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A new species of *Calamaria* Boie, 1827 (Squamata: Calamariidae) from Hunan Province, South Central China

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ABSTRACT

A new species of Southeast Asian reed snake *Calamaria* Boie, 1827, *Calamaria hunanensis* sp. nov., is described from Hunan Province, China. Genetic analysis based on the mitochondrial cyt *b* gene indicates that the new species forms a deeply divergent clade, with a minimum interspecific genetic distance of 16.1% from *C. jinggangensis*. Morphologically, it differs from other species of the genus in the following characters: 181 ventral scales and 23 subcaudals in the male; preocular present; eye diameter slightly smaller than the eye-mouth distance; mental not touching anterior shields; posterior chin shields in contact at the anterior third; 6 scales surrounding paraparietal; end of tail sharp-pointed, dorsal scales reduced to four rows above the 4th subcaudal scale anterior to the tail tip; dorsal body basically brown, with nine narrow indistinct deep brown stripes; light nuchal collar present; lateral margins of ventral scales pigmented dark brown, matching the coloration of the adjacent outermost dorsal scales; and ventral body yellow.

Keywords: *Calamaria hunanensis*; Taxonomy; Morphology; Phylogeny

INTRODUCTION

The genus *Calamaria* Boie, 1827, a group of semi-fossorial

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reed snakes, is one of the most diverse serpent lineages in Asia with 69 recognized species, distributed from northeastern India and south China, through mainland Southeast Asia to the Maluku Islands of Indonesia (Inger & Marx, 1965; Uetz et al., 2026). In China, prior to 2018, only three species were recognized: *C. pavimentata* Duméril, Bibron & Duméril, 1854; *C. septentrionalis* Boulenger, 1890; and *C. yunnanensis* Chernov, 1962 (Yang & Zheng, 2018; Zhao et al., 1998, Zhao, 2006). Subsequent discoveries, including *C. andersoni* Yang & Zheng, 2018 from Yunnan, *C. arcana* Yeung, Lau & Yang, 2022 from Guangdong, Hunan and Fujian, *C. jinggangensis* Cai, Jiang, Wu, Huang, Fei & Ding, 2023 from Jiangxi and Guizhou, *C. synergis* Zhang, Xu, Nguyen, Poyarkov, Vogel, Wang & Huang, 2025 from Yunnan, and *C. berezowskii* Günther, 1896 revalidated from Sichuan, have elevated the number of Chinese *Calamaria* to eight species (Cai et al., 2023; Liang et al., 2024; Yang & Zheng, 2018; Yeung et al., 2022; Zhang et al., 2023, 2025a, 2025b). These recent taxonomic revisions and findings revealed an underestimated species diversity within this genus, particularly in southern China and adjacent mountainous areas (Cai et al., 2023; Liang et al., 2024; Yeung et al., 2022; Zhang et al., 2024, 2025a, 2025b).

In 2022, we conducted a series of field surveys under Suining County Biodiversity Resources Survey Project in Suining County, Shaoyang City, Hunan Province, south-central China (Figure 1), and collected one specimen of the genus *Calamaria*. It was assigned to the genus by following characters: dorsal scales in 13 rows throughout body, internasals and prefrontals fused, and parietal broadly in

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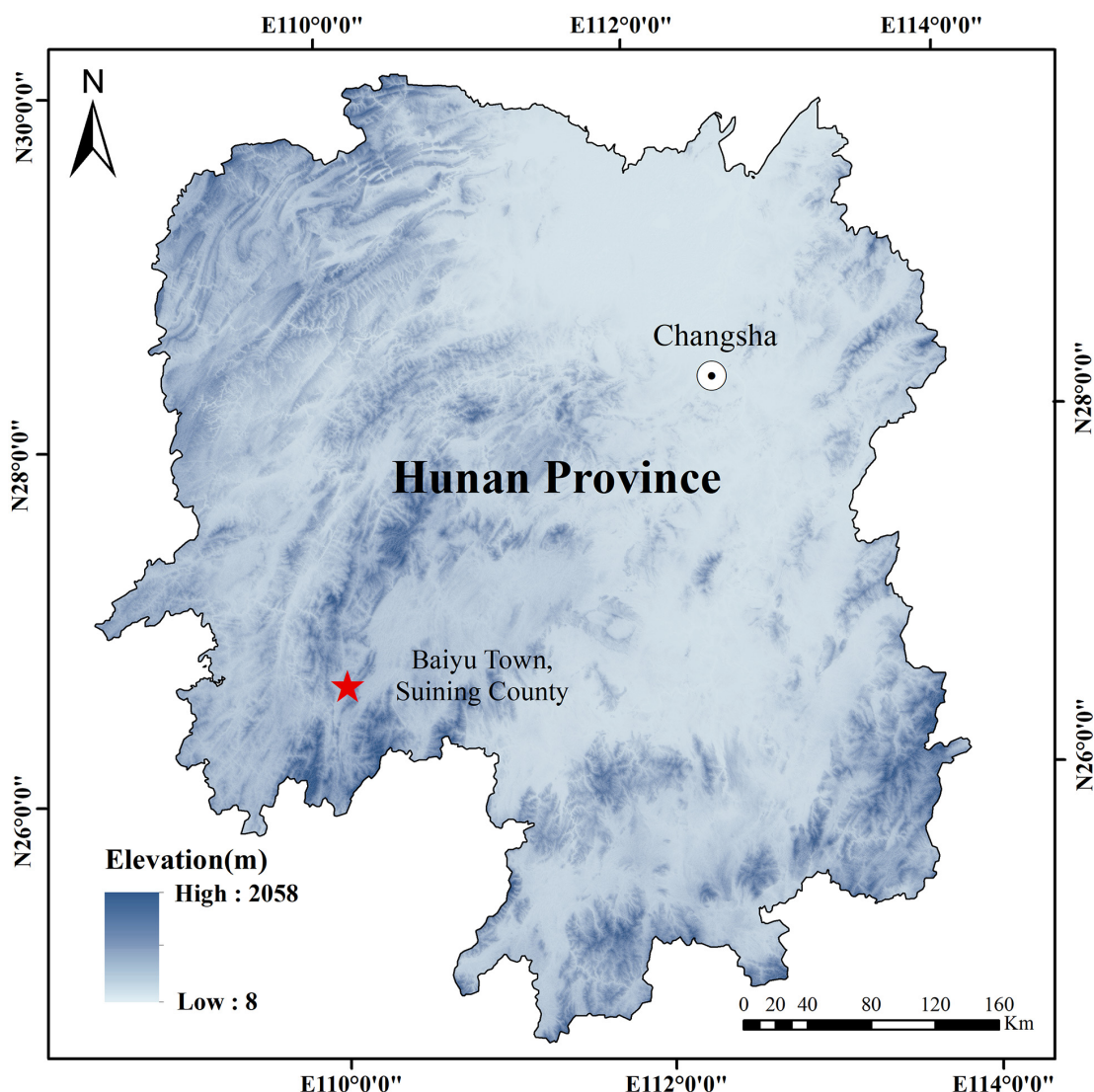


Figure 1 Distribution of *Calamaria hunanensis* sp. nov.

