A New Species of the Genus *Calamaria* (Squamata: Colubridae) from Yunnan Province, China

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A new reed snake of the genus *Calamaria* Boie, 1827, *Calamaria andersoni*, new species, is described on the basis of a single male specimen collected from Yingjiang County, Yunnan Province, China. The new species can be distinguished from other congeners by the unique combination of the following morphological characters: nine modified maxillary teeth; four supralabials, second and third supralabials entering orbit; one preocular; mental not touching anterior chin shields; six shields and scales surrounding the paraparietal; dorsal scales in 13:13:13 rows; 171 ventral scales; 23 subcaudals; tail slowly tapering anteriorly, then abruptly tapering at tip; dorsal scales reduced to four rows on tail at last two subcaudals; dorsum of body and tail brownish with indistinct narrow black stripes on sides; dark collar on neck absent; light rings/blotches on neck and tail absent; and ventral scales with dark outermost corners. *Calamaria andersoni*, new species, is the fourth species of the genus recorded from China.

THE colubrid genus *Calamaria* Boie, 1827 is one of the largest groups of Asian snakes and extends from the Ryukyu Islands of Japan, southwest through mainland China and Southeast Asia, to Sumatra and adjacent islands, eastward to Sulawesi (Inger and Marx, 1965; Grismer et al., 2004). Currently, the genus *Calamaria* contains 61 species worldwide, with most species only occurring in the Great Sunda Islands (Inger and Marx, 1965; Uetz et al., 2017). In southern China, only three species of *Calamaria* are known at present, namely *C. pavimentata* Duméril, Bibron, and Duméril, *C. septentrionalis* Boulenger, and *C. yunnanensis* Chernov (Zhao et al., 1998; Zhao, 2006). In adjacent Vietnam, five new species of *Calamaria* have been described in the past decade, greatly increasing the number of species of *Calamaria* from four to nine in the country (Ziegler and Quyet, 2005; Ziegler et al., 2008; Nguyen et al., 2009, 2010; Orlov, 2009; Orlov et al., 2010).

During recent field surveys in the western part of Yunnan Province, China in May 2016, we collected an unknown colubrid specimen which could be assigned to the genus *Calamaria* based on 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). Subsequent morphological and molecular analyses revealed that this individual is distinct from three species of *Calamaria* known from China and all other recognized congeners by a combination of morphological characters and molecular divergences, and represents an undescribed species. Herein, we describe this specimen as a new species of *Calamaria*.

MATERIALS AND METHODS

Sampling.—The specimen examined in this study was preserved in 75% ethanol and deposited at the Biological Museum of Sun Yat-sen University (SYS), Guangzhou.

DNA extraction and sequencing.—Genomic DNA was extracted from the muscle tissue using a TIANamp Genomic DNA Kit (Tiangen Biotech). We amplified fragments of mitochondrial 12S rRNA, 16S rRNA, and cytochrome *b* (cyt-*b*) genes using the primer pairs L1091/H1478 (Chen et al., 2013), L3975/ H4551 (Simon et al., 1994), and L14910/H16064 (Lawson et al., 2005), respectively. PCR amplifications were performed in a reaction volume of 25 μl containing 100 ng of template DNA, 0.3 mM of each PCR primer, and 10 μl 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 purified products were sequenced with respective forward and reverse primers using BigDye Terminator Cycle Sequencing Kit (Thermo Fisher Scientific Inc., Waltham, MA) according to the guidelines of the manufacturer. The PCR products were sequenced on an ABI Prism 3730 automated DNA sequencer at the ShangHai Majorbio Bio-pharm Technology Co., Ltd.

Phylogenetic analyses.—In addition to our new specimen of *Calamaria*, we included sequences of all species of *Calamaria* for which 12S rRNA, 16S rRNA, and cyt-*b* sequences were available from GenBank for genetic analysis (Table 1). *Elaphe quatuorlineata, Hierophis spinalis,* and *Lycodon rufozonatus* were used as outgroups. The sequences were aligned by Muscle 3.6 (Edgar, 2004) under default settings, checked by eye, and adjusted if necessary. 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 for 12S and 16S sequences, and HKY+I model for cyt-*b* sequences 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 using MEGA 6.06 (Tamura et al., 2013).

