specimens (Appendix 1) and literatures.

Institution acronyms

FMNH: Field Museum of Natural History, Chicago. **GXNU**: Guangxi Normal University. **KFBG**: Kadoorie Farm and Botanic Garden. **KU**: University of Kansas Biodiversity Institute. **LSUHC**: La Sierra University Herpetological Collections. **LSUMZ**: Louisiana State University Museum of Natural Science. **MVZ**: Museum of Vertebrate Zoology. **RMB**: Rafe M. Brown field tag (specimen deposited in Museum Zoologicum Bogoriense, Indonesia). **ROM**: Royal Ontario Museum, Canada. **SYS**: Biological Museum of Sun Yat-sen University, Guangzhou, China. **TNHC**: Texas Natural History Collections. **USMHC**: Universiti Sains Malaysia Herpetological Collection, Malaysia. **ZFMK**: Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany. **ZISP**: Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia.

Molecular results

The DNA dataset contains 35 samples with a total of 1105 base pairs. The phylogenetic topologies that resulted from Bayesian Inference (BI) and Maximum likelihood analysis (ML) are generally consistent in phylogenetic structure (Fig. 2). Phylogenetically, the species *Calamaria* from Mt. Gongga strongly clustered into a single lineage with high nodal support (Fig. 2, BPP 1.00 / UFB 100). Subsequently, it clustered with *C. pavimentata* KFBG14507 from Ningming, Guangxi (BPP 0.96 / UFB 94), and was clustered into the same clade with *C. arcana*, *C. jinggangensis*, *C. septentrionalis*, *C. pavimentata*, *C. andersoni* and *C. yunnanensis*.

The uncorrected pairwise *p*-distances of Cyt *b* sequences between the specimen of Mt. Gongga and the other 12 congeners included in the study were 17.1–31.2%, with the minimum value observed in the comparison with sequences of *C. arcana* (P = 17.1%) (Table 2). This divergence is clearly among interspecies level since these levels of divergences are distinctly higher than those observed between two other well distinguished species of *C. andersoni* and *C. yunananensis* (P = 12.2–12.6%). The

Table 2. Uncorrected *p*-distances between *Calamaria* species based on 1105 base pairs from the mitochondrial genes Cyt *b*. The serial numbers in Table 2 are consistent with those in Table 1.

No.	Taxa	1–6	7	8–10	11–12	13–16	17–20	21–22	23	24–25	26–27	28	29	30–32
1–6	*C. septentrionalis*	0.000–0.038												
7	*C. pavimentata*	0.174–0.194												
8–10	*C. andersoni*	0.130–0.148	0.246	0–0.019										
11–12	*C. yunnanensis*	0.159–0.164	0.237	0.122–0.126	0.000									
13–16	*C. arcana*	0.090–0.116	0.179–0.199	0.181–0.206	0.183–0.209	0.007–0.034								
17–20	*C. jinggangensis*	0.095–0.103	0.180	0.161–0.171	0.158	0.062–0.074	0.000							
21–22	*C. muelleri*	0.202–0.223	0.266	0.228–0.238	0.261–0.273	0.178–0.198	0.169–0.178	0.007						
23	*C. lumbricoidea*	0.243–0.248	0.258	0.244–0.256	0.251	0.228–0.239	0.228	0.164						
24–25	*C. palavanensis*	0.187–0.222	0.231–0.242	0.227–0.243	0.254–0.266	0.207–0.229	0.173–0.192	0.167–0.182	0.166–0.181	0.022				
26–27	*C. gervaisii*	0.178–0.207	0.243–0.258	0.202–0.212	0.243–0.244	0.197–0.223	0.159–0.163	0.173–0.183	0.197–0.223	0.112–0.174	0.100			
28	*C. schlegeli*	0.232–0.243	0.263	0.258	0.271	0.217–0.228	0.217	0.182–0.192	0.205	0.170–0.175	0.186–0.218			
29	*C. nebulosa*	0.186–0.196	0.214	0.172	0.173	0.162–0.182	0.176	0.208–0.219	0.193	0.202–0.207	0.187–0.234	0.197		
30–32	*C. berezowskii*	**0.187–0.203**	**0.190**	**0.216–0.226**	**0.208**	**0.171–0.200**	**0.202**	**0.223–0.234**	**0.255**	**0.254–0.277**	**0.254–0.271**	**0.312**	**0.176**	**0.000**

