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A new species of *Calamaria* (Squamata: Colubridae) from Guangdong Province, southern China

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Abstract

A new species of the genus *Calamaria* Boie, 1827, *Calamaria arcana* **sp. nov.**, is described based on a single male specimen collected from Mt. Dadongshan, Guangdong, southern China. The new species can be distinguished from all known congeners by the significant genetic divergence in the mitochondrial cytochrome-*b* gene fragment (*p*-distance ≥ 13.9%), and morphologically by the combination of the following characters: (1) ten modified maxillary teeth; (2) four supralabials, second and third supralabials entering orbit; (3) preocular present; (4) mental not touching anterior chin shields; (5) six scales and shields surrounding the paraparietal; (6) 170 ventral scales; (7) 22 paired subcaudals; (8) tail not gradually tapering, abruptly tapering at the tip; (9) dorsal scales reduced to five rows above last subcaudal at tail; (10) dorsum of body and tail brownish; (11) dark collar on nuchal region absent; (12) two outermost dorsal scale rows light yellow with upper margins partly dark pigmented; (13) ventral scales immaculate, without dark outermost corners and pigmentation anteriorly; and (14) absence of distinct dark longitudinal line or scattered spots on the underside of tail. *Calamaria arcana* **sp. nov.**, represents the fifth species of the genus recorded in China. Following the IUCN Red List Categories and Criteria, we propose the new species to be listed as Data Deficient.

Keywords

Calamaria arcana sp. nov., Calamariinae, integrative taxonomy, morphology, phylogeny, snake, Southeast Asia

Introduction

Southeast Asian reed snakes of the genus *Calamaria* Boie, 1827 are small semi-fossorial snakes ranging from north-east India to the Maluku Islands of east Indonesia (Inger and Marx 1965; Uetz et al. 2022). Members of the genus can be easily identified based upon the combination of the following morphological characters: dorsal scales in 13 rows throughout body; internasals and prefrontals fused; parietal broadly in contact with supralabials (Inger and Marx 1965). Currently, the genus *Calamaria* contains 66 species worldwide, with most species occurring only in the Greater Sunda Islands and Philippines (Inger and Marx 1965; Uetz et al. 2022). In mainland Southeast Asia and China, 18 species of *Calamaria* are known at present, including *C. abramovi* Orlov, 2009; *C. albiventer* (Gray, 1834); *C. andersoni* Yang & Zheng, 2018; *C. buchi* Marx & Inger, 1955; *C. concolor* Orlov, Nguyen, Nguyen, Ananjeva & Ho, 2010; *C. dominici* Ziegler, Tran & Nguyen,

2019; *C. gialaiensis* Ziegler, Nguyen & Nguyen, 2009; *C. lovii* Boulenger, 1887; *C. lumbricoidea* Boie, 1827; *C. nebulosa* Lee, 2021; *C. pavimentata* Duméril, Bibron & Duméril, 1854; *C. prakkei* Lidth De Jeude, 1893; *C. sangi* Nguyen, Koch & Ziegler, 2009; *C. schlegeli* Duméril, Bibron & Duméril, 1854; *C. septentrionalis* Boulenger, 1890; *C. strigiventris* Poyarkov, Nguyen, Orlov & Vogel, 2019; *C. thanhi* Ziegler & Quyet, 2005; and *C. yunnanensis* Chernov, 1962 (Smith 1943; Inger and Marx 1965; Darevsky and Orlov 1992; Zhao et al. 1998; Ziegler and Quyet 2005; Yang and Rao 2008; Ziegler et al. 2008; Nguyen et al. 2009; Orlov 2009; Nguyen et al. 2010; Orlov et al. 2010; Yang and Zheng 2018; Poyarkov et al. 2019; Quah et al. 2019; Ziegler et al. 2019; Lee 2021).

During long-term and extensive biodiversity surveys conducted by Kadoorie Farm and Botanic Garden in Southern China between 1997 and 2002, a *Calamaria* specimen was collected from Mt. Dadongshan of Guangdong Province, and initially identified as *Calamaria pavimentata* in the survey report (Kadoorie Farm and Botanic Garden 2003). Recent molecular analysis and detailed morphological examinations revealed that the specimen showed remarkable morphological differences from *C. pavimentata* and all other members of the genus, and represented a separately evolving lineage within the genus *Calamaria*. Herein, we describe the specimen as a new species of *Calamaria* incorporating morphological and molecular data.

Materials and methods

Sampling

The specimen were preserved in 80% ethanol and deposited at the Herpetological Collection of Kadoorie Farm and Botanic Garden (**KFBG**), Hong Kong SAR, under voucher no. KFBG 14611.

DNA extraction and sequencing

Genomic DNA was extracted from the muscle tissue using a Qiagen DNeasy Blood & Tissue Kit (Qiagen Biotech). We amplified fragments of mitochondrial 12S rRNA, 16S rRNA, and cytochrome-*b* (cyt-*b*) genes using the primer pairs L1091/H1478 (Kocher et al. 1989), L3975/ H4551 (Simon et al. 1994), and L14910/H16064 (Lawson et al. 2005), respectively following Yang and Zheng (2018). PCR amplifications were performed in a reaction volume of 25 ul containing 100 ng of template DNA, 0.3 mM of each PCR primer, and 10 ul Premix EX TaqTM (Takara). The PCR conditions were an initial denaturing step at 95°C for 4 min, followed by 35 cycles of denaturing at 94°C for 30 s, annealing at 52°C for 30 s, an extension step at 72°C for 1 min; and a final extension of 72°C for 7 min. PCR products were purified with spin columns. The PCR Amplification products were checked on 1.5% agarose gel and then sent to BGI-Hong Kong for purification and bidirectional sequencing. The purified products were sequenced with respective PCR forward and reverse primers using ABI BigDye Terminator Kit v3.1 (Thermo Fisher Scientific Inc., Waltham, MA) according to the guidelines of the manufacturer. The PCR products were sequenced on an ABI 3730xl DNA Analyzer at the BGI Tech Solutions (Hong Kong) Co., Ltd.