Red star indicates the type locality, Baiyu Town, Suining County, Hunan Province, China

contact with supralabials (Inger & Marx, 1965). Morphological and phylogenetic analyses revealed the specimen from Baiyu Town to be an undescribed species. It is described as a new species herein.

MATERIALS AND METHODS

Sampling

The collected specimen was fixed and preserved in 75% ethanol. Muscle tissue was excised and preserved in 95% ethanol, and stored at -20°C . The voucher specimen was deposited in the Biological Museum of Central South Inventory and Planning Institute of National Forestry and Grassland Administration, Changsha, Hunan, China (Institute abbreviation CSIPI).

Genetic analysis

Genomic DNA was extracted from muscle tissue excised from the specimen using the TSINGKE TSP202-200 Trelief® Hi-Pure Animal Genomic DNA Kit (Tsingke Biotechnologies, China). 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 2X San Taq PCR Master Mix (with Blue Dye), 10 μl ddH₂O, 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 40 cycles: denaturing at 94°C for 30 sec, annealing at 47°C for 45 sec, an extension step at 72°C for 90 sec; followed by a final extension of 72°C for 10 min. The PCR products were sent to Tsingke Biotechnologies Co., Ltd. (Beijing, China), where the purified PCR products were sequenced using the same forward and reverse primers. Sequences generated in this study were deposited in GenBank under accession no. PZ322771. Sequences used for genetic comparison were downloaded from GenBank (Table 1).

A total of 46 sequences were included in the analysis (Table 1). Among them, one was generated in this study, 42 were *Calamaria*'s sequences from Southeast Asia and China and the three outgroup taxa *Elaphe quatuorlineata* (Lacépède, 1789), *Lycodon rufozonatus* Cantor, 1842 and *Orientocoluber spinalis* (Peters, 1866) were downloaded from GenBank. Outgroups were chosen following Yang & Zheng (2018) and Yeung et al. (2022). Sequences were aligned by MEGA11

Table 1 Information of samples used in this study.

No.	Taxa	Voucher	Locality	cyt b	Reference
1	<i>Calamaria septentrionalis</i>	HS 11119 (CHS 116)	Tunxi, Huangshan, Anhui, China	MK201273	Li et al., 2020
2	<i>C. septentrionalis</i>	HS 12055 (CHS 118)	Huangshan, Anhui, China	MK201274	Li et al., 2020
3	<i>C. septentrionalis</i>	HSR19100	Mt. Huangshan, Anhui, China	OQ354842	Cai et al., 2023
4	<i>C. septentrionalis</i>	KFBG14506	Hainan Island, China	MH445956	Yang & Zheng, 2018
5	<i>C. septentrionalis</i>	FTB 2839	unknown locality	KR814699	Pyron unpublished data
6	<i>C. septentrionalis</i>	RE 30 (CHS 302)	Mangshan, Hunan, China	MK201384	Li et al., 2020
7	<i>C. septentrionalis</i>	DL2021610-1	Huangsha, Guangxi, China	OQ354838	Cai et al., 2023
8	<i>C. septentrionalis</i>	HS11145	Mt. Nanling, Guangdong, China	OQ354840	Cai et al., 2023
9	<i>C. septentrionalis</i>	ROM35605	Nguyễn Bình, CaoBang, Vietnam	AF471081	Lawson et al., 2005
10	<i>C. septentrionalis</i>	ROM35597	Cao Bang, Vietnam	KX694890	Alencar et al., 2016
11	<i>C. jinggangensis</i>	DL20200725	Mt. Jinggangshan, Jiangxi, China	OQ354830	Cai et al., 2023
12	<i>C. jinggangensis</i>	DL20200625-4	Mt. Jinggangshan, Jiangxi, China	OQ354833	Cai et al., 2023
13	<i>C. jinggangensis</i>	DL20200625-3	Mt. Jinggangshan, Jiangxi, China	OQ354832	Cai et al., 2023
14	<i>C. jinggangensis</i>	DL20200625-2	Mt. Jinggangshan, Jiangxi, China	OQ354831	Cai et al., 2023
15	<i>C. jinggangensis</i>	LFR2023028	Rongjiang, Guizhou, China	OR529438	Zhang et al., 2024
16	<i>C. arcana</i>	KFBG14611	Mt. Dadongshan, Guangdong, China	ON482335	Yeung et al., 2022
17	<i>C. arcana</i>	GP9975	Yongxing, Hunan, China	OP980549	Cai et al., 2023
18	<i>C. arcana</i>	DLR199	Mt. Wuyi, Fujian, China	OQ354834	Cai et al., 2023
19	<i>C. arcana</i>	HS17082	Mt. Dawu, Guangdong, China	OQ354835	Cai et al., 2023
20	<i>C. andersoni</i>	HSR20101	Dehong, Yunnan, China	OQ354844	Cai et al., 2023
21	<i>C. andersoni</i>	HSR20181	Tengchong, Yunnan, China	OQ354845	Cai et al., 2023
22	<i>C. andersoni</i>	SYSr001699	Yingjiang, Yunnan, China	MH445955	Yang & Zheng, 2018
23	<i>C. yunnanensis</i>	ROM41547	Simao, Yunnan, China	KX694891	Zaher et al., 2009
24	<i>C. yunnanensis</i>	YPx503	Unknown	JQ598922	Grazziotin et al., 2012
25	<i>C. synergis</i>	AHNU ZR24046	Mt. Jinuo, Xishuangbanna, Yunnan, China	PV745121	Zhang et al., 2025a
26	<i>C. synergis</i>	AHNU ZR25021	Mt. Jinuo, Xishuangbanna, Yunnan, China	PV745122	Zhang et al., 2025a
27	<i>C. hunanensis</i> sp. nov.	CSIPI ZNY202207179	Baiyu, Suining, Shaoyang, Hunan, China	PZ322771	This study
28	<i>C. nebulosa</i>	FMNH258666	Phongsaly, Laos	MN338524	Quah et al., 2019
29	<i>C. berezowskii</i>	GXNU DLR194	Mt. Gongga, Sichuan, China	PP747047	Liang et al., 2024
30	<i>C. berezowskii</i>	GXNU DLR195	Mt. Gongga, Sichuan, China	PP747048	Liang et al., 2024
31	<i>C. berezowskii</i>	GXNU 20221215002	Mt. Gongga, Sichuan, China	PP747049	Liang et al., 2024
32	<i>C. pavimentata</i>	KFBG14507	Ningming, Guangxi, China	MH445957	Yang & Zheng, 2018
33	<i>C. lumbricoidea</i>	USMHC1560	Air Itam Dam, Penang, Peninsular, Malaysia	MN338526	Quah et al., 2019
34	<i>C. cf. lumbricoidea</i>	KU 315159	Pasonanca NP, Zamboanga, Philippines	MT819388	Weinell et al., 2020
35	<i>C. cf. lumbricoidea</i>	KU 334479	Mt. Lumot, Gingoog, Misamis, Philippines	MT819389	Weinell et al., 2020
36	<i>C. muelleri</i>	TNHC58955	Gowa, South Sulawesi, Indonesia	MT819390	Weinell et al., 2020
37	<i>C. muelleri</i>	RMB1283	Gowa, South Sulawesi, Indonesia	MT819391	Weinell et al., 2020
38	<i>C. schlegeli</i>	LSUHC10278	Perak, Peninsular, Malaysia	MN338525	Quah et al., 2019
39	<i>C. alcalai</i>	PNM 9873	Sitio Palbong, Barangay Batong Buhay, Sablayan, Mindoro, Philippines	MT819383	Weinell et al., 2020
40	<i>C. gervaisii</i>	KU324661	Puguis, Benguet, Philippines	MT819384	Weinell et al., 2020
41	<i>C. gervaisii</i>	KU334485	Municipality, Ilocos Sur, Philippines	MT819385	Weinell et al., 2020
42	<i>C. palavanensis</i>	KU309445	Barangay Irawan, Palawan, Philippine	MT819386	Weinell et al., 2020
43	<i>C. palavanensis</i>	KU311411	Mt. Mantalingahan, Palawan, Philippine	MT819387	Weinell et al., 2020
44	<i>Elaphe quatuorlineata</i>	LSUMZ40626	Turkey, European Turkey	AY486931	Nagy et al., 2004
45	<i>Lycodon rufozonatus</i>	LSUMZ44977	Unknown	AF471063	Lawson et al., 2005
46	<i>Orientocoluber spinalis</i>	MVZ211019	Yinnan, Ningxia, China	AY486924	Nagy et al., 2004