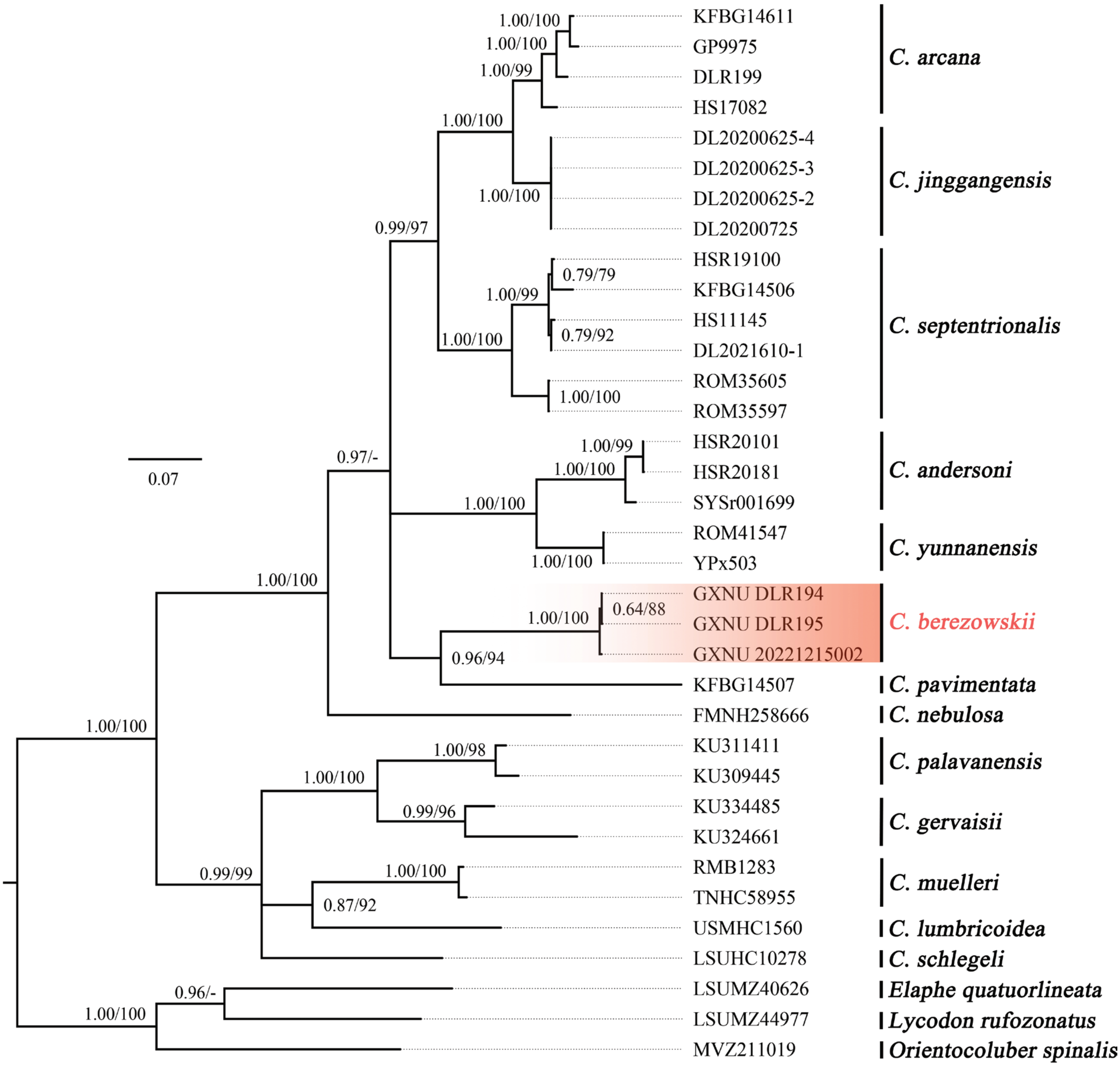


Figure 2. Phylogenetic relationships of *Calamaria* species based on Cyt *b*. Bayesian posterior probabilities (BPP) and Ultrafast bootstrap approximation (UFB) were denoted beside each node (those lower than 75/0.7 were denoted as "-").

Calamaria specimens from Mt. Gongga, Luding, Sichuan represent an evolutionary distinct lineage.

Morphological results

Morphology of the three *Calamaria* specimens from Luding County, Sichuan province matches the original description of *C. berezowskii* Günther 1896 and the morphological characters of lectotype ZISP 8823 (Figs 3, 4, 5, 6). They share the following characters: one preocular and one postocular; presence of a more or less distinct yellowish collar; similar number of ventral scales (149–165 vs 155–167) and subcaudal scales (16–25 vs 14–22); presence of a faint dark line on each side of the back; tail blunt and conical at the end. Besides, Luding County and the type locality Pingwu County are both located on the eastern slope of Qinghai-Tibet Plateau bordering the Sichuan Basin, with a geographic distance of about 350 km (Fig. 1). Hence, the Luding specimens were identified as conspecific with *C. berezowskii*.

Morphological comparisons based on some characters between *Calamaria berezowskii* and other known species of the genus from mainland Southeast Asia and China were listed in Table 3. It can be distinguished by a combination of the following characters: four supralabials; preocular present; rostral shield width greater than height; mental not touching anterior chin shields; eye diameter less than distance from eye to mouth edge; less than 1/2 of the posterior chin shield meets in the midline; dorsal scales reduced to six rows at tail; presence of indistinct light rings in the nuchal region or a more or less distinct yellowish collar; and the two outermost dorsal scale rows light khaki, with upper margins partly dark pigmented.

Table 3. Morphological comparisons of species of *Calamaria* from mainland Southeast Asia and China. "1+***" indicates preventrals + ventrals. Entries marked with '–' are not available.

Species	Comparison between rostral height and width	Comparison between eye diameter and eye-mouth distance	Number of scales surrounding paraparietal	Preocular: present (1) or absent (0)	Supralabials	Mental touching (1) or separated from (0) anterior chin shields	Posterior chin shields meeting in midline (1), diverging or meeting only in anteriorly (0)	End of tail	Modified maxillary teeth	Ventrals		Subcaudals		Dorsal scales reducing to four rows above position of subcaudal on tail	Total length	
										Male	Female	Male	Female		Male	Female
C. berezowskii	H < W	<	6	1	4	0	1	Obtuse point	–	2+149–155	153–2+165	22–25	16	Not reduced	248–290	123–305
C. pavimentata	H ≥ W	≥	5–6	1	–	0	1	Sharp point	8–9	125–168	137–206	3–33	8–20	Last–13th subcaudal	84–313	115–485
C. septentrionalis	H < W	≥	6	1	–	1	1	Broadly rounded	8–9	148–166	168–188	15–19	6–11	Not reduced	111–344	117–384
C. yunnanensis	H < W	–	6	0	4	0	–	Obtuse point	8–9	167–184	199	15–20	19	3rd–last subcaudal	245–300	516
C. andersoni	H < W	>	6	1	4	0	0	Obtuse point	9	171	–	23	–	2nd–last subcaudal	351.4	–
C. arcana	H < W	>	6	1	4	0	1	Obtuse point	10	170–176	192	20–22	12	Not reduced	144–303.2	36.5
C. jinggangensis	H < W	>	6	1	4	0	1	Obtuse point	9	159–160	179	20–22	12–14	Not reduced	314–353+	329–364
C. lumbricoidea	H < W / H >W	≥	4–5	1	5	1	1	Sharp point	9–11	144–196	137–229	17–27	13–21	Last–11th subcaudal	149–498	120–642
C. albiventer	H < W	>	5	1	5	1	1	Sharp point	9	143–144	147–162	21–22	15–19	5th–8th subcaudal	205	170–361
C. schlegeli	–	–	5–6	0–1	5	0	0–1	Blunt	9–10	129–161	136–180	25–44	19–37	3rd–25th subcaudal	–	
C. lovii gimletii	–	–	6	0	–	0	1	Blunt	8–9	161–202	215–249	14–20	10–12	Last–5th subcaudal	–	
C. prakkei	H = W	>	5	1	5	1	1	Sharp point	7–8	126–132	142–144	31–32	24–25	7th–15th subcaudal	172–245	230–256
C. buchi	H > W	≤	5	1	5	1	1	Obtuse point	9	–	221–236	–	13–14	3rd–4th subcaudal	–	389–466
C. thanhi	H < W	>	6–7	0	4	0	0	Gradually to a point	9	184	198	28	21	Not reduced	461	455
C. gialaiensis	H < W	>	5	1	4	1	0	Rounded	9	3+191	–	23	–	Last subcaudal	457	–
C. sangi	H < W	>	5–6	1	4	1	0	Obtuse point	9	2+190		19		Last–3rd subcaudal	373.3	
C. abramovi	H = W	<	6	1	4	0	0	Sharp point	8	159	174	26	20	Last subcaudal	–	482
C. concolor	H < W	<	5	1	5	1	1	Obtuse point	8	3+209	–	19	–	Last subcaudal	578	–
C. dominici	H < W	>	6	1	4–5	1	0	Obtuse point	9	–	1+174	–	17–18	5th–6th subcaudal	–	
C. strigiventris	H < W	<	6	1	4	0	0	Abruptly to point	9–11	130–157	158–180	29–33	20–30	Last–6th subcaudal	–	
C. nebulosa	H < W	>	6	0	4	0	0	Obtuse point	9	–	3+179	–	22	Last subcaudal	–	354