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

Species	Locality	Voucher no.	GenBank no. (12S, 16S, cyt-*b*)
(1) *Calamaria,* new species	Yingjiang, Yunnan, China	SYS r001699	MH445958, MH445961, MH445955
(2) *C. septentrionalis*	Hainan Island, China	KFBG 14506	MH445960, MH445962, MH445956
(3) *C. septentrionalis*	Unknown	FTB2839	KR814612, KR814637, KR814699
(4) *C. septentrionalis*	Cao Bang, Vietnam	ROM 35605	AF471081
(5) *C. septentrionalis*	Cao Bang, Vietnam	ROM 35597	KX694584, KX694624, KX694890
(6) *C. pavimentata*	Ningming, Guangxi, China	KFBG 14507	MH445959, MH445963, MH445957
(7) *C. yunnanensis*	Simao, Yunnan, China	ROM 41547	KX694572, KX694625, KX694891
(8) *C. yunnanensis*	Unknown	—	JQ598801, JQ598863, JQ598922
(9) *Elaphe quatuorlineata*	Turkey, European Turkey	LSUMZ 40626	AY122798, AF215267, AY486931
(10) *Lycodon rufozonatus*	Unknown	LSUMZ 44977	AF233939, HM439978, AF471063
(11) *Hierophis spinalis*	Yinnan, Ningxia, China	MVZ 211019	AY541508, AY376773, AY486924

Morphological characters.—Terminology and measurements follow Inger and Marx (1965) and Nguyen et al. (2010). Measurements were taken by JHY to the nearest 0.1 mm with dial calipers; ventral scales were counted after Dowling (1951). Sex was determined by the presence of hemipenis. Comparative morphological data of species of *Calamaria* were obtained from examined specimens of *Calamaria* and published 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).

RESULTS

Molecular relationships.—Based upon the phylogenetic analyses of the combined fragments of 12S rRNA, 16S rRNA, and cyt-*b* genes, the unknown *Calamaria* sp. from Yingjiang County was found to be genetically distinct and the sister taxon of *C. yunnanensis* (Fig. 1). Based on uncorrected pairwise divergence of the combined fragments, the *Calamaria* sp. from Yingjiang County differs from the three known species of *Calamaria* (*C. pavimentata*, *C. septentrionalis,* and *C. yunnanensis*) by relatively high genetic distances $p =$ 10.7% to 23.4% (Table 2). These levels of divergences are typically indicative of differentiation at the species level in vertebrates (Vences et al., 2005; Chambers and Hebert, 2016). As the observed molecular differences are complemented by differences in morphology (see below), we therefore consider the *Calamaria* sp. from Yingjiang County to be a new species.

Calamaria andersoni, new species

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Anderson's Reed Snake
Figures 2, 3

Holotype.—SYS r001699, male, China, Yunnan Province, Yingjiang County, Tongbiguan Town, 24°32′53.30″N, 97°35′59.10″E, approx. 1520 m above sea level, Jian-Huan Yang, 6 May 2016.

Diagnosis.—*Calamaria andersoni* can be distinguished from all other species of *Calamaria* by the combination of the following characters: nine modified maxillary teeth; four supralabials, second and third supralabials entering orbit; one preocular; mental not touching anterior chin shields; six shields and scales surrounding the paraparietal; dorsal scales in 13:13:13 rows; 171 ventral scales; 23 subcaudals; tail slowly tapering anteriorly, then abruptly tapering at tip; dorsal scales reduced to four rows on tail at last two subcaudals; dorsum of body and tail brownish with indistinct narrow black stripes on sides; dark collar on neck absent; light rings/blotches on neck and tail absent; and ventral scales with dark outermost corners.

Description of holotype.—Rostral wider than high, portion visible from above shorter than prefrontal suture; prefrontal slightly shorter than frontal, not entering orbit, and touching first two supralabials; frontal hexagonal, about 3.4 times maximum width of supraocular; paraparietal surrounded by six shields and scales; preocular 1/1 (left/right, hereinafter), small, slightly higher than postocular; postocular 1/0, higher than wide, not as high as eye diameter; eye diameter larger than eye–mouth distance; distance from anterior corner of eye to nostril 2.0 mm and to the tip of snout 3.1 mm; pupil rounded; supralabials 4/4, second and third entering orbit, fourth longest, relative supralabial width 4>2>1>3; mental 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 third subcaudal, to seven rows above 13th subcaudal, to six rows above 16th subcaudal, to five rows above 21st subcaudal, and to four rows above the last two subcaudals on tail; ventral scales 171, subcaudals 23, divided, followed by a shield covering tail tip; anal scale single.