(Tamura et al., 2021). The following analyses were conducted by using PhyloSuite (Zhang et al., 2020). Before phylogenetic analysis, ambiguously aligned fragments in the alignment were removed using Gblocks (Talavera & Castresana, 2007) with the following parameter settings: minimum number of sequences for a conserved/flank position (20/20), maximum number of contiguous non-conserved positions (8), minimum length of a block (10), and allowed gap positions (with half). ModelFinder (Kalyaanamoorthy et al., 2017) was used to select the best-fit model using BIC criterion. The best-fit model

according to BIC: GTR+F+I+G4. Bayesian Inference (BI) phylogenies were inferred using MrBayes v.3.2.6 (Ronquist et al., 2012) under GTR+I+G+F model (2 parallel runs, 10 000 000 generations), in which the initial 25% of sampled data were discarded as burn-in. Maximum likelihood (ML) phylogenies were inferred using IQ-TREE (Nguyen et al., 2015) under the TVM+I+G4+F model with 5 000 ultrafast bootstraps (Minh et al., 2013). Genetic distances were estimated using MEGA11 with an uncorrected pairwise distance model (Tamura et al., 2021).

Morphological analysis

Terminology and descriptions generally follow Inger & Marx (1965) and Liang et al. (2024). 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 the posterior margin of the cloaca; tail length (TaL), from posterior margin of cloaca to the tip of tail. Other measurements were taken with a digital caliper to the nearest 0.1 mm: head length 1 (HL1), from tip of the snout to posterior end of parietal suture; head length 2 (HL2), from tip of the snout to the line connecting left and right jaw joints; head width (HW), measured at the widest part of the head on dorsal view; head height (HH), maximal highest part of the head; 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). Enlarged shield(s) anterior to the first ventral scale were regarded as preventral(s). The number of dorsal scale rows (DSR) is given at one head length behind head, at midbody, and at one head length before the 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.

Relevant morphological data of other *Calamaria* species from Southeast Asia and China were obtained from examined specimens (Appendix) and from literature listed in Table 2.

Institutional acronyms

FMNH: Field Museum of Natural History. **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). **ROM**: Royal Ontario Museum. **SYS**: Biological Museum of Sun Yat-sen University. **TNHC**: Texas Natural History Collections. **USMHC**: Universiti Sains Malaysia Herpetological Collection. **ZFMK**: Zoologisches Forschungsmuseum Alexander Koenig. **ZISP**: Zoological Institute, Russian Academy of Sciences, St. Petersburg.

RESULTS

Genetic analysis

The cyt *b* dataset contains 46 samples with 1026 base pairs. Phylogenetic trees generated using BI and ML analyses resulted yielded similar topologies for the candidate new species (Figure 2). The specimen CSUPI ZNY202207179, eight species recorded from China, and *C. nebulosa* Lee, 2021 formed a large clade with strong support (BPP 1.00, UFB 100). However, the phylogenetic position of the specimen within this large clade remained largely unresolved. The specimen formed a deeply divergent clade sister to *C. nebulosa* with weak support (BPP 0.70, UFB<80). Genetic distance analysis showed that the specimen was highly divergent from other species, with a minimum divergence of 16.1% from *C. jinggangensis*. This divergence is clearly at the interspecific level, larger than those between *C. septentrionalis* and *C. jinggangensis* (10.8%–12.3%), and *C. arcana* and *C. jinggangensis* (5.3%–6.8%) (Supplementary Table S1).

Morphological comparison

The specimen CSUPI ZNY202207179 (male) differs from all recognized species in the number of ventrals and subcaudals, the shape of rostral, mental and posterior chin shields, the tail tip, dorsal scales reducing to four rows above 4th subcaudal scale anterior to tail tip, and coloration. For the detailed description and morphological comparisons, see the following section.

Table 2 References for morphological data of species in *Calamaria*

Species	References
<i>Calamaria nebulosa</i>	Lee, 2021
<i>C. jinggangensis</i>	Cai et al., 2023; Liang et al., 2024; Zhang et al., 2024; This study
<i>C. abramovi</i>	Orlov, 2009; Wallach et al., 2014
<i>C. albiventer</i>	Lee, 2021; Inger & Marx 1965; Wallach et al., 2014
<i>C. andersoni</i>	Yang & Zheng, 2018; Zhang et al., 2025b
<i>C. synergis</i>	Zhang et al., 2025a
<i>C. arcana</i>	Cai et al., 2023; Yeung et al., 2022; Zhang et al., 2023; This study
<i>C. berezowskii</i>	Günther 1896; Liang et al., 2024
<i>C. buchi</i>	Inger & Marx 1965; Nguyen et al., 2009; Ziegler et al., 2008
<i>C. concolor</i>	Orlov et al., 2010; Wallach et al., 2014
<i>C. dominici</i>	Ziegler et al., 2019
<i>C. gialaiensis</i>	Lee et al., 2021; Wallach et al., 2014; Ziegler et al., 2008
<i>C. lovii gimletti</i>	Grismer et al., 2004; Inger & Marx 1965; Lee et al., 2021; Quah et al., 2018
<i>C. lumbricoidea</i>	Inger & Marx, 1965; Lee et al., 2021; Weinell et al., 2020
<i>C. pavementata</i>	Inger & Marx, 1965; Nguyen et al., 2009; Ziegler et al., 2008
<i>C. prakkei</i>	Inger & Marx 1965; Wallach et al., 2014
<i>C. sangi</i>	Nguyen et al., 2009; Wallach et al., 2014
<i>C. schlegeli</i>	Inger & Marx, 1965; Quah et al., 2018; Weinell et al., 2020
<i>C. septentrionalis</i>	Inger & Marx, 1965; Poyarkov et al., 2019; Ziegler et al., 2008
<i>C. strigiventris</i>	Poyarkov et al., 2019
<i>C. thanhi</i>	Nguyen et al., 2009; Wallach et al., 2014; Ziegler & Quyet, 2005; Ziegler et al., 2007, 2008
<i>C. yunnanensis</i>	Chernov, 1962; Lee et al., 2021; Stuart & Heatwole, 2008; Yang & Rao, 2008