Taxonomy

Calamaria berezowskii Günther, 1896

Figs 3, 4, 5, 6

Calamaria pavimentata — populations in Sichuan of Zhao et al. (1998) and Zhao (2006); Zhao (2003). Synonym.

Description of lectotype ZISP 8823. Adult male, collected from Lun-ngan-fu (龙安府 Long'an Fu, now 龙安镇 Long'an Town of 平武县 Pingwu County) of Szechuen (Sichuan Province of China) (Günther 1896).

HL: 8.0 mm, 3.0% of SVL; HW: 5.0 mm, HW/HL: 62.5%; HH: 4.0 mm, HH/HL: 50.0%; EyeD: 0.7 mm, larger than eye-mouth distance 1.0 mm; EyeD 8.8% of HL.

SVL: 271 mm; TaL: 19 mm; TL: 290 mm; Tal/TL: 6.6%.

DSR: 13–13–13 scales; VEN: 155; SC: 22, divided, followed by a shield covering tail tip; anal scale single.

Rostral as broad as high; pupil rounded; preocular 1/1 (left/right, hereafter); postocular 1/1; supralabials 4/4, the second and third supralabials entering orbit, the fourth longest; infralabials 5/5. Visible yellowish collar on left and right sides of the neck present. Dorsal color brown, with a faint dark line along each side of the back; ventral surface uniform white. Tail rather obtuse, with a conical end. 2–3 pairs light color spots at the base of the tail.

Description of referred specimen GXNU DLR195. Adult female, collected from Mt. Gongga, Moxi Town, Luding County, Ganzi Tibetan Autonomous Prefecture, Sichuan Province, China (29.645105°N, 102.111076°E, 1736 m a.s.l.) collected by Xu Zhang on 31 August 2018.

Body elongated, cylindrical; head small, not distinct from nape; tail short, similar in form to head; tail not flattened, tapering and bluntly pointed at tip.

HL: 8.3 mm, 2.9% of SVL; HW: 3.9 mm, HW/HL: 47.0%; HH: 4.0 mm, HH/HL: 48.2%; EyeD: 0.7 mm, larger than eye-mouth distance 1.1 mm; EyeD 8.4% of HL.

SVL: 288 mm; TaL: 17 mm; TL: 305 mm; Tal/TL: 5.6%. Body thickness about 4.70 to 6.71 mm; base of tail 3.18 mm thick.

Table 3. Continued.

Species	TaL / TL ratio (given as %)		Coloration of dorsum	Coloration of venter	Dorsal scales with light spots present (1) or absent (0)	Dorsum with light nuchal collar (1) or absent (0)	Tail with light rings: present (1) or absent (0)	References
	Male	Female						
C. berezowskii	6.6–10.5	5.6–6.5	Blackish-brown or brown	Light khaki or white	0	1	0–1	Günther 1896; this study
C. pavimentata	6.9–16.9	3.7–8.5	Brown	Yellow	0	0–1	0–1	Inger and Marx 1965; Ziegler et al. 2008; Nguyen et al. 2009
C. septentrionalis	6.3–8.6	2.6–4.3	Dark brown to black	Yellow	1	1	1	Inger and Marx 1965; Ziegler et al. 2008; Poyarkov et al. 2019
C. yunnanensis	5.4–8.2	5	Bluish-grey or olive-brown	Red or yellow	0	0–1	1	Chernov 1962; Stuart and Heatwole 2008; Yang and Rao 2008; Lee 2021
C. andersoni	9.2	–	Brownish	Yellow	1	0	0	Yang and Zheng 2018
C. arcana	7.2–11.8	4.7	Grey-brown with somewhat iridescent (in life) or caramel-brown (in alcohol)	Orangish-red (in life) or light yellowish-beige (in alcohol)	0	0	1	Yeung et al. 2022; Zhang et al. 2023; Cai et al. 2023
C. jinggangensis	7.1–10.1	3.6–4.6	Brownish black with iridescent (in life) or brownish black (in alcohol)	Dark orange (in life) or light khaki (in alcohol)	0	0	0–1	Cai et al. 2023; this study
C. lumbricoidea	6.3–11.4	3.9–8.3	Dark brown to black	Yellow	0–1	0	0–1	Inger and Marx 1965; Weinell et al. 2021; Lee 2021
C. albiventer	8.8–9.3	4.7–8.8	Brown	Red	1	1	0	Inger and Marx 1965; Wallach et al. 2014; Lee 2021
C. schlegeli	11.1–21.3	7.3–14.4	Dark brown to black	White or yellow	0	0	0–1	Inger and Max 1965; Quah et al. 2018; Weinell et al. 2021
C. lovii gimletii	6.2–8.4	3.0–3.7	Dark brown	Yellow	0	0–1	0–1	Inger and Marx 1965; Quah et al. 2018; Grismer et al. 2004; Lee 2021
C. prakkei	16.5–16.7	9.6–10.5	Brown	Yellow	1	1	–	Inger and Marx 1965; Wallach et al. 2014
C. buchi	–	3.9–4.1	Black	Yellow	1	0	0	Inger and Marx 1965; Ziegler er al. 2008; Nguyen et al. 2009
C. thanhi	9.9	6.8	Dark blue to grey	Yellow	0	0	1	Ziegler and Quyet 2005; Ziegler et al. 2007; Ziegler et al. 2008; Nguyen et al. 2009; Wallach et al. 2014
C. gialaiensis	8.1	–	Grey-brown	Yellow	0	0	1	Ziegler et al. 2008; Wallach et al. 2014; Lee 2021
C. sangi	6.2		Greyish-brown	yellow	0	1	0	Nguyen et al. 2009; Wallach et al. 2014
C. abramovi	13.3	7.1	Black	Black and yellow	1	0	0	Orlov 2009; Wallach et al. 2014
C. concolor	7.3	–	Brown	Cream	0	0	0	Orlov et al. 2010; Wallach et al. 2014
C. dominici	_	6.2	Black	Yellow and black	0	0	0	Ziegler et al. 2019
C. strigiventris	13.8–17.9	8.4–11.5	Slate grey to grey-brown	Yellow and black	0	0	0	Poyarkov et al. 2019
C. nebulosa	–	7.9	Bluish-grey	Yellow	1	0	0	Lee 2021