Phylogenetic analyses

In addition to our new specimen of *Calamaria*, we included sequences of all species of *Calamaria* in mainland Southeast Asia and China for which 12S rRNA, 16S rRNA, and cyt-*b* sequences were available from GenBank for genetic analysis (for accession numbers see Table 1). *Elaphe quatuorlineata*, *Orientocoluber spinalis*, and *Lycodon rufozonatus* were used as outgroups (Yang and Zheng 2018). Successful sequences from samples were checked and edited in Geneious Prime 2022.0.1 software (Kearse et al. 2012). DNA sequences were aligned in MEGA 10.1.8 (Kumar et al. 2018) by the Clustal W algorithm with default parameters (Thompson et al. 1997). Phylogenetic trees were constructed based on a concatenated dataset 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 analyses were performed in MrBayes 3.2.6 (Ronquist et al. 2012) under the GTR+G model as suggested by the Akaike Information Criterion implemented in jModelTest 2.1.2 (Darriba et al. 2012). We employed two separate MCMC runs, each with four Metropolis-coupled chains. The analyses were run for 2.5 million generations, with parameters and topology sampling every 1000 generations. The stationary phase was detected using Tracer 1.6 (Rambaut et al. 2014). The first 1000 trees were discarded as burn-in, and posterior probabilities were determined from the remaining trees. We also calculated the uncorrected pairwise genetic distances (*p*-distance) using MEGA 10.1.8 (Kumar et al. 2018).

Morphometrics

Terminology and measurements follow Inger and Marx (1965) and Ziegler et al. (2008). Measurements were taken by JHY and HYY to the nearest 0.1 mm with digital calliper (Mitutoyo CD-6 CS); ventral scales were counted after Dowling (1951). Sex was determined by the presence of hemipenis. The measurements were as follows:

EyeD eye horizontal diameter; **Eye–Mouth D** eye–mouth distance, measured from the anterior point of the

Table 1. Samples and sequences used for phylogenetic analysis in this study.

Species	Voucher no.	Locality	GenBank no. (12S, 16S, cyt-*b*)
(1) *Calamaria arcana* **sp. nov.**	KFBG 14611	Mt. Dadongshan, Guangdong, China	ON464176, ON464177, ON482335
(2) *C. septentrionalis*	KFBG 14506	Hainan Island, China	MH445960, MH445962, MH445956
(3) *C. septentrionalis*	FTB2839	Unknown	KR814612, KR814637, KR814699
(4) *C. septentrionalis*	ROM 35597	Cao Bang, Vietnam	KX694584, KX694624, KX694890
(5) *C. septentrionalis*	ROM 35605	Cao Bang, Vietnam	—, —, AF471081
(6) *C. pavimentata*	KFBG 14507	Ningming, Guangxi, China	MH445959, MH445963, MH445957
(7) *C. yunnanensis*	ROM 41547	Simao, Yunnan, China	KX694572, KX694625, KX694891
(8) *C. yunnanensis*		Unknown	JQ598801, JQ598863, JQ598922
(9) *C. andersoni*	SYS r001699	Yingjiang, Yunnan, China	MH445958, MH445961, MH445955
(10) *C. nebulosa*	FMNH 258666	Phongsaly, Laos	—, MW699929, MN338524
(11) *C. lumbricoidea*	USMHC 1450	Penang, Peninsular Malaysia	—, —, MN338526
(12) *C. schlegeli*	LSUHC 10278	Perak, Peninsular Malaysia	—, —, MN338525
(13) *Elaphe quatuorlineata*	LSUMZ 40626	Turkey, European Turkey	AY122798, AF215267, AY486931
(14) *Lycodon rufozonatus*	LSUMZ 44977	Unknown	AF233939, HM439978, AF471063
(15) *Orientocoluber spinalis*	MVZ 211019	Yinnan, Ningxia, China	AY541508, AY376773, AY486924

eye to the mouth; **HL** head length (from tip of snout to posterior margin of the mandible); **HW** maximum head width; **SVL** snout–vent length (from tip of snout to posterior margin of cloacal plate); **TaL** tail length (from posterior margin of cloacal plate to tip of tail); **TL** total length (from tip of snout to tip of tail); **TaL/TL** ratio tail length/total length.

Comparative morphological data from other species of *Calamaria* found in mainland Southeast Asia and China were obtained from examined specimens (Appendix 1) and literature (Smith 1943; Inger and Marx 1965; Darevsky and Orlov 1992; Zhao et al. 1998; Ziegler and Quyet 2005; Yang and Rao 2008; Ziegler et al. 2008; Orlov 2009; Nguyen et al. 2010; Orlov et al. 2010; Yang and Zheng 2018; Poyarkov et al. 2019; Ziegler et al. 2019; Lee 2021).

Results

Molecular results

The fragments of mitochondrial 12S rRNA, 16S rRNA, and cytochrome-*b* (cyt-*b*) genes of specimen KFBG 14611 were successfully extracted. Both the ML tree and BI analyses based on the concatenated alignment strongly support the placement of KFBG 14611 from Mt. Dadongshan in the genus *Calamaria* (Fig. 1) and KFBG 14611 was found to be the sister taxon of *C. septentrionalis* with high levels of nodal support (100/1.0 for BI and ML values, respectively). Together these taxa formed a genetically distinct lineage and was clustered into the same clade with *C. andersoni*, *C. yunnanensis*, *C. pavimentata* and *C. nebulosa* (Fig. 1).