Head length 6.5 mm; head width 5.2 mm; snout–vent length 319.1 mm; tail length 32.3 mm; body thickness 5.3 to 6.8 mm; body thickness index 0.0150–0.0193; tail root 5.0 mm thick; tail not as thick as body, slowly tapering anteriorly, abruptly tapering at tip; tail length/total length ratio 9.2%; maxillary teeth modified, 9/9.

Coloration in life.—Brownish above, somewhat iridescent. Dorsal head and upper parts of supralabials brown scattered with small black flecks and blotches; underside of head light yellow with brownish flecks on the infralabials and chin shields; body and tail brown above, each dorsal scale with small black flecks; dorsal scales solid black on upper corners of 1st row and lower corners of 2nd row, upper corners of 2nd row and lower corners of 3rd row, as well as upper corners of 4th row and lower corners of 5th row, forming three indistinct narrow black stripes on each side of body; dark collar at nuchal region absent; light rings/blotches on neck and tail absent; ventral scales of body and tail orange-yellow, with

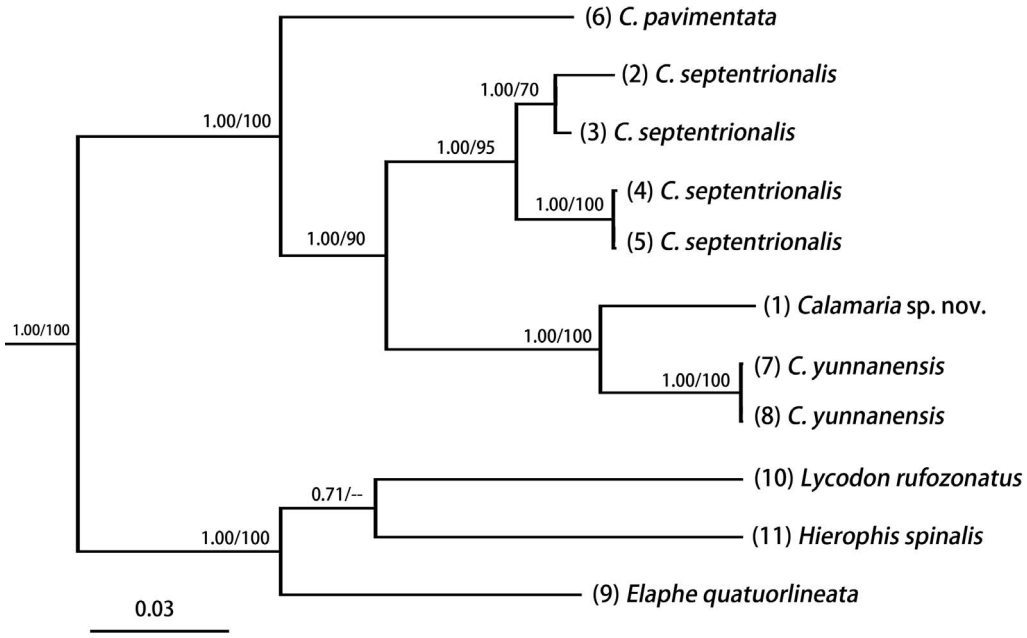

Fig. 1. Bayesian inference (BI) tree derived from partial sequences of the combined fragments of 12S rRNA, 16S rRNA, and cyt-*b* genes. Numbers at nodes are Bayesian posterior probabilities followed by bootstrap support values for maximum likelihood analyses.

black outermost corners; underside of tail with small black flecks on median line at posterior 1/4 parts.

Coloration in preservation.—Color in preservative brown above; narrow black stripes still visible; venter faded to light yellow.