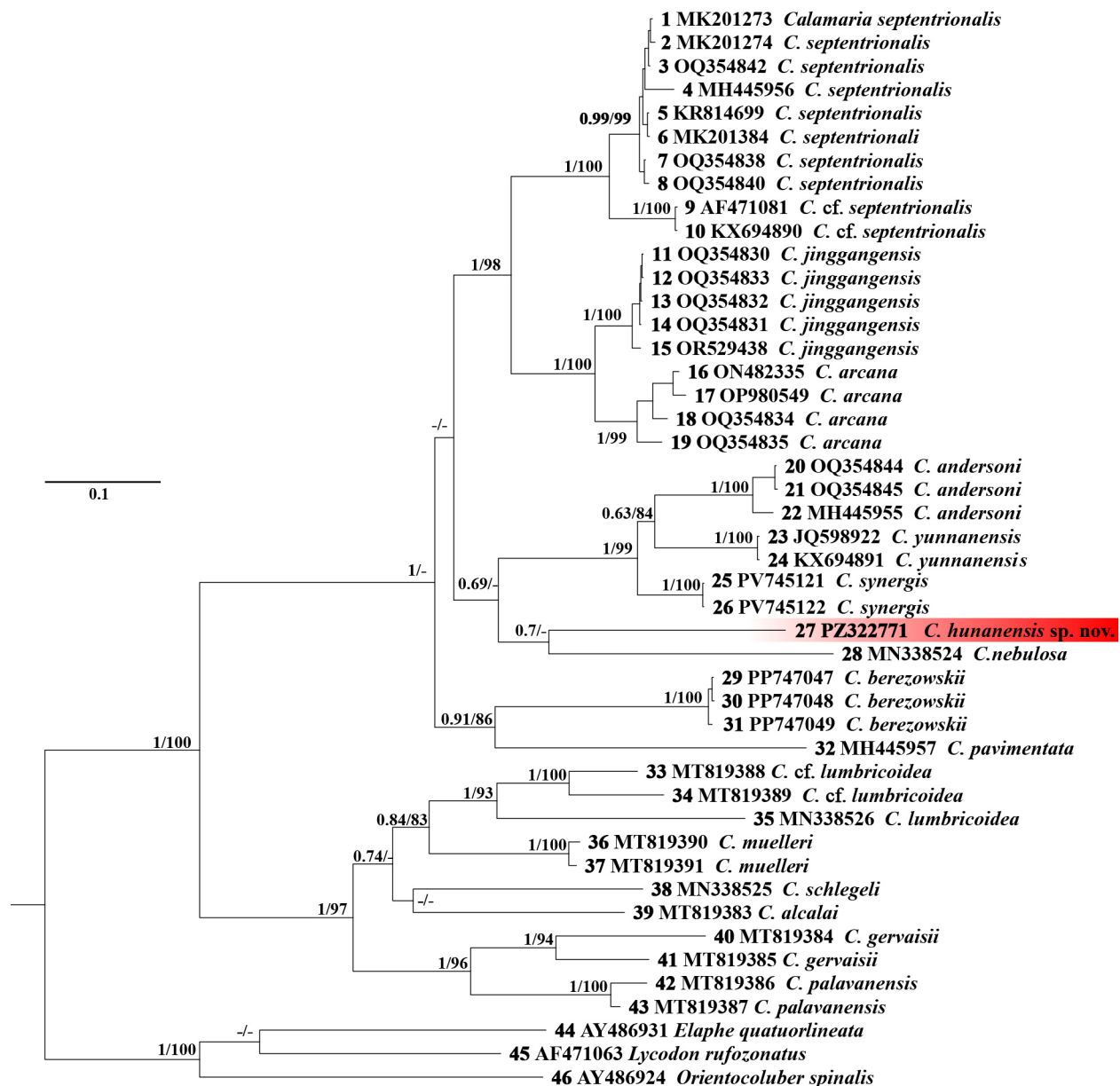


Figure 2 Phylogenetic relationships of *Calamaria* species based on cyt *b* resulted from Bayesian Inference

Bayesian posterior probabilities (BPP) and Ultrafast bootstrap approximation (UBF) were denoted beside each node (those lower than 0.6/80 were denoted as "-").

The specimen CSIP1 ZNY202207179 (male) represents a species new to science based on genetic and morphological evidence, and it is described herein.

Taxonomic account

Calamaria hunanensis sp. nov. Zhang, Zhu, Guo & Shi

Figure 3, 4

<https://zoobank.org/NomenclaturalActs/9F07FA8D-91D9-44B4-AEE4-B53760650679>

Holotype: CSIP1 ZNY202207179, an adult or subadult male, collected on 7 July 2022 at 16:00 by Mengfei Zhang, Ronghui Fan, and Ling Tao on a forest path in Baiyanchong, Zengjiaqiao Village, Baiyu Town, Suining County, Shaoyang City, Hunan Province, China (湖南省邵阳市绥宁县白玉乡曾家桥村白岩冲 E110°21'44.1377", N26°41'43.5740", 690 m a.s.l.).

Etymology: The specific epithet '*hunanensis*' refers to Hunan Province, where the type locality is located. The Chinese

name is suggested as '湖南两头蛇'. The English common name is suggested as 'Hunan Reed Snake'.

Diagnosis: Male with 181 ventral scales and 23 subcaudals; preocular present; eye diameter slightly smaller than eye-mouth distance; mental scale not touching anterior shields; posterior chin shields in contact at anterior third part; 6 scales surrounding paraparietal; tail tip sharp-pointed, with dorsal scales reduced to the 4th rows above the 4th subcaudal scale anterior to the tail tip; dorsal body basically brown, with nine narrow indistinct deep brown stripes; light nuchal collar present; lateral margins of ventral scales pigmented dark brown, matching the coloration of the adjacent outermost dorsal scales; ventral body yellow.