VEN: 165 (+2 preventrals); SC: 16, all paired; anal shield entire, ventral scales immaculate.

DSR: 13–13–13 scales, dorsal scales smooth and immaculate.

Rostral shield width (2.22 mm) is larger than height (1.62 mm), internasals and prefrontals fused 2 scales; prefrontal length (2.28 mm) is less than frontal length (2.61 mm), not entering orbit, and touching first two supralabials; frontal hexagonal, longer (2.61 mm) than wide (2.50 mm); six paraparietals; parietal scales long, tangent to supraocular, postocular scales, supralabials; one preocular present; parietal broadly in contact with the last supralabials; pupil rounded; supralabials 4/4, second and third entering orbit, the fourth largest (length 2.34/2.32 mm) and tangent to the postoculars; mental not touching anterior chin shields; infralabials 5/5, first three touching anterior chin shields; anterior chin shields are slightly longer than posterior chin shields, presence of mental groove; less than 1/2 of the posterior chin shield meets in the midline; dorsal scales reduced to 6 rows above last subcaudal at tail; anal scale is complete and single.

Coloration in life. The dorsal color was blackish brown, with a faint dark line along each side of the back which is about 3 scales wide apart; the outermost corners of the ventral scales were brownish, and the ventral surface was lighter; presence of distinct yellowish collar; absence of light ring at the base of the tail.

Coloration in preservative. The specimen was preserved in alcohol. Dorsal body blackish brown, ventral surface light khaki. Dorsal head and neck coincide with the dorsal body, with a pair of light spots on each side of the neck and on the back of head; dorsum without distinct blotches; venter immaculate, without any dark stripes or scattered spots; ventral surface of tail with a dark longitudinal stripe and blotches; ventral scales with dark outermost corners.

Variations for population from Mt. Gongga, Luding County. Measurements of other specimens are given in Table 4. Male with relatively longer tail (TaL/TL are distinctly larger in the adult male). Adult female GXNU DLR194 displays many scattered brown spots on the venter, and with distinct light-yellow blotches on the left and right sides of the neck, but in adult male GXNU DLR195 the venter is immaculate without any dark spots. The population of Mt. Gongga, Luding County differs slightly from the type specimen in dorsal and ventral coloration (dorsal body blackish brown vs brown, ventral surface light khaki vs white), and differs from the lectotype ZISP 8823 in color spots at the base of the tail (absent vs present) (Figs 3, 4, 5).

Table 4. Main morphological characters of *Calamaria berezowskii*. Abbreviations are listed in the Materials and Methods. "–" indicates missing data. Data of lectotype and syntype of *C. berezowskii* by Günther 1896 and Nikolai Orlov.

Voucher No.	ZISP 8823	–	GXNU DLR194	GXNU DLR195	GXNU 20221215002
Type of specimen	Lectotype	Syntype	–	–	–
Sex	♂	–	♂	♀	♀
Ontogenetic	Adult	Adult	Adult	Adult	Juvenile
Preocular	1/1	1	1/1	1/1	–
Postocular	1/1	1	1/1	1/1	–
Supralabials	4/4	4	4/4	4/4	4/4
Infralabials	5/5	–	5/5	5/5	5/5
Dorsals	13–13–13	–	13–13–13	13–13–13	13–13–13
Ventrals	155	167	2+149	2+165	153
Subcaudals	22	14	25	16	16
Tailspot	2+1	–	Absent	Absent	Absent
TL (mm)	290	245	248	305	123
SVL (mm)	271	220	222	288	115
TaL (mm)	19	25	26	17	8
TaL/TL	0.066	0.102	0.105	0.056	0.065
HL (mm)	8.0	7.0	8.1	8.3	–
HW (mm)	5.0	–	4.1	3.9	–
HH (mm)	4.0	–	3.5	4.0	–
HW/HL	0.625	–	0.506	0.470	–
HH/HL	0.500	–	0.432	0.482	–
EyeD (mm)	0.7	–	0.6	0.7	–
Eye-MouthD (mm)	1.0	–	0.9	1.1	–