Comparisons.—We compared *Calamaria andersoni* with 14 species of *Calamaria* from China and mainland southeast Asia (including Malay Peninsula), i.e., *C. abramovi* Orlov; *C. albiventer* (Gray); *C. buchi* Marx and Inger; *C. concolor* Orlov, Nguyen, Nguyen, Ananjeva, and Ho; *C. gialaiensis* Ziegler, Nguyen, and Nguyen; *C. lovii* Boulenger; *C. lumbricoidea* Boie; *C. pavimentata*; *C. prakkei* Lidth De Jeude; *C. sangi* Nguyen, Koch, and Ziegler; *C. schlegeli* Duméril, Bibron, and Duméril; *C. septentrionalis*; *C. thanhi* Ziegler, and Quyet; and *C. yunnanensis*. Unrelated Sundaland clade taxa from the Great Sunda Islands are omitted from comparisons for simplicity.

Calamaria andersoni can be readily distinguished from *C. albiventer*, *C. lumbricoidea*, *C. prakkei,* and *C. schlegeli* by having a lower number of supralabials (4 vs. 5) and the pattern of supralabials shields touching the orbit (second and third supralabials touching orbit vs. third and fourth supralabials touching orbit in *C. albiventer*, *C. lumbricoidea*, *C. prakkei,* and *C. schlegeli*). Additionally, these four species only occur south of the Isthmus of Kra in Peninsular Malaysia.

Calamaria andersoni 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*), fewer ventral scales in male (171 vs. 159 in *C. abramovi*), fewer subcaudals in male (23 vs. 26 in *C. abramovi*), and a distinctly different coloration (body black with yellow-orange spots on venter in *C. abramovi*).

Calamaria andersoni 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*), six shields and scales surrounding paraparietal (vs. five in *C. buchi*), and ventral scales with dark outermost corners (vs. ventral scales immaculate yellow in *C. buchi*).

Calamaria andersoni differs from *C. concolor* by having four supralabials (vs. five in *C. concolor*), eye diameter larger than distance from eye to mouth edge (reverse condition in *C. concolor*), mental not touching anterior chin shields (touching in *C. concolor*), tail not flatted (versus slight flatted

Table 2. Uncorrected *p*-distances (%) among the sequences based on fragments of 12S rRNA,16S rRNA, and cyt-*b* genes.

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
(1) *Calamaria,* new species	—										
(2) *C. septentrionalis*	17.7	—									
(3) *C. septentrionalis*	16.6	1.9	—								
(4) *C. septentrionalis*	17.0	5.3	4.6	—							
(5) *C. septentrionalis*	17.0	5.3	4.6	0.0	—						
(6) *C. pavimentata*	23.4	18.0	16.8	19.3	19.3	—					
(7) *C. yunnanensis*	10.7	16.8	16.6	16.2	16.2	20.7	—				
(8) *C. yunnanensis*	10.7	16.8	16.6	16.2	16.2	20.7	0.0	—			
(9) *Elaphe quatuorlineata*	25.5	24.5	23.8	24.5	24.5	25.3	24.9	24.9	—		
(10) *Lycodon rufozonatus*	26.5	24.7	24.1	24.9	24.9	26.6	28.4	28.4	16.9	—	
(11) *Hierophis spinalis*	26.3	26.3	25.6	24.9	24.9	28.7	27.1	27.1	19.0	19.4	—