Description of holotype: Adult or subadult male. SVL 243 mm, TaL 21 mm, TL 264 mm, HL1 5.6 mm, HL2 7.6 mm, HW 3.9 mm, HH 2.5 mm, EyeD 0.7 mm, Eye-MouthD 0.8 mm, frontal length 1.9 mm, prefrontal length 2.0 mm. TaL/TL ratio 0.08, EyeD/Eye-MouthD ratio 0.88, HW/HL ratio 0.70, HH/HL

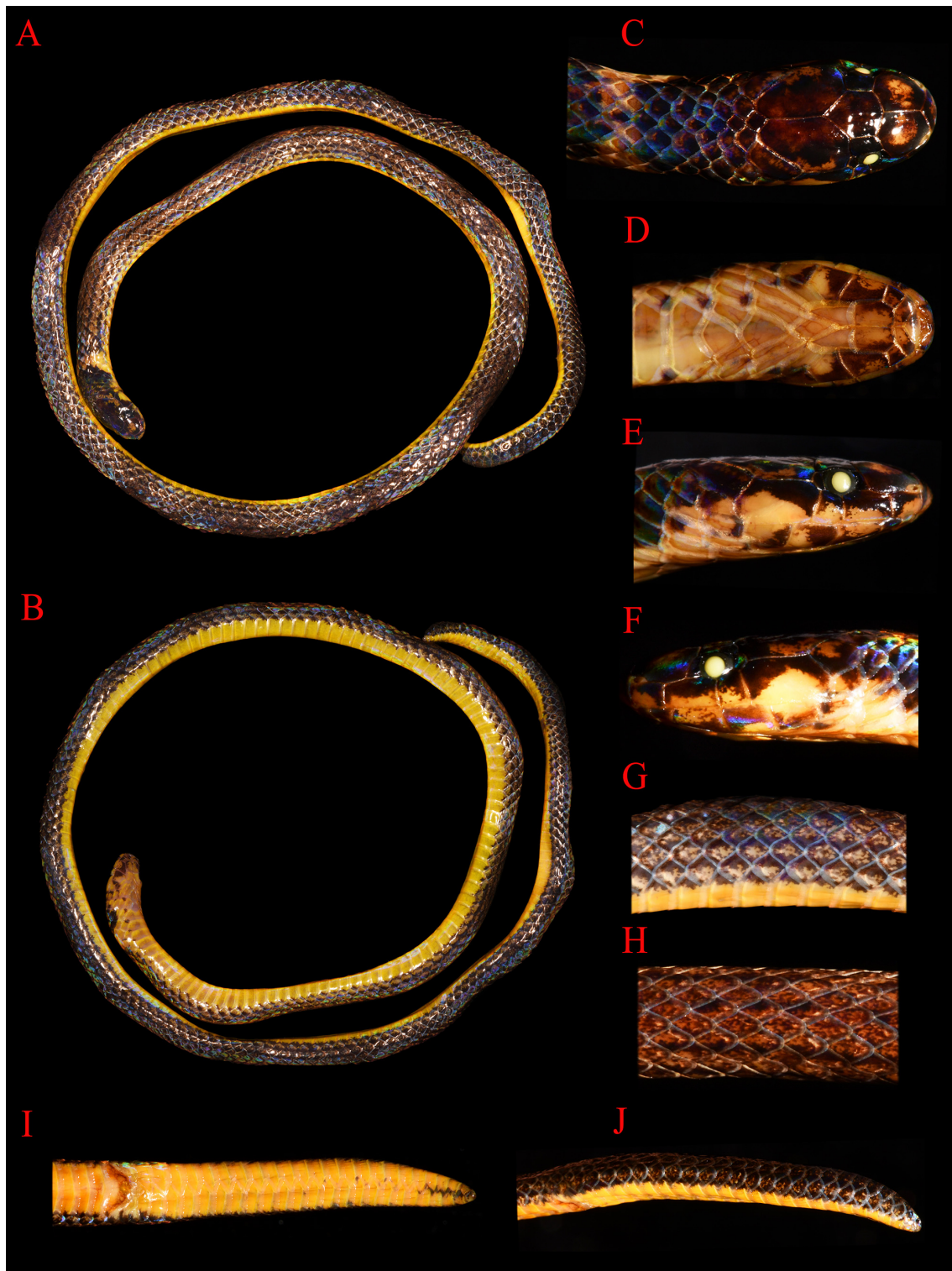


Figure 3 Holotype of *Calamaria hunanensis* sp. nov. CSIP ZNY202207179 in a fresh condition

A: dorsal view; B: ventral view; C: dorsal head and neck; D: ventral head; E: right lateral head; F: left lateral head; G: lateral middle body; H: dorsal middle body; I: ventral tail; J: lateral tail. Photographed by Keji Guo.

ratio 0.45.

Head near oval-shaped, indistinct from neck; snout blunt, weakly depressed; nostrils small and elliptical, located on anterolateral head; eyes small, round, diameter slightly smaller than eye-mouth distance; pupil round; rostral scale near shield-like in frontal view, wider than high, portion visible from above 1/2 of the prefrontal suture, suture bordering prefrontal and rostral scale widely "V"-shaped; prefrontal

scales subhexagonal, slightly longer than frontal; frontal scale shield-shaped, subhexagonal, length 1.2 times its width, equal to parietal suture in length; posterior angle formed by the frontal/parietal sutures producing the posterior vertex of the frontal at a right angle ($\sim 90^\circ$); parietals subhexagonal, longer than wide, scale length longer than frontal; parietal suture 1.5 times of prefrontal suture; anterior parietal angle formed by the sutures between the parietal/frontal and the suture