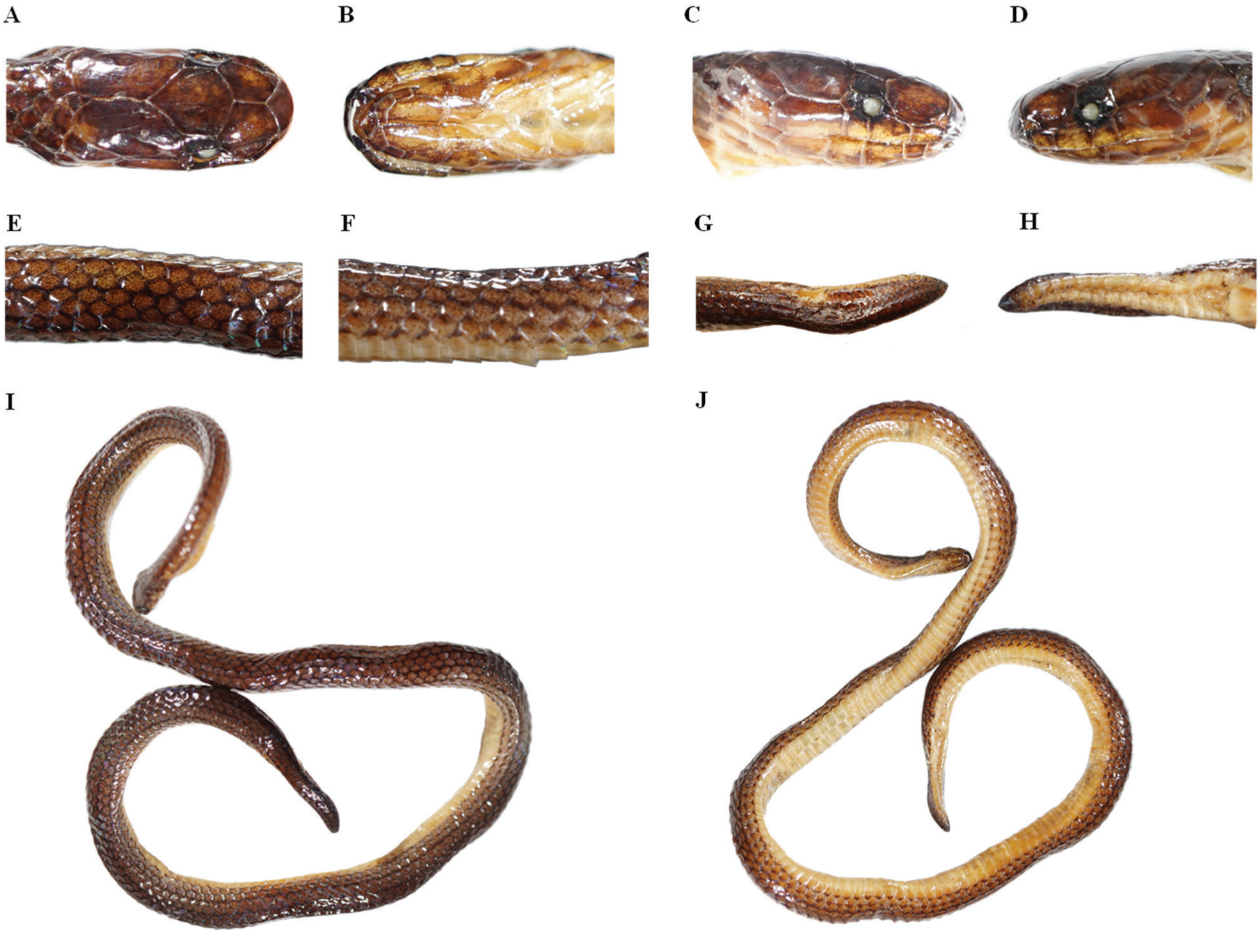


Figure 3. *Calamaria berezowskii* (GNXU DLR195): **A.** Dorsal head; **B.** Ventral head; **C.** Right view of head; **D.** Left view of head; **E.** Dorsal middle body; **F.** Lateral middle body; **G.** Dorsal tail; **H.** Ventral tail; **I.** Dorsal view; **J.** Ventral view.

Detailed morphological comparisons. Detailed morphological comparisons between *Calamaria berezowskii* and 20 congeners of the genus from China and mainland Southeast Asia are:

Calamaria berezowskii is distinguished from *C. albiventer*, *C. lumbricoidea*, *C. prakkei*, *C. schlegeli*, and *C. concolor* by having fewer supralabials (4 vs 5–6), and the 2nd and 3rd supralabials shields touching the orbit (vs.

Figure 4. *Calamaria berezowskii* (GXNU DLR194) in life.

3rd and 4th supralabials touching orbit), mental not touching anterior chin shields (vs. touching in *C. albiventer*, *C. lumbricoidea*, *C. prakkei* and *C. concolor*).

Calamaria berezowskii is distinguished with *C. lovii*, *C. nebulosa*, *C. thanhi* and *C. yunnanensis* by having presence of preocular scale (vs. absence of preocular scale), eye diameter less than distance from eye to mouth edge (vs. reverse condition in *C. thanhi* and *C. nebulosa*), fewer ventral scales in males (149–155 vs 161–202 in *C. lovii*, 149–155 vs 184 in *C. thanhi* and 149–155 vs 167–184 in *C. yunnanensis*), more subcaudal scale in males (22–25 vs 14–20 in *C. lovii*, 22–25 vs 15–20 in *C. yunnanensis*).

Calamaria berezowskii differs from *C. sangi*, *C. gialaiensis* and *C. buchi* by having mental not touching anterior chin shields (vs. touching), dorsal scales reduced to six rows at tail (vs. reduced to four rows), fewer ventral scales (149–167 vs 190 in *C. sangi*, 149–167 vs 191 in *C. gialaiensis*, 149–167 vs 221–236 in *C. buchi*).

Calamaria berezowskii is distinct from *C. arcana* and *C. jinggangensis* by having the eye diameter less than the distance from the eye to mouth edge (vs. reverse condition in *C. arcana* and *C. jinggangensis*), fewer ventral scales (149–167 vs 170–192 in *C. arcana* and *C. jinggangensis*), more subcaudals in females (16 vs 12–14 in *C. arcana* and *C. jinggangensis*), a distinctly different coloration (dark orange or orangish-red in fresh specimens in *C. arcana* and *C. jinggangensis*), presence of dark outermost corners on ventral scales (vs. absence in *C. arcana* and *C. jinggangensis*), a faint dark line along each side of the back present (vs. opposite condition in *C. arcana* and *C. jinggangensis*), a dark longitudinal line or scattered spots on the underside of tail present (vs. absent in *C. arcana* and *C. jinggangensis*).