The uncorrected pairwise divergence of the cyt-*b* fragments between the specimen KFBG 14611 and other seven congeners included in the study were ≥ *p* = 13.9%, with the minimum value observed in the comparison with sequences of *C. septentrionalis* (*P* = 13.9–14.8%) (see Table 2). These levels of divergences are distinctly higher than those observed between two other well-distinguished species, *C. andersoni* and *C. yunananensis* (*P* = 10.2–10.5%), and are also typically indicative of differentiation at the species level in vertebrates (John and Avise 1998; Kartavtsev 2011). As the observed molecular differences are complemented by significant differences in morphology from all other congeners (see below), we therefore consider the unknown *Calamaria* specimen as a new species.

Taxonomic account

Calamaria arcana sp. nov.

https://zoobank.org/CFCB3C7F-842E-4D6D-A11F-C77189B11224

Figs 2, 3, 4, 5G–I, 6G–I

Calamaria pavimentata – Kadoorie Farm and Botanic Garden (2003); Li et al. (2011).

Suggested common names. Lingnan Reed Snake (English), 岭南两头蛇 (Chinese)

Holotype. KFBG 14611 (Figs 2, 3, 4, 5G–I, 6G–I), male, Mt. Dadongshan, Nanling National Nature Reserve, Lianzhou County, Qingyuan City, Guangdong Province, China (中国广东南岭国家级自然保护区大东山管理站). The specimen was found at between the hydroelectric station near Dadongshan Management Station (24°55’58.5”N 112°42’51.8”E) and Shizi Ping (石仔坪), 800–900 m above sea level, collected by Michael W.N. Lau on 7 July 2000 at 9:20 am.

Table 2. Uncorrected *p*-distances (%) among the sequences based on the cyt-*b* gene fragment. The numbers of base substitutions per site from between sequences are shown.

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)
(1) *Calamaria arcana* **sp. nov.**	—														
(2) *C. septentrionalis*	14.8	—													
(3) *C. septentrionalis*	13.9	2.0	—												
(4) *C. septentrionalis*	14.3	5.2	4.6	—											
(5) *C. septentrionalis*	14.4	6.0	4.9	0.0	—										
(6) *C. pavimentata*	22.2	18.6	17.7	19.2	19.8	—									
(7) *C. yunnanensis*	21.2	16.4	16.2	15.9	15.9	20.6	—								
(8) *C. yunnanensis*	20.9	16.2	16.0	15.9	15.7	20.4	0.0	—							
(9) *C. andersoni*	20.7	16.8	16.0	16.7	16.6	22.0	10.5	10.3	—						
(10) *C. nebulosa*	21.1	18.8	19.3	19.8	19.6	21.6	19.4	19.2	19.4	—					
(11) *C. lumbricoidea*	27.1	25.4	24.8	24.5	24.8	27.1	26.3	25.8	26.0	24.4	—				
(12) *C. schlegeli*	27.5	23.1	23.2	24.1	23.5	24.7	25.3	24.9	24.6	21.4	19.9	—			
(13) *Elaphe quatuorlineata*	27.7	25.2	24.5	25.3	25.6	27.8	25.7	25.3	27.5	26.6	25.4	25.9	—		
(14) *Lycodon rufozonatus*	28.4	25.7	24.4	26.3	26.2	28.1	29.7	29.2	28.4	28.5	24.7	25.3	17.8	—	
(15) *Orientocoluber spinalis*	28.9	26.9	26.3	26.5	27.2	31.0	28.3	27.8	28.3	26.8	26.1	26.0	20.1	20.2	—

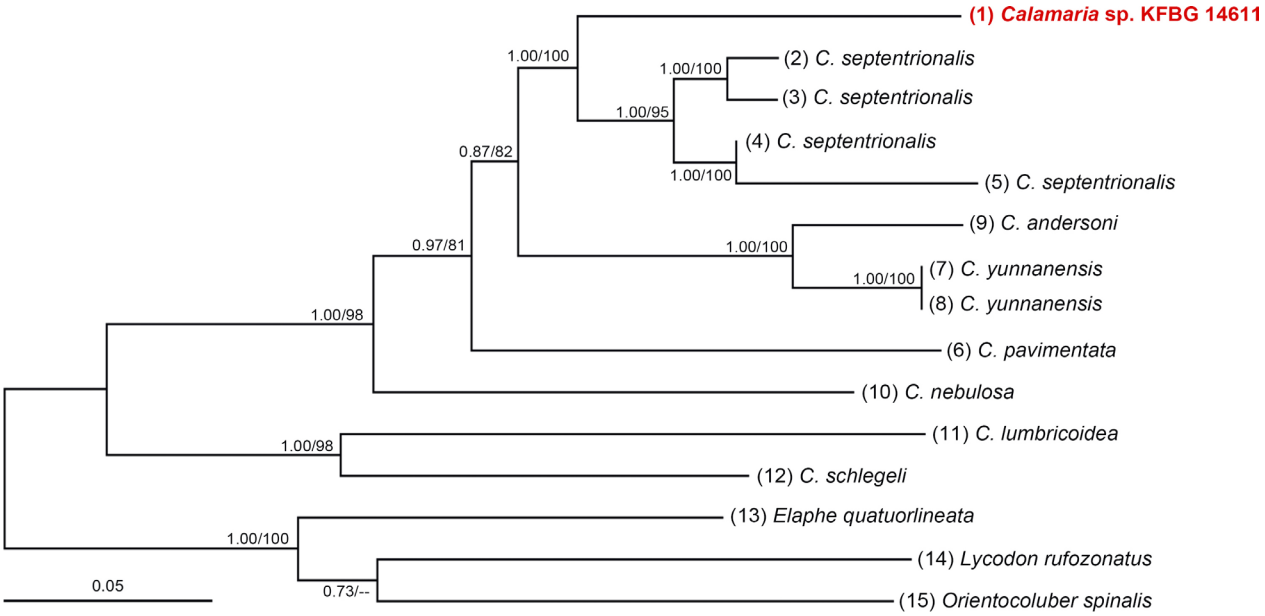

Figure 1. Bayesian inference (BI) tree derived from partial sequences of the combined fragments of 12S rRNA, 16S rRNA, and cyt-*b* genes. Numbers above branches are Bayesian posterior probabilities (>70% retained), and numbers below branches indicate bootstrap support values for maximum likelihood analyses (>70 retained).