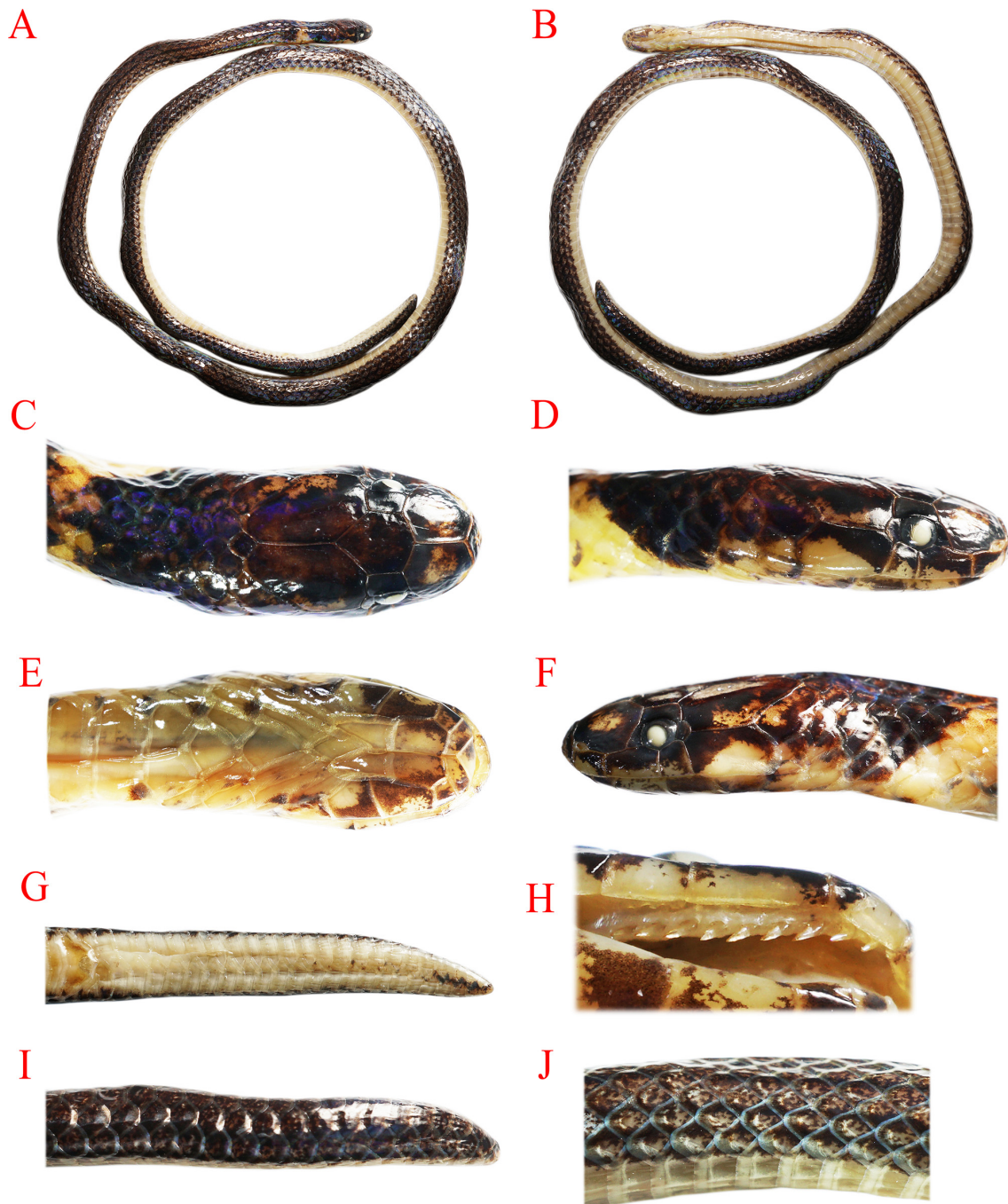


Figure 4 Holotype of *Calamaria hunanensis* sp. nov. CSIP1 ZNY202207179 in preservative

A: dorsal view; B: ventral view; C: dorsal head and neck; D: right lateral head and neck; E: ventral head; F: right lateral head and neck; G: ventral tail; H: maxillary teeth; I: lateral tail; J: lateral dorsal middle body. Photographed by Shengchao Shi.

between the supraocular/parietal broad obtuse, pointing laterally ($\sim 130^\circ$); supraoculars trapezoid, width of frontal around 1.9 times the maximum width of supraoculars; paraparietal shaped like a temporal scale with 6/6 scales and shields in contact; postocular near rectangular, height about two thirds of eye diameter; preocular single, small, long triangular; nasals small and soft, nearly separated by nostril into anterior and posterior parts, bordering prefrontal, rostral and first supralabial; supralabials 4/4, 1st and 3rd smallest, anterior half of 1st in contact with nasal, posterior half of 1st and anterior part of 2nd in contact with prefrontal, middle part of 2nd in contact with preocular, posterior part of 2nd and anterior two thirds of 3rd in contact with eye, posterior third of 3rd in contact with postocular, 4th largest and rectangular, in

contact with postocular, parietal, paraparietal and one temporal; infralabials 5/5, 1st–3rd touching anterior part of chin shields, 3rd largest, slightly larger than 4th and 5th, contacting near half of posterior chin shields; 4th partly in contact with posterior chin shields; mental triangular, size similar to 1st infralabial, not touching the anterior chin shields; anterior chin shields paired, near rectangular, completely in contact with each other; posterior chin shields in contact at anterior 1/3 part, separated by the first gular scale, posterior portion pointed; three gular scales before first ventral scale, 1st rhomboid, 2nd and 3rd fan-shaped.

Body elongated, cylindrical in cross section; tail thick, roughly equal in diameter to the rest of body; tail slightly



Figure 5 Habitat of the type locality of the new species

A: macrohabitat, mixed planted coniferous forest and bamboo forest on mountain; B: microhabitat, forest path in planted coniferous forest. Photographed by Qi Li.

tapering from base to middle portion, then abruptly tapering from the 19th subcaudal to the tip of tail, ending in a sharp point in both dorsal and ventral views (Figure 5I). Dorsal scale rows smooth, in 13 rows throughout; dorsal scales reducing to six rows above the 6th subcaudal scale, and reducing to four rows above the 19th subcaudal scale (4th anterior to the tail tip); ventrals 181; subcaudals 23, all paired; cloacal plate single; maxillary teeth 9/9, unmodified, arranged in a continuous series.

Colouration of the holotype in a fresh condition: Dorsum basically brown, the first and second row of dorsal scales light yellow with dark brown pigments forming thin faint deep brown stripes along the body, a total of 9 thin faint dark brown stripes present along dorsal body from nuchal collar to rest of body, reducing to 5 on dorsal tail. The stripes are half the width of a dorsal scale, the outer three stripes on lateral body are basically connected while the inner three are less distinct and disconnected (Figure 3G, I). Ventral body bright yellow; lateral margins of ventral scales pigmented with dark brown, matching the coloration of the adjacent outermost dorsal scales (Figure 3G). Dorsal head dark brown; prefrontals yellowish in the centre; parietals mostly deep brown with a small narrow yellowish patch bordering the 4th supralabials; iris dark; supralabials deep brown above and yellow below, with yellow patches on the 4th supralabials that are nearly triangular. Ventral head creamy yellow stained with light rusty pigments; infralabials and anterior chin shields with distinct rusty patches; several rusty dots present on the ventrolateral

neck, five on the right and one on the left. Nuchal collar bright yellow on the lateral neck, deeper and narrower on the dorsal neck. The colouration of the dorsal tail consistent with dorsal body; ventral tail bright yellow with a short dark brown stripe on the tip of tail involving last five pairs of subcaudals (Figure 3I).

Colouration of the holotype in preservative. Described based on the specimen preserved in 75% ethanol for 2 years. Dorsum basically brown, the yellow parts of body faded to cream white. Pupil milky white (Figure 4).

Comparisons.

Calamaria hunanensis **sp. nov.** differs from all other congeners from China and mainland Southeast Asia in morphology, detailed comparisons were presented in Table S2.

Calamaria hunanensis **sp. nov.** is possibly sister to *C. nebulosa* in the phylogeny based on the cytb gene. It is morphologically different from the latter in the following characters: preocular present (vs. absent); eye diameter slightly smaller than the eye-mouth distance (vs. larger); 1st and 3rd supralabials smallest (1st and 2nd smallest); 3rd infralabial largest (vs. 5th largest); end of tail sharp-pointed, dorsal scales reducing to four rows above 4th subcaudal scale anterior to the tail tip (vs. obtuse-pointed, reducing to four rows above the last subcaudal scale); light nuchal collar present (vs. absent).

Calamaria hunanensis **sp. nov.** differs from *C. jinggangensis* in: eye diameter slightly smaller than the eye-mouth distance (vs. larger); posterior chin shields in contact at the anterior 1/3 part (vs. almost completely meeting at the midline); end of tail sharp-pointed (vs. obtuse-pointed); dorsal scales reducing to four rows above 4th subcaudal scale anterior to the tail tip (vs. reducing to 6 rows above the last subcaudal); lateral margins of ventrals pigmented dark brown (vs. immaculate); more ventral scales in males (181 vs. 158–160).

Calamaria hunanensis **sp. nov.** differs from *C. abramovi* Orlov, 2009 by dorsal scales reducing to four rows above the 4th subcaudal scale anterior to the tail tip (vs. above the last subcaudal); coloration of dorsal body brown (vs. black); coloration of venter yellow (vs. black and yellow); light nuchal collar present (vs. absent); more ventral scales in males (181 vs. 159).