Calamaria berezowskii differs from *C. abramovi* by having the rostral wider than high (vs. width equal to high in *C. abramovi*), fewer ventral scales in males (149–155 vs 159 in *C. abramovi*), dorsal scales reduced to six rows at tail (vs. reduced to four rows in *C. abramovi*), tail ends in obtuse point (vs. sharp point in the end of tail in *C. abramovi*), and a distinctly different coloration (body black with yellow-orange spots on venter in *C. abramovi*).

Calamaria berezowskii differs from *C. andersoni* by having eye diameter smaller than distance from eye to mouth edge (vs. reverse condition in *C. andersoni*), fewer ventral scales in males (149–155 vs 171 in *C. andersoni*), dorsal scales reduced to six rows at tail (vs. reduced to four rows in *C. andersoni*), light blotches on neck present (vs. absent in *C. andersoni*).

Calamaria berezowskii differs from *C. septentrionalis* by having eye diameter less than distance from eye to mouth edge (vs. reverse condition in *C. septentrionalis*),

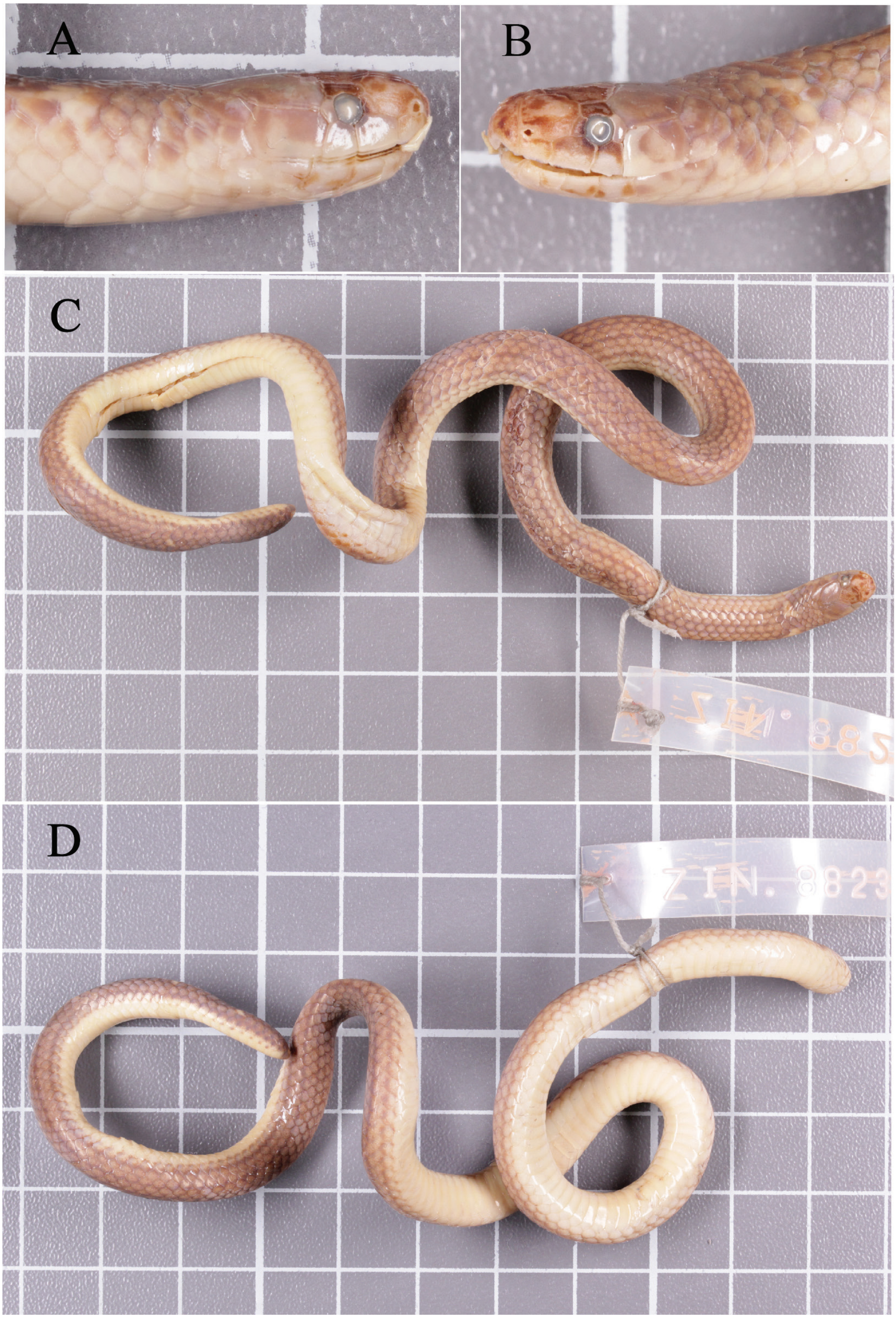
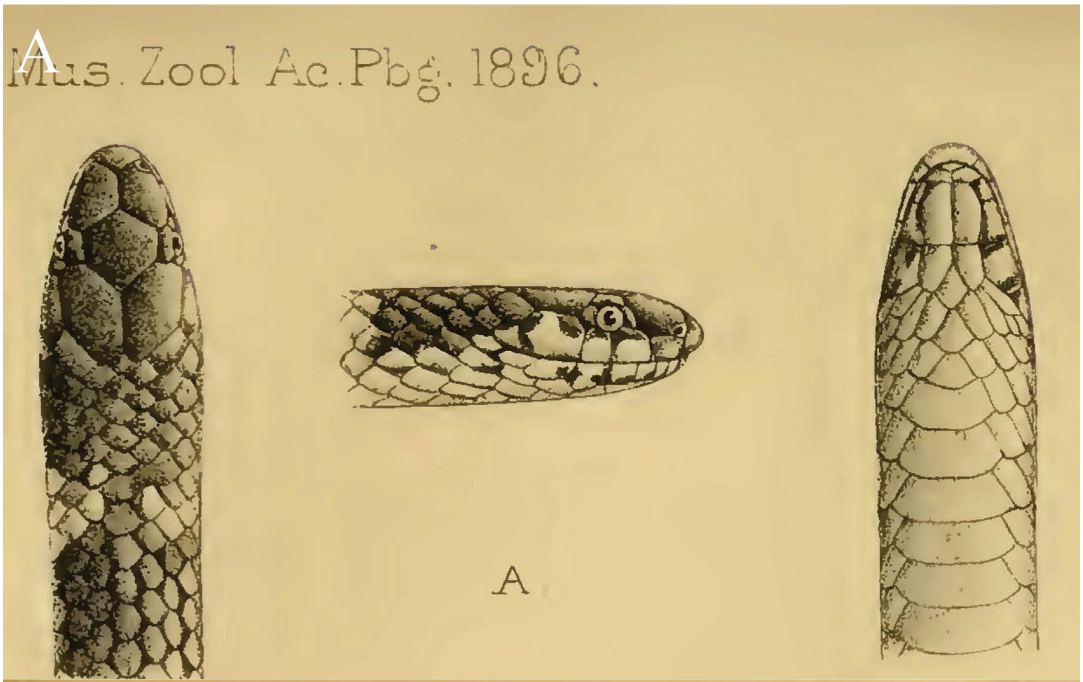