Etymology. The species epithet "arcana" is the nominative form the Latin adjective "arcanus" meaning "hidden", "secret" and is given in feminine form to match the female genitive declension of the genus name *Calamaria*. It refers to the fact that this species is difficult to find with only a single specimen discovered during our long term survey in southern China. For common name we suggested as "Lingnan reed snake" in English and "Ling Nan Liang Tou She (岭南两头蛇)" in Chinese, referring to the Lingnan (岭南, means 'South of the Nanling Mountains') region where the new species is found. Lingnan is a geographic area referring to the lands in the south of the Nanling Mountains, covering the modern Chinese provinces of Guangdong, Guangxi and Hainan, as well as northern Vietnam.

Diagnosis. *Calamaria arcana* **sp. nov.** is assigned to the genus *Calamaria* by its molecular phylogenetic position and the following morphological characters: dorsal scales in 13 rows throughout body, possessing elongate, cylindrical bodies, along with internasals and prefrontals fused, four supralabials and five infralabials (Inger and Marx 1965; Ziegler et al. 2008).

Calamaria arcana **sp. nov.** could be distinguished from all other species of *Calamaria* by the combination of the following characters: (1) ten modified maxillary teeth; (2) four supralabials, second and third supralabials entering orbit; (3) preocular present; (4) mental not touching anterior chin shields; (5) six scales and shields surrounding the paraparietal; (6) 170 ventral scales; (7) 22 paired subcaudals; (8) tail not gradually tapering, abruptly tapering

Figure 2. Holotype of *Calamaria arcana* **sp. nov.** (KFBG 14611) in life: **A** dorsolateral view **B** ventral view **C** lateral view of left side of head. Photographs by Michael W.N. Lau.

at the tip; (9) dorsal scales reduced to five rows above last subcaudal at tail; (10) dorsum of body and tail brownish; (11) dark collar on nuchal region absent; (12) two outermost dorsal scale rows light yellow with upper margins partly dark pigmented; (13) ventral scales immaculate, without dark outermost corners and pigmentation anteriorly; and (14) absence of distinct dark longitudinal line or scattered spots on the underside of tail.

Description of holotype. Male. Rostral wider than high (height 1.8 mm, width 2.2 mm), portion visible from above about equal to prefrontal suture; prefrontal shorter