Calamaria hunanensis **sp. nov.** differs from *C. albiventer* (Gray, 1834) in having 6 scales surrounding paraparietal (vs. 5); 4 supralabials (vs. 5); mental touching anterior chin shields (vs. separated from anterior chin shields); posterior chin shields in contact at the anterior 1/3 part (vs. meeting at the midline); more ventral scales in males (181 vs. 143–144); dorsal scales reducing to four rows above the 4th subcaudal scale anterior to tail tip (vs. above the 5th–8th subcaudal scales); dorsal body with 9 thin faint dark brown stripes (vs. 2 light stripes on each side of the body); coloration of venter yellow (vs. red); dorsal scales without light spots (vs. spots present).

Calamaria hunanensis **sp. nov.** differs from *C. andersoni* by: eye diameter smaller than eye-mouth distance (vs. larger); thin faint, dark brown stripes present along dorsal body (vs. uniformly dark brown to blackish); nuchal collar present (vs. absent); and more ventral scales in males (181 vs. 166–171).

Calamaria hunanensis **sp. nov.** differs from *C. arcana* (Figure S1) by: dorsal scales reducing to four rows above 4th

subcaudal scale anterior to tail tip (vs. reduced to five rows above the last subcaudal); coloration of venter yellow (vs. orangish-red); a light nuchal collar present (vs. absent or indistinct); and lateral margins of ventral scales pigmented dark brown, matching the coloration of the adjacent outermost dorsal scales (vs. two outermost dorsal scale rows light yellow with upper margins partly dark pigmented while other dorsal scales grey-brown). The tail lacks a light ring at the root of the tail (vs. a narrow interrupted light ring present).

Calamaria hunanensis **sp. nov.** differs from *C. berezowskii* by: more ventral scales in males (181 vs. 149–155) but fewer subcaudals in males (23 vs. 25); posterior chin shields in contact at the anterior 1/3 part (vs. meeting at the midline); and dorsal scales reducing to four rows above 4th subcaudal scale anterior to tail tip (vs. dorsal scales reduced to six rows above last subcaudal at the tail).

Calamaria hunanensis **sp. nov.** differs from *C. buchi* by: rostral wider than high (vs. higher than wide); 6 scales surrounding paraparietal (vs. 5 scales); 4 supralabials (vs. 5); length of parietal shorter than distance from rostral to posterior tip of frontal (vs. greater); ventrals with brown lateral tips (vs. immaculate); coloration of first and second row of dorsal scales light yellow with deep brown pigments forming thin faint deep brown stripes along body (vs. first row of scales yellowish throughout length of body, second row yellowish in anterior part of body); and mental not touching anterior shields (vs. touching).

Calamaria hunanensis **sp. nov.** differs from *C. concolor* Orlov, Truong, Tao, Ananjeva & Cuc, 2010 by 4 supralabials (vs. 5); 6 scales surrounding paraparietal (vs. 5 scales); fewer ventral scales in males (181 vs. 209); more subcaudals (23 vs. 19); dorsal scales reducing to four rows above 4th subcaudal scale anterior to tail tip (vs. above last subcaudal); mental not touching anterior shields (vs. touching); a total of 9 thin faint dark brown stripes present on the body (vs. body uniformly brown above); and a light nuchal collar present (vs. absent).

Calamaria hunanensis **sp. nov.** differs from *C. dominici* Ziegler, Tran & Nguyen, 2019 by: mental not touching anterior shields (vs. touching); eye diameter slightly smaller than the eye-mouth distance (vs. larger); dorsum basically brown, with 9 thin faint deep brown stripes (vs. dorsum dark with irregular yellow blotches); ventral body yellow (vs. ventral side dark with few yellow blotches and bands); and light nuchal collar present (vs. absent).

Calamaria hunanensis **sp. nov.** differs from *C. gilaensis* Ziegler, Sang & Truong, 2008 by 6 scales surrounding paraparietal (vs. 5 scales); mental not touching anterior shields (vs. touching); end of tail sharp-pointed (vs. rounded); dorsal scales reducing to four rows above 4th subcaudal scale anterior to tail tip (vs. above last subcaudal); and light nuchal collar present (vs. absent).

Calamaria hunanensis **sp. nov.** differs from *C. lovii gimletti* Boulenger, 1905 by preocular present (vs. absent); posterior chin shields in contact at anterior 1/3 part (vs. meeting in midline); end of tail sharp-pointed (vs. rounded); more subcaudals (23 vs. 14–20).

Calamaria hunanensis **sp. nov.** differs from *C. lumbricoidea* Boie, 1827 by 4 supralabials (vs. 5); 6 scales surrounding paraparietal (vs. 5 scales); mental not touching anterior shields (vs. touching); posterior chin shields in contact at anterior 1/3 part (vs. meeting in midline); dorsal scales reducing to four rows above 4th subcaudal scale anterior to tail tip (vs. above last 11th subcaudal); light nuchal collar

present (vs. absent).

Calamaria hunanensis **sp. nov.** differs from *C. pavimentata* by rostral wider than high (vs. height equal to or larger than width); posterior chin shields in contact at anterior 1/3 part (vs. meeting at the midline); more ventral scales in males (181 vs. 125–168); and dorsal scales reducing to four rows above 4th subcaudal scale anterior to tail tip (vs. above last 13th subcaudal).

Calamaria hunanensis **sp. nov.** differs from *C. prakkei* Lidth De Jeude, 1893 by 6 scales surrounding paraparietal (vs. 5 scales); 4 supralabials (vs. 5); mental not touching anterior shields (vs. touching); posterior chin shields in contact at anterior 1/3 part (vs. meeting at the midline); 9 maxillary teeth (vs. 7–8); more ventral scales in males (181 vs. 126–132); fewer subcaudals (23 vs. 31–32); larger body size (264 mm vs. 172–245 mm); and a smaller Tail/Total Length ratio (8.0% vs. 16.5–16.7%).

Calamaria hunanensis **sp. nov.** differs from *C. sangi* Nguyen, Koch & Ziegler, 2009 by mental not touching anterior shields (vs. touching); end of tail sharp point (vs. obtuse point); maxillary teeth unmodified (vs. modified); ventral tail with short dark brown stripe on the tip of tail (vs. with a longitudinal stripe along the median sutures of subcaudal scales).

Calamaria hunanensis **sp. nov.** differs from *C. schlegeli* Duméril, Bibron & Duméril, 1854 by: 4 supralabials (vs. 5); end of tail sharp-pointed (vs. blunt); more ventral scales in male (181 vs. 126–161); fewer subcaudals in male (23 vs. 25–44); smaller Tail/Total Length ratio (8.0% vs. 11.1–21.3%); and a light nuchal collar present (vs. absent).

Calamaria hunanensis **sp. nov.** differs from *C. septentrionalis* (Figure S2) by: mental not touching anterior shields (vs. touching); posterior chin shields in contact at the anterior 1/3 part (vs. meeting at the midline); end of tail sharp-pointed (vs. broadly blunt); more ventral scales in males (181 vs. 148–166); more subcaudals in males (23 vs. 15–19); dorsal scales reducing to four rows above 4th subcaudal scale anterior to the tail tip (vs. not reduced); teeth unmodified (vs. modified); ventral body yellow (vs. orange); ventral tail with short dark brown stripe on the tip of tail (vs. with distinct longitudinal stripe along the median sutures of subcaudal scales).