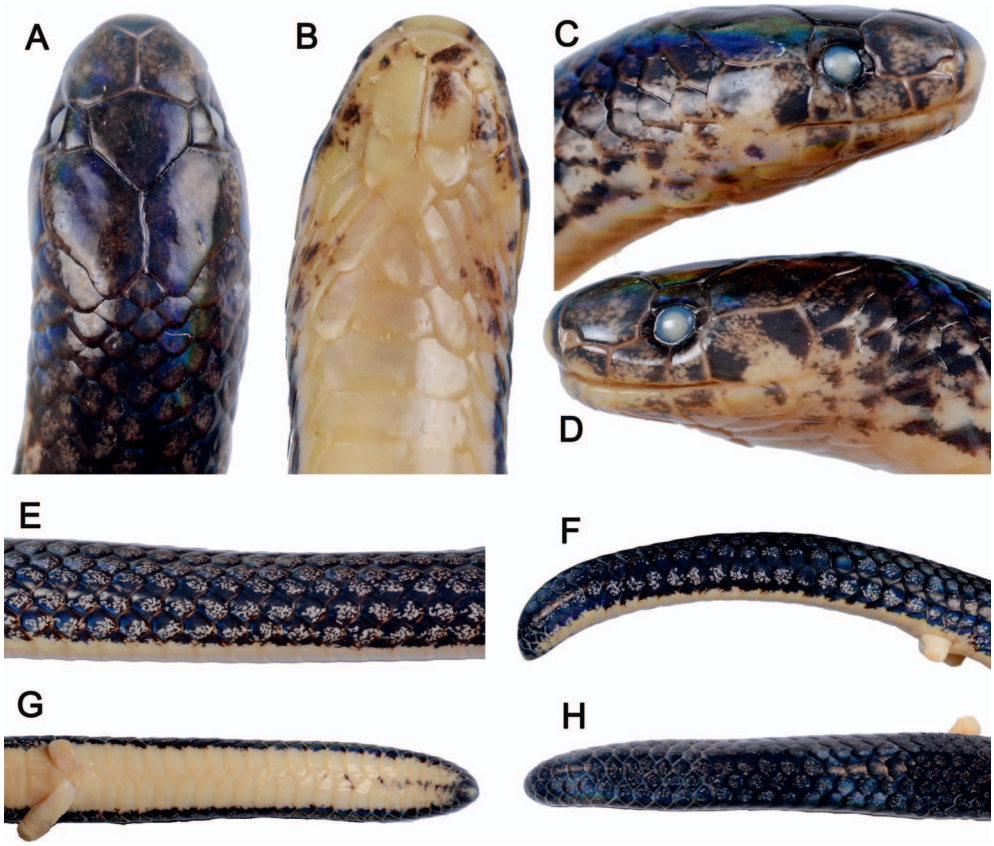

Fig. 2. Holotype of *Calamaria andersoni*, new species, in preservative: (A) dorsal view of head; (B) ventral view of head; (C) dorsolateral view of right side of head; (D) dorsolateral view of left side of head; (E) dorsolateral view of mid-body; (F) lateral view of tail; (G) ventral view of tail; (H) dorsal view of tail. Photographs by Jian-Huan Yang.

laterally in *C. concolor*), fewer ventral scales in male (171 vs. 209 in *C. concolor*), and a distinctly different coloration (body uniform brown above and immaculate cream below in *C. concolor*).

Calamaria andersoni differs from *C. gialaiensis* by having mental not touching anterior chin shields (vs. touching in *C. gialaiensis*), lower number of ventral scales in male (171 vs. 191 in *C. gialaiensis*), absence of dark collar in nuchal region (vs. an indistinct dark collar present in nuchal region in *C. gialaiensis*), absence of a distinct dark longitudinal line under of tail (vs. present in *C. gialaiensis*), tail not as thick as body (vs. tail as thick as body, not tapering, with rounded end in *C. gialaiensis*), light blotches/rings absent at tail (vs. presence of two pairs of light blotches on tail in *C. gialaiensis*).

Calamaria andersoni differs from *C. lovii* by having a preocular scale (vs. absent in *C. lovii*), absence of light blotches/rings on neck (vs. present in *C. lovii*), the end of tail abruptly tapering at tip (vs. tail tip bluntly rounded in *C. lovii*). *Calamaria andersoni* further differs from the subspecies *C. l. ingermarxorum* from southern Vietnam by having mental not touching anterior chin shields (vs. touching in *C. l. ingermarxorum*), fewer ventral scales in males (171 vs. 205 in *C. l. ingermarxorum*), and ventral scales with dark outermost corners (vs. ventral scales immaculate yellow in *C. l. ingermarxorum*); *Calamaria andersoni* further differs from the subspecies *C. l. gimletti* from Malay Peninsula by having higher number of subcaudals in male (23 vs. 14–20 in *C. l. gimletti*).

Calamaria andersoni differs from *C. pavimentata* by having rostral shield width larger than length (vs. reverse condition in *C. pavimentata*), higher number of ventral scales in males (171 vs. 125–168 in *C. pavimentata*), the end of tail abruptly tapering at tip (tail tapering gradually to a point in *C. pavimentata*), absence of dark collar at nuchal region (vs. a broad dark collar present in nuchal region in *C. pavimentata*), absence of light blotches/rings on neck and tail (vs. light blotches/rings on neck and tail present on tail in *C. pavimentata*).