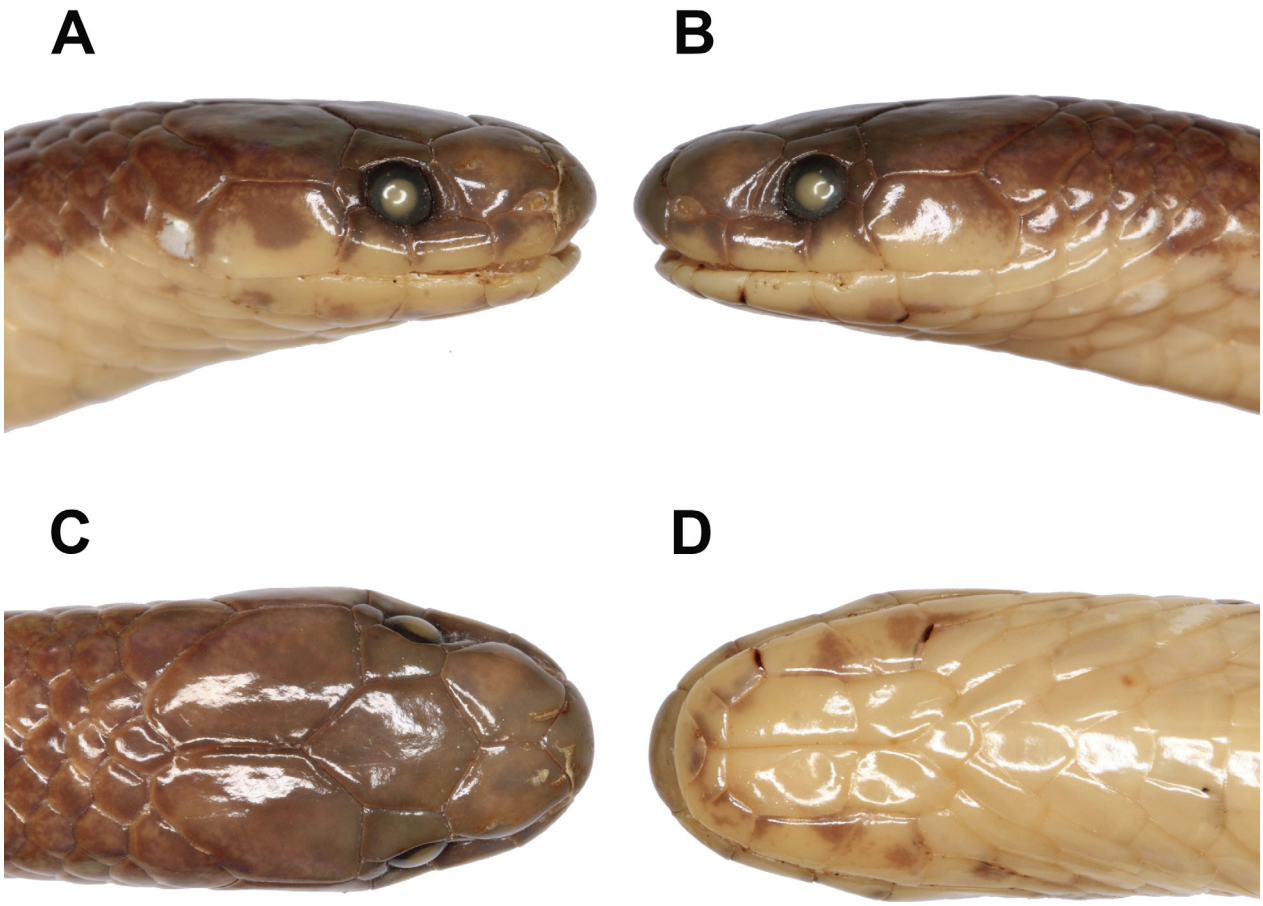

Figure 3. Head of the holotype of *Calamaria arcana* **sp. nov.** (KFBG 14611): **A** right side **B** left side **C** dorsal view **D** ventral view.

than frontal, not entering orbit, and touching first two supralabials; frontal hexagonal, about 2.2 times maximum width of supraocular; paraparietal surrounded by six shields and scales; a single preocular present, higher than postocular; postocular one, higher than wide, not as high as eye diameter; eye diameter 1.4 mm, larger than eye-mouth distance 0.8 mm; distance from anterior corner of eye to nostril 1.9 mm and to the tip of snout 3.1 mm; pupil rounded; supralabials 4/4 (left/right, hereafter), second and third entering orbit, fourth longest, third about 0.9 times of second in length, first shorter than third and as wide as second; mental semicircular to triangular, not touching anterior chin shields; infralabials 5/5, first three touching anterior chin shields; first pair of chin shields meeting in midline, second pair touching anteriorly and separated posteriorly by first gular scales; three gulars in midline between second chin shields and first ventrals; dorsal scales in 13 rows throughout body, reducing to eight rows above 3rd subcaudal, to seven rows above 8th subcaudal, to six rows above 10th subcaudal, and to five rows above the last subcaudal on tail; ventral scales 170, subcaudals 22, divided, followed by a shield covering tail tip; anal scale single.

Head length 7.1 mm; head width 5.1 mm; SVL 281.5 mm; TaL 21.7 mm; TL 303.2 mm; body thickness about 5.8 to 6.4 mm; body thickness index at mid body 0.021; tail root 4.5 mm thick; tail not as thick as body, slightly tapering then abruptly tapering at tip; TaL/TL ratio 7.2%; maxillary teeth modified, 10/10.

Coloration of the holotype in life. Dorsal surface of body grey-brown above, somewhat iridescent; dark collar at nuchal region absent; indistinct light ring behind head present, nearly invisible; a narrow interrupted light ring at root of tail present, followed by few light spots on middle and tip of tail. Underside of head orangish-beige with black flecks on the infralabials; ventral scales of body and tail immaculate orangish-red, without dark pigmentation on outermost corners and anterior margins; underside of tail without dark longitudinal line or blotches.

Coloration in preservation. Color preserved in ethanol fade to caramel-brown above. Dorsal head and upper parts of supralabials brown, lower parts of supralabials light yellow; underside of head light yellow with brownish spots on the infralabials; presence of an indistinct narrow yellow ring about 5 scales wide on neck, nearly invisible; dorsum of body caramel-brown, with light flecks on each dorsal scale; two outermost dorsal scale rows light yellow with upper margins partly dark pigmented; a narrow interrupted light ring at root of tail, light spots on middle and tip of tail still visible; ventral scales of body and tail uniformly light yellowish-beige; underside of tail without dark median line or blotches.

Comparisons. We compared *Calamaria arcana* **sp. nov.** with 18 congeners of the genus *Calamaria* from China and mainland Southeast Asia listed above. Unrelated taxa

Figure 4. Holotype of *Calamaria arcana* **sp. nov.** (KFBG 14611): **A** dorsal view **B** ventral view.

from the Greater Sunda Islands are omitted from comparisons for the sake of simplicity.

Calamaria arcana **sp. nov.** can be readily distinguished from *C. albiventer*, *C. lumbricoidea*, *C. prakkei* and *C. schlegeli* by having lower number of supralabials (four vs. five), and the pattern of supralabials shields touching the orbit (2nd and 3rd supralabials touching orbit vs. 3rd and 4th supralabials touching orbit). Additionally, these four species only occur south of the Isthmus of Kra in Peninsular Malaysia where is extremely far from the type locality of *C. arcana* **sp. nov.**

Calamaria arcana **sp. nov.** can be further easily distinguished with *C. lovii*, *C. nebulosa*, *C. thanhi* and *C. yunnanensis* by the presence of a preocular scale (vs. absence of preocular scale).

Calamaria arcana **sp. nov.** differs from *C. abramovi* by having eye diameter larger than distance from eye to mouth edge (vs. reverse condition in *C. abramovi*), posterior chin shields meeting in midline (vs. separated in *C. abramovi*), dorsal scales reduced to five rows at tail (vs. reduced to four rows in *C. abramovi*), and a distinctly different coloration (body black and with yellow-orange spots on venter in *C. abramovi*).

Calamaria arcana **sp. nov.** differs from *C. andersoni* by having dorsal scales reduced to five rows at tail (vs. reduced to four rows in *C. andersoni*), light color spots at root of tail present (vs. absent in *C. andersoni*. Fig. 6), ventral scales immaculate, without dark outermost corners (vs. presence of dark outermost corners on ventral scales in *C. andersoni*, Fig. 6), two outermost dorsal scale rows light yellow with upper margins partly dark pigmented (vs. all dorsal scale rows in same coloration in *C. andersoni*, Fig. 5).