Calamaria hunanensis **sp. nov.** differs from *C. strigiventris* Poyarkov, Nguyen, Orlov & Vogel, 2019 by: more ventral scales in males (181 vs. 130–157); fewer subcaudals in males (23 vs. 29–33); smaller Tail/Total Length ratio (8.0% vs. 13.8–17.9%); a light nuchal collar present (vs. absent); ventral body yellow (vs. ventral body bright yellow with three interrupted longitudinal black stripes, one wide in the middle and a pair on the lateral edge of the ventrals).

Calamaria hunanensis **sp. nov.** differs from *C. synergis* Zhang, Xu, Nguyen, Poyarkov, Vogel, Wang & Huang, 2025 by: rostral wider than high (vs. higher than wide); 6 scales surrounding paraparietal (vs. 5 scales); end of tail sharp-pointed (vs. obtuse point); and more ventral scales in males (181 vs. 161–166).

Calamaria hunanensis **sp. nov.** differs from *C. thanhi* Ziegler & Quyet, 2005 by: eye diameter slightly smaller than the eye-mouth distance (vs. larger); preocular present (vs. absent); dorsal scales reducing to four rows above 4th subcaudal scale anterior to tail tip (vs. not reduced); a light nuchal collar present (vs. absent); light ring on tail absent (vs. present).

Calamaria hunanensis **sp. nov.** differs from *C. yunnanensis* by: preocular present (vs. absent); maxillary teeth unmodified (vs. modified); posterior chin shields in contact at the anterior 1/3 part (vs. meeting at the midline); the third infralabial contacting near half of the posterior chin shields (vs. not contacting); more subcaudals in males (23 vs. 15–20); and ventral tail with short dark brown stripe on the tip of tail (vs. dark brown midventral stripe starting from the tail tip, breaking up near the start of the cloacal plate.)

Distribution and Ecology.

The new species is only known from the type locality Suining County, Shaoyang City, Hunan Province, China. It was found on a path in a planted pine forest on 7 July 2022 at an elevation of 690 m (Figure 5). The native vegetation of this area consists of subtropical evergreen broad-leaved forests. *Lycodon flavozonatus* (Pope, 1928) was found to be sympatric and could be a potential predator of the *C. hunanensis* sp. nov. The new species is rare at the type locality.

DISCUSSION

The new species is weakly supported as the sister taxon to *Calamaria nebulosa*. This phylogenetic uncertainty is likely due to limited information from a single gene for a group with multiple deeply divergent clades. The distinct morphological differences and deep divergence between the new species and other known species support its validity as a new species. The deep genetic divergence between the new species and other species implies that there might be more new species of the clade to be found in a large area from Guizhou and Guangxi, China, to Laos.

Calamaria quadrimaculata Duméril, Bibron & Duméril, 1854 is a synonym of *C. pavimentata* (Inger & Max, 1965). It was originally found in Java but was also recorded from mountains north of Kiujiang (Jiujiang, Jiangxi Province, China) later (Günther, 1864; Pratt, 1892). It differs from the new species by having ventrals 136–145 (vs. 181), subcaudals 13 (vs. 23), a neck with a broad blackish-brown collar edged with white anteriorly and posteriorly (vs. nuchal collar bright yellow without white edges); lower parts uniform white (vs. bright yellow), and an upper part of the tail with two pairs of white spots, one at its base, and the other on its extremity (vs. no light color) (Günther, 1864). Since no morphological description was provided by Pratt (1892) and the morphological description of the Java population (e.g. numbers of ventrals and subcaudals, the two pairs of white spots on the upper part of tail) resemble those of *C. septentrionalis*, whose type locality is Kiujiang, we could not exclude the possibility that the record of *C. quadrimaculata* from Kiujiang actually is *C. septentrionalis*.

The description of the new species brings the number of *Calamaria* in Hunan Province to three: *C. arcana*, *C. hunanensis* **sp. nov.** and *C. septentrionalis* (Shen et al., 2014; Wu et al., 2016; Zhang et al., 2023). Although several field surveys were conducted in the type locality and nearby, only one specimen of the new species was found. The population status, natural history and distribution area for the new species largely remain unknown. Further investigation on this rare species to determine its conservation status is strongly recommended.

Updated key to species of *Calamaria* from China.

- 1a Preocular absent *C. yunnanensis*
1b Preocular present 2

- 2a Dorsal scales reduced to 4 rows on tail at last subcaudals 3
2b Dorsal scales reduced to > 4 rows on tail at last subcaudals 6
3a Light rings/blotches on neck absent *C. andersoni*
3b Light rings/blotches on neck present 4
4a Light rings/blotches on tail absent *C. synergis*
4b Light rings/blotches on tail present 5
5a Tail tapering gradually to a point *C. pavimentata*
5b Tail not tapering, broadly rounded on tip
C. septentrionalis
6a End of tail sharp-pointed *C. hunanensis* **sp. nov.**
6b End of tail obtuse-pointed 7
7a Usually posterior chin shield meeting in the midline, collar or spots in nuchal region absent *C. jinggangensis*
7b Less than half of posterior chin shield meeting in the midline, indistinct light ring or one pair of spots in nuchal region present 8
8a Ventral scales 170–176 in males, 192 in females
C. arcana
8b Ventral scales 149–155 in males, 153–165 in females..... *C. berezowskii*

APPENDIX

Examined specimens

Calamaria arcana GXNU DLR199, adult female from Mt. Wuyi, Fujian, China.

Calamaria septentrionalis GXNU DL2021610-1 adult male from Guilin, Guangxi, China.

NOMENCLATURAL ACTS REGISTRATION

This study, along with the nomenclatural act it encompasses, has been registered with ZooBank, the official registry of the International Code of Zoological Nomenclature (ICZN). The publication can be accessed via the following identifier: <https://zoobank.org/References/8A22B6E8-3DD8-4095-9472-2D20E6080E0F>.

SCIENTIFIC FIELD SURVEY PERMISSION INFORMATION

Sample collections was approved by Forestry Bureau of Suining County (No. 2022-033) following the Wildlife Protection Law of the People's Republic of China.

DATA AVAILABILITY

Supplementary tables 1 and 2 of A new species of *Calamaria* Boie, 1827 (Squamata: Calamariidae) from Hunan Province, South Central China <https://doi.org/10.57760/sciencedb.34958>

Supplementary figures 1 and 2 of A new species of *Calamaria* Boie, 1827 (Squamata: Calamariidae) from Hunan Province, South Central China <https://doi.org/10.57760/sciencedb.34956>.

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHOR'S CONTRIBUTIONS

K. G., and S. S. designed the study; M.Z., W. Z., S. F., Q. L., conducted field work; M.Z., W. Z., Y. W., S. S. prepared the manuscript, performed morphological analyses, performed molecular and phylogenetic analyses; L. D., V. G., Z. C., Y. N., provided comparative materials and revised the manuscript. All authors read and approved the final version of the

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