Figure 5. Lectotype ZISP 8823 of *Calamaria berezowskii*: **A.** Right view of head; **B.** Left view of head; **C.** Dorsal view; **D.** Ventral view.



B

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Calamaria berezowskii, sp. n.

(Pl. I, fig. A.)

Rostral shield reverted to, and distinctly visible on, the upper side of the head. Vertical as broad as long, about four times as wide as the supraocular; one ante-, and one post-ocular. Four upper labials; the first pair of lower labials meeting behind the symphysial. Two pairs of chin-shields, without a central shield between them, the anterior not much longer than the posterior. Orbit narrower than its distance from the labial margin. Ventrals 155, 167; subcaudals 22, 14. Tail rather obtuse, with a conical end. Upper parts brown, with a faint dark line along each side of the back, and another running along the meeting edges of the two outer series of scales. Lower parts, and the lower half of the outer-most row of scales uniform white. A more or less distinct yellowish collar, about five scales distant from the head. Two pairs of small white spots may be present or absent on the tail.

Total length	290 mill.	245 mill.
Length of head . . .	8 „	7 „
„ „ tail	18 „	25 „

Two specimens from Lun-ngan-fu (Berezowski, 1893).

This species is allied to its compatriot, *Calamaria septentrionalis*, but has an entirely different rostral shield. In this respect it comes nearer to *Calamaria siamensis*, which has a narrower vertical shield, and variegated abdomen.

Figure 6. Original description of *Calamaria berezowskii* Günther, 1896: **A.** Drawing; **B.** Description.

mental separated from anterior chin shields (vs. reverse condition in *C. septentrionalis*), not gradually tapering at tip (vs. tail tip broadly rounded in *C. septentrionalis*), higher number of subcaudals (22–25 vs 15–19 in males and 16 vs 6–11 in females), absence of black stripe on venter of tail (vs. venter of tail with broad and distinct median black stripe in *C. septentrionalis*).

Calamaria berezowskii differs from *C. pavimentata* by having the rostral shield width larger than high (vs. reverse condition in *C. pavimentata*), the eye diameter less than the distance from eye to mouth edge (vs. reverse condition in *C. pavimentata*), tail not tapering anteriorly and abruptly pointed at tip (vs. tail tapering gradually to a point in *C. pavimentata*), dorsal scales reduced to six rows at tail (vs. reduced to four rows in *C. pavimentata*), dorsum of body with two faint dark lines (vs. distinct narrow and dark longitudinal lines on dorsum in *C. pavimentata*).

Calamaria berezowskii differs from *C. dominici* by having eye diameter less than eye-mouth distance (vs. opposite situation in *C. dominici*), mental separated from anterior chin shields (vs. mental touching tip of right anterior chin shield in *C. dominici*), dorsal scales reduced to six rows at tail (vs. reduced to four rows in *C. dominici*), fewer ventral scales in females (153–165 vs 174 in *C. dominici*), absence of blotches on dorsum (vs. present irregular yellow blotches on dorsum in *C. dominici*), venter immaculate without dark stripes (vs. ventral side dark with yellow blotches and bands).

Calamaria berezowskii differs from *C. strigiventris* by having posterior chin shields meeting in midline (vs. diverging or meeting only in anteriorly in *C. strigiventris*), dorsal scales reduced to six rows at tail (vs. reduced to four rows in *C. strigiventris*), lower number of subcaudals (22–25 vs 29–33 in males and 16 vs 20–30 in females in *C. strigiventris*), venter immaculate (vs. presence of three interrupted longitudinal black stripes in *C. strigiventris*), tail not flattened, tapering and bluntly pointed at tip (vs. slowly tapering anteriorly, then abruptly tapering to a point in *C. strigiventris*).

Diagnosis. *Calamaria berezowskii* can be distinguished from all other congeneric species by having the following combination of morphological characters: 1) rostral shield width larger than high; 2) prefrontal shorter than frontal, touching the first and second supralabials; 3) frontal hexagonal, length longer than width; 4) mental not touching anterior chin shields; 5) two pairs of chin-shields, the anterior not much longer than the posterior, less than 1/2 of the posterior chin shield meets in the midline; 6) eye diameter less than eye-mouth distance; 7) single preocular, single postocular; 8) four supralabials, second and third supralabials entering orbit; 9) five infralabials, first three touching anterior chin shields, first pair of supralabials touching each other; 10) six scales and shields surrounding the paraparietals; 11) dorsal scales smooth, DSR 13–13–13 (n = 4); 12) dorsal scales reduced to six rows above last subcaudal at tail; 13) 149 (+2 preventrals)–155 ventrals in the males (n = 2), 153–165 (+2 preventrals) in the females (n = 2); 14) 22–25 subcaudals in the males (n = 2), 16 in the females (n = 2), all paired; 15) anal plate single and intact; 16) dorsum of body and tail blackish-brown or brown, with a faint dark line along each side of the back; 17) a more or less distinct yellowish collar; 18) 2–3 pairs of small white spots may be present or absent on the tail; 19) ventral scales of body light khaki or white, with a dark longitudinal line or scattered spots on the underside of tail; 20) two outermost dorsal scale rows light khaki with upper margins partly dark pigmented; 21) tail relatively short (5.6–10.5% of the total length), not flattened, slowly tapering to obtuse, with a conical end.