Calamaria arcana **sp. nov.** differs from *C. buchi* by having rostral shield width larger than length (vs. reverse condition in *C. buchi*), eye diameter larger than distance from eye to mouth edge (vs. equal to or shorter in *C. buchi*), mental not touching anterior chin shields (vs. touching in *C. buchi*), dorsal scales reduced to five

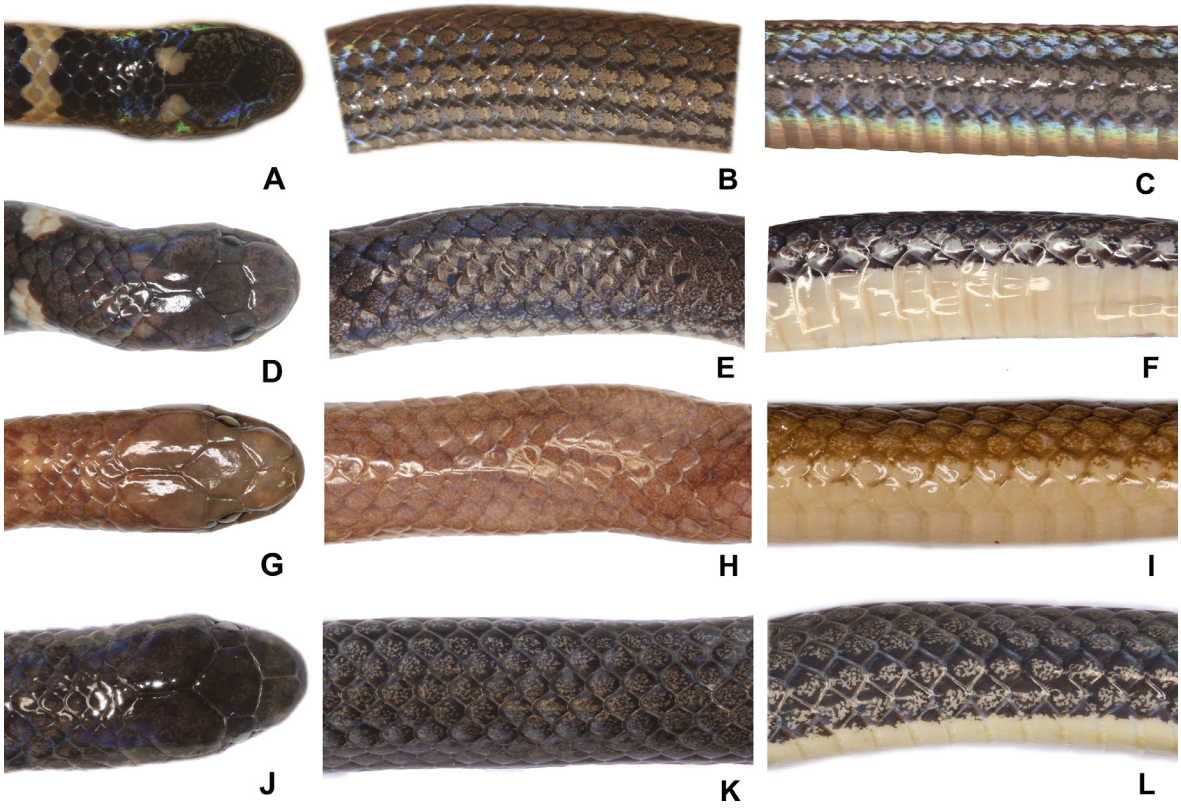

Figure 5. Comparative characters of the head shape and body coloration of *Calamaria arcana* **sp. nov.**, *C. andersoni*, *C. septentrionalis*, *C. pavimentata*. **A–C** dorsal view of head and body, and dorsolateral view of body of *C. pavimentata* (KFBG 14507). **D–F** dorsal view of head and body, and dorsolateral view of body of *C. septentrionalis* (KFBG 14506). **G–I** dorsal view of head and body, and dorsolateral view of body of holotype of *C. arcana* **sp. nov.** (KFBG 14611). **J–L** dorsal view of head and body, and dorsolateral view of body of *C. andersoni* (SYS r001699).

rows at tail (reduced to four rows in *C. buchi*), and six shields and scales surrounding paraparietal (vs. five in *C. buchi*).

Calamaria arcana **sp. nov.** differs from *C. concolor* by having lower number of supralabials (4 vs. 5 in *C. concolor*), eye diameter larger than distance from eye to mouth edge (vs. reverse condition in *C. concolor*), mental not touching anterior chin shields (vs. touching in *C. concolor*), tail not flatted (vs. slight flatted laterally in *C. concolor*), dorsal scales reduced to five rows at tail (vs. reduced to four rows in *C. concolor*), and lower number of ventral scales in males (170 vs. 209 in *C. concolor*).

Calamaria arcana **sp. nov.** differs from *C. dominici* by having dorsal scales reduced to five rows at tail (vs. reduced to four rows in *C. dominici*), absence of distinct yellow blotches on dorsum (vs. present in *C. dominici*), venter immaculate without any dark stripes or spots (vs. ventral side dark with yellow blotches and bands).

Calamaria arcana **sp. nov.** differs from *C. gialaiensis* by having mental not touching anterior chin shields (vs. touching in *C. gialaiensis*), lower number of ventral scales (170 in males vs. 191 in unknown gender), dorsal scales reduced to five rows at tail (vs. reduced to four rows in *C. gialaiensis*), absence of a dark longitudinal line under of tail (vs. present in *C. gialaiensis*), and absence of dark collar in nuchal region (vs. an indistinct dark collar present in nuchal region in *C. gialaiensis*).

Calamaria arcana **sp. nov.** differs from *C. sangi* by having mental not touching anterior chin shields (vs. touching in *C. sangi*), dorsal scales reduced to five rows at tail (vs. reduced to four rows in *C. sangi*), lower number of ventral scales (170 vs. 190 in *C. sangi*), absence of dark collar in nuchal region (vs. presence of dark collar in nuchal region in *C. sangi*), and venter immaculate (vs. venter cream with dark transverse bands in *C. sangi*).