Calamaria andersoni differs from *C. sangi* by having mental not touching anterior chin shields (vs. touching in *C. sangi*), lower number of ventral scales (171 vs. 190 in *C. sangi*), absence of dark collar in nuchal region (vs. a dark collar present in nuchal region in *C. sangi*), light blotches/rings on tail absent (vs. presence of an indistinct thin yellow ring on tail), venter without dark transverse bands (vs. venter cream with narrow dark transverse bands in *C. sangi*).

Calamaria andersoni differs from *C. septentrionalis* by having the end of tail abruptly tapering at tip (vs. tail tip broadly rounded in *C. septentrionalis*), higher number of ventral scales in males (171 vs. 148–166 in *C. septentrionalis*), higher number of subcaudals in males (23 vs. 15–19 in *C. septentrionalis*), dorsal scales reduced to four rows at tail (vs. dorsal scales not reduced to four rows at tail in *C. septentrionalis*), absence of light blotches/rings on neck and tail (vs. light blotches/rings on neck and tail present on tail in *C. septentrionalis*).

Calamaria andersoni differs from *C. thanhi* by having a preocular scale (vs. absent in *C. thanhi*), dorsal scales reduced to four rows at tail (vs. dorsal scales reduced to five rows at tail in *C. thanhi*), dorsum of body without light bands (vs. dorsum of body with four yellowish bands in *C. thanhi*), absence of light blotches/rings on tail (vs. present in *C. thanhi*).

Calamaria andersoni differs from *C. yunnanensis* by having a preocular scale (vs. absent in *C. yunnanensis*, Fig. 4), fewer ventral scales in males (171 vs. 179–201 in *C. yunnanensis*), higher number of subcaudals in males (23 vs. 15–22 in *C. yunnanensis*), all dorsal scale rows with dark pigmentation (vs. the outermost one or two dorsal scale rows immaculately