Etymology. Named after collector of type specimen, Russian traveler and zoologist. Michael Berezowski. For common name, we suggest "川西两头蛇" (Chinese), "Berezowski's Reed Snake" (English).

Distribution and ecology. Terra typica of *Calamaria berezowskii* is Lun-ngan-fu (now Long'an Town of Pingwu County) (Günther 1896). It is known from western Sichuan Province, China, including Pingwu County, Shimian County, Baoxing County, Luding County, Mt. Emei and Pingshan County (Zhao et al. 1998; Zhao 2003; this study). These localities are all on the eastern edge of the Qinghai-Tibet Plateau bordering Sichuan Basin. In Luding County, it was found at an elevation of 1680–1827 meters, surrounded by mountainous evergreen broad-leaved forest belt and evergreen broad-leaved deciduous broad-leaved mixed forest belt (Fig. 7). It has a typical subtropical-based vertical natural belt spectrum, with big altitude differences, and abundant rare plant and animal resources (Wang et al. 2023).

Discussion

Morphologically, specimens of *C. berezowskii* collected in Luding County, Sichuan Province are a close match to the type specimens collected in Pingwu County, Mianyang City of Sichuan Province (Günther 1896). For example, there was no significant difference in the number of ventral and subcaudal scales; presence of a more or less distinct yellowish collar; one preocular and one postocular; presence of faint dark line along each side of the dorsum. Geographically, Luding County and Pingwu County are both located in the western region of the Sichuan Province, which lies on the eastern slope of the Qinghai-Tibet Plateau bordering the Sichuan Basin. Even though the samples of *Calamaria* used by this study do not originate from the type locality, the morphology of the voucher specimens matches the original description by Günther (1896), so we confirm that the *Calamaria* specimens collected in Luding County, Sichuan Province are *C. berezowskii* and recover the validity of *C. berezowskii*.

Due to their subterranean mode of life, digging behavior and mysterious habits, *Calamaria* species are not often encountered in the wild. However, in the last five years, five species of *Calamaria* were described, which

Figure 7. Macrohabitat of *Calamaria berezowskii* in Luding County, Sichuan Province, China.

once more shows that the diversity of the genus *Calamaria* is still highly underestimated. In addition, the formal redescription of *C. berezowskii* brings the total number of species of the genus *Calamaria* in China to seven, namely *C. pavimentata*, *C. septentrionalis*, *C. yunnanensis*, *C. andersoni*, *C. arcana*, *C. jinggangensis*, and *C. berezowskii* (Cai et al. 2023; Uetz et al. 2023). At the same time, finding additional specimens of *C. berezowskii* from the type locality and surrounding areas would allow for a greater understanding of its intraspecific variation.

C. berezowskii was found at an elevation of 1680–1827 meters, which is higher than the altitude of all previously known *Calamaria* in China (vs.175–1520 m a.s.l.). The discovery of this new species increases the elevation of all known *Calamaria* species in China and suggests that the actual habitat of *Calamaria* species may span a greater range of elevations. The Hengduan Mountains in southwest China are a biodiversity hot spot, harboring a high percentage of endemic biota (Hu et al. 2012; Wu et al. 2013; Sun et al. 2017; Qiao et al. 2022). For snakes, the high altitude, steep terrain and varying climatic conditions of the Hengduan Mountains are important factors limiting their distribution and are conducive to species migration and isolation, accelerating gene flow and promoting species adaptation to the environment and species formation. Therefore, studying this species is beneficial for understanding the influence of geographical factors and the ecological environment on species formation and evolution.

We update the key to *Calamaria* in China, which is based on Zhao (2006), Yeung et al. (2022) and Cai et al. (2023). The identification of this key is only for reference. Species should be identified by comparing more morphological characteristics or molecular sequences.

Key to the identification of the genus *Calamaria* from China

1	Preocular absent	*Calamaria yunnanensis*
–	Preocular present	2
2	Dorsal scales reduced to four rows on tail at last subcaudals	3
–	Dorsal scales reduced to more than four rows on tail at last subcaudals	5
3	Light rings/blotches on neck and tail absent	*Calamaria andersoni*
–	Presence of light rings/blotches on neck or tail	4
4	Tail tapering gradually to a point	*Calamaria pavimentata*
–	Tail not tapering, broadly rounded on tip	*Calamaria septentrionalis*

5 Most of the posterior chin shield meets in the midline, absence of collar or spots in nuchal region .... *Calamaria jinggangensis*
– Less than 1/2 of the posterior chin shield meets in the midline, present indistinct light ring or one pair of spots in nuchal region 6
6 Ventral scales immaculate, without dark outermost corners and pigmentation anteriorly.................... *Calamaria arcana*
– Two outermost dorsal scale rows light khaki, with upper margins partly dark pigmented............. *Calamaria berezowskii*

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Appendix 1

Examined specimens (n = 6)

Calamaria berezowskii (n = 4). China: Sze-chuen (now Sichuan Province): Lun-ngan-fu (Long'an Fu, now Long'an Town of Pingwu County): ZISP 8823 (lectotype, adult male); Sichuan: Mt. Gongga, Moxi Town, Luding County, Ganzi Tibetan Autonomous Prefecture: GXNU DLR195 (adult female); GXNU DLR194 (adult male); GXNU 20221215002 (juvenile female).

Calamaria jinggangensis (n = 2). China: Guangxi: Quanzhou County, Guilin: GXNU 20210909007 (adult male); Longsheng County, Guilin: GXNU 20220613012 (adult male).