Calamaria arcana **sp. nov.** differs from *C. strigiventris* by having eye diameter larger than distance from eye to mouth edge (vs. reverse condition in *C. strigiventris*), lower number of subcaudals in males (22 vs. 29–33 in males), higher number of ventrals in males (170 vs. 130–157), dorsal scales reduced to five rows at tail (vs. reduced to four rows in *C. strigiventris*), venter immaculate (vs. presence of three interrupted longitudinal black stripes in *C. strigiventris*).

Calamaria arcana **sp. nov.** differs from *C. pavimentata* by having rostral shield width larger than length (vs. reverse condition in *C. pavimentata*), tail slowly tapering anteriorly and abruptly tapering at tip (vs. tail tapering gradually to a point in *C. pavimentata*, Fig. 6), dorsal scales reduced to five rows at tail (vs. reduced to four

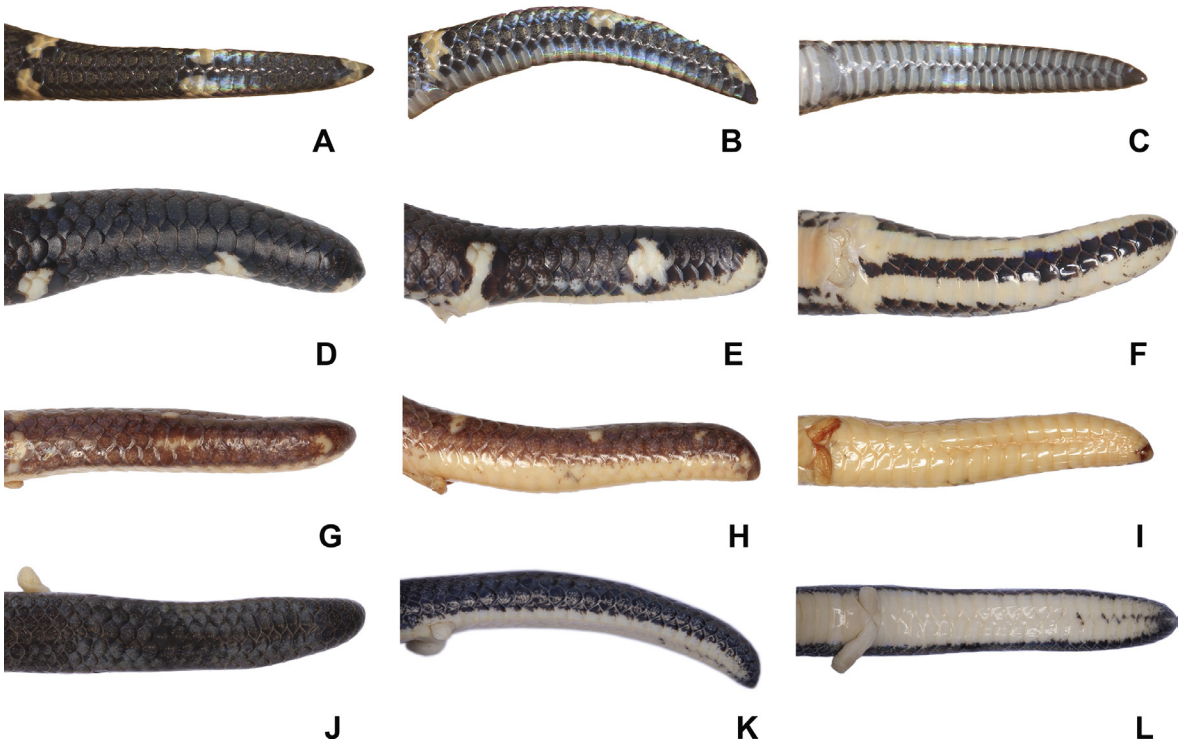

Figure 6. Comparison of the tail shape and coloration among *Calamaria arcana* **sp. nov.**, *C. andersoni*, *C. septentrionalis* and *C. pavimentata*. **A–C** dorsal, lateral and ventral views of the tail of *C. pavimentata* (KFBG 14507). **D–F** dorsal, lateral and ventral views of the tail of *C. septentrionalis* (KFBG 14506). **G–I** dorsal, lateral and ventral views of the tail of holotype of *C. arcana* **sp. nov.** (KFBG 14611). **J–L** dorsal, lateral and ventral views of the tail of *C. andersoni* (SYS r001699).

rows in *C. pavimentata*), absence of dark collar at nuchal region (vs. present in *C. pavimentata*, Fig. 5), absence of distinct narrow and dark longitudinal lines on dorsum (vs. present in *C. pavimentata*, Fig. 5), absence of dark outermost corners on ventral scales (vs. ventral scales with dark outermost corners in *C. pavimentata*, Fig. 6).

Calamaria arcana **sp. nov.** can be distinguished from the sister taxon of *C. septentrionalis* by having tail abruptly tapering at the tip of end (vs. tail not tapering in *C. septentrionalis*), higher number of maxillary teeth (10 vs. 8–9 in *C. septentrionalis*), higher number of ventral scales in males (170 vs. 148–166 in *C. septentrionalis*), higher numbers of subcaudals in males (22 vs. 15–19 in *C. septentrionalis*), dorsal scales reduced to five rows at tail (vs. reduced to four rows in *C. septentrionalis*), absence of black line or spots on venter of tail (vs. venter of tail with broad and distinct median black stripe), absence of dark outermost corners on ventral scales (vs. present in *C. septentrionalis*, Fig. 6), two outermost dorsal scale rows light yellow with upper margins partly dark pigmented (vs. all dorsal scale rows in same coloration in *C. septentrionalis*, Fig. 5).

Natural history, distribution and conservation status. The holotype was found active on a cloudy morning along a trail in tall shrubland and secondary forest.

Calamaria arcana **sp. nov.** is currently only known from a single specimen from its type locality, Mt. Dadongshan, Nanling National Nature Reserve, Guangdong Province, China (Fig. 7). It can be expected that the new species should be distributed across adjacent mountain areas, but in view of the uncertainty of its extent of occurrence, and little information on its ecology and threats, we recommend the new species be listed as Data Deficient (DD) according the International Union for Conservation of Nature (IUCN) Red List categories and Criteria (IUCN 2022).

Discussion

The description of *Calamaria arcana* **sp. nov.** brings the total number of *Calamaria* species in China to five, namely *C. andersoni*, *C. arcana*, *C. pavimentata*, *C. septentrionalis* and *C. yunnanensis*. While new species of *Calamaria* were continuously described from adjacent country Vietnam in the past decades (Poyarkov et al. 2019; Ziegler et al. 2019), it can be expected that the diversity of the genus in southern China is still highly underestimated. Probably due to their fossorial behaviour and secretive burrowing habits, species of the genus *Calamaria* are not commonly encountered in the wild, which may explain why most species in the region are known only from very limited specimens, and often described based on a single specimen (see Yang and Zheng 2018; Lee 2021 for examples). Molecular sequences of the many congeners were also limited which impedes further in-deep research on the systematic and phylogeny of the genus.

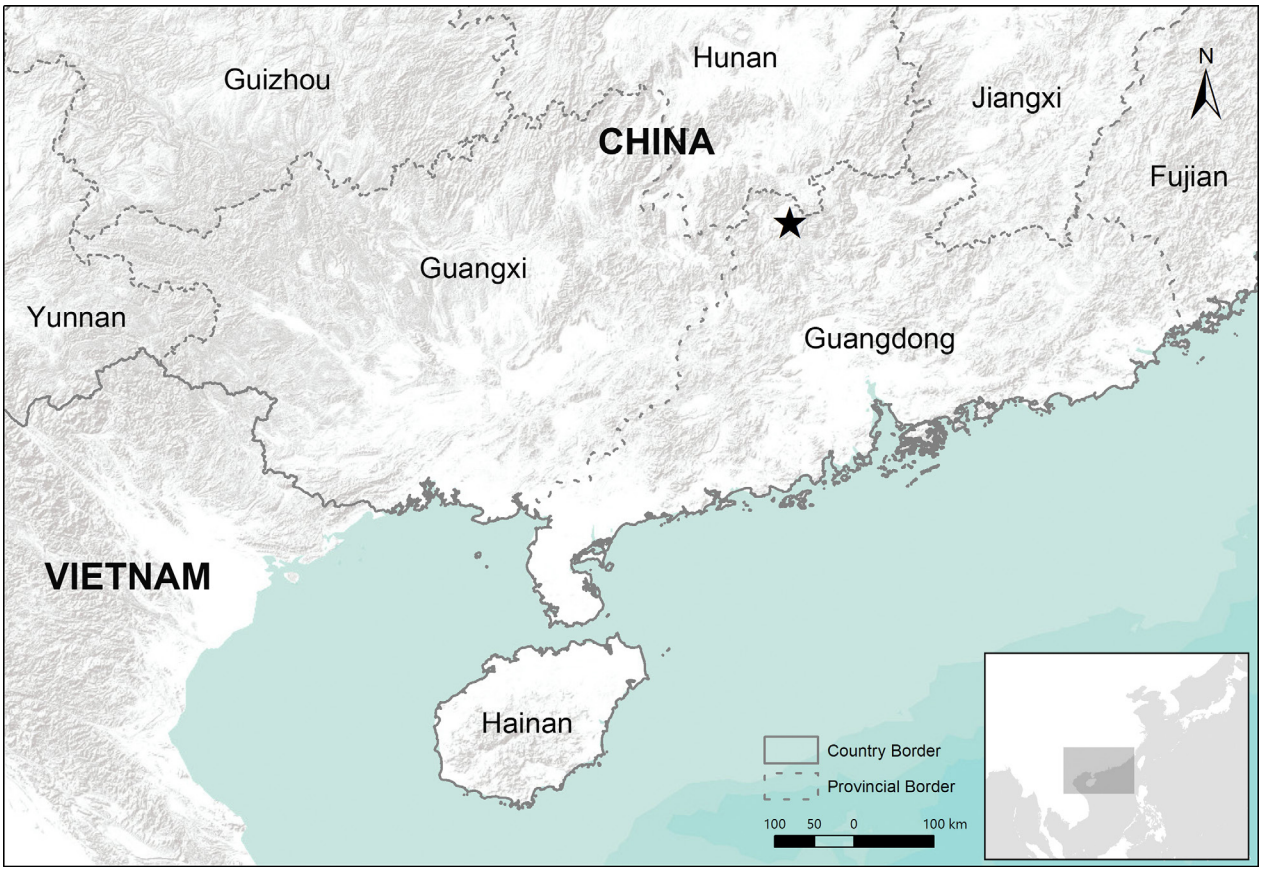

Figure 7. Map of Southern China showing the type locality of *Calamaria arcana* **sp. nov.**; Mt. Dadongshan, Lianzhou County, Guangdong Province, China (star).

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Appendix 1

Examined specimens

Calamaria septentrionalis (*N* = 3). China: Hainan: Bawangling Nature Reserve: KFBG 14506; China: Hong Kong: KFBG 14613; China: Guangdong: Baiyong Nature Reserve: KFBG 14614.

Calamaria pavimentata (*N* = 3). China: Guangxi: Nonggang Nature Reserve: KFBG 14507, SYS r001725–1726.

Calamaria andersoni (*N* = 1). China: Yunnan: Yingjiang County: SYS r001699.