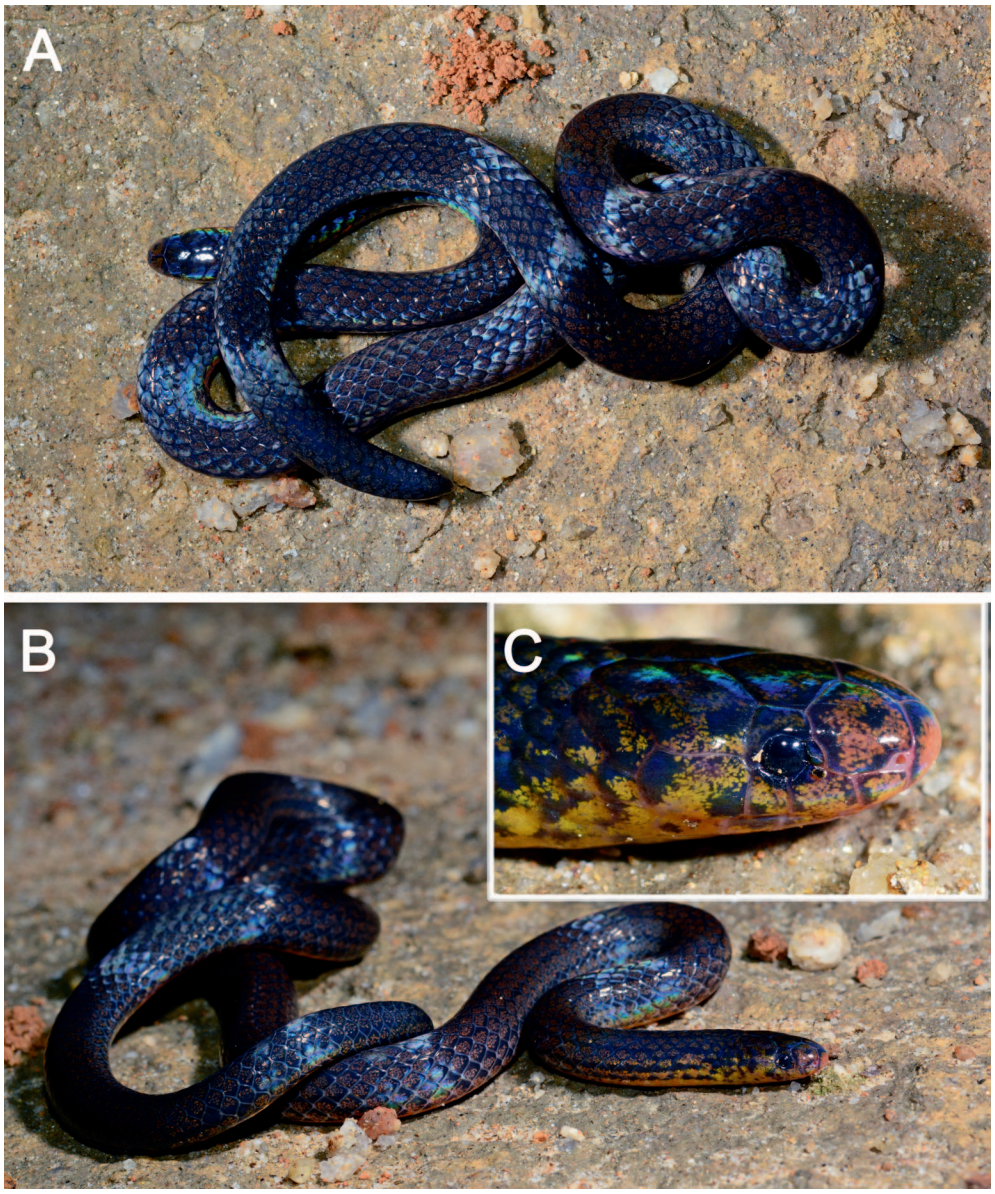

Fig. 3. Holotype of *Calamaria andersoni*, new species, in life: (A) dorsal view; (B) lateral view; (C) lateral view of right side of head. Photographs by Jian-Huan Yang.

Fig. 4. *Calamaria yunnanensis* from Jingdong County, Yunnan Province, China (type locality). Insert photo depicts the head scalation (absence of preocular). Photographs by Chung-Wei You.

light colored in *C. yunnanensis*, Fig. 4); ventral scales with dark outermost corners (vs. ventral scales immaculate in *C. yunnanensis*, Fig. 4).

Distribution.—*Calamaria andersoni* is currently only known from a single specimen from its type locality in Yingjiang County of Yunnan Province, China (Fig. 5).

Etymology.—We name this new species in honor of John Anderson (1833–1900) for his great pioneering work on the biodiversity inventory and research of the region. From Scotland, Anderson visited the western part of Yunnan Province, China twice during 1867–1875 and published the first monograph of the fauna in the region (Anderson, 1878). As a common name, we suggest "Anderson's Reed Snake."

DISCUSSION

With the description of *Calamaria andersoni*, the number of species of *Calamaria* in China is up to four, namely *C. andersoni*, *C. pavimentata*, *C. septentrionalis,* and *C. yunnanensis*. The type locality of *Calamaria andersoni* is ca. 5 km from the international border with Myanmar's Kachin State; we

Fig. 5. Map of western part of Yunnan Province, China showing the type locality (dark star) of *Calamaria andersoni*, new species.

can expect that the new species also occurs in the Kachin State of Myanmar. The extensive forests of northern Myanmar are biologically still insufficiently explored, and only a single species of *Calamaria*, *C. pavimentata*, was documented from the country (Uetz et al., 2017); it is likely that further survey efforts in northern Myanmar will lead to discoveries of additional records of species of *Calamaria*, including species new to science.

According to the phylogenetic tree, the four samples of *C. septentrionalis* formed two highly divergent lineages with high support values in both ML and BI analyses: one composed of two samples from northern Vietnam, and the other composed of a specimen from Hainan Province, China and a specimen with unknown locality (Fig. 1). There were relatively high genetic divergences (p = 4.6–5.3%) observed between these two lineages, which indicates the possibility that these two lineages belong to different species. Further taxonomic studies on this widely recorded species are needed to clarify the placement of different geographic populations.

Probably due to their secretive burrowing habits, species of the genus *Calamaria* are not frequently encountered in the wild in Indochina and southern China, and most of the species in the region are known only from very limited specimens, often described based on a single specimen (see Orlov et al., 2010 and Nguyen et al., 2010 for examples and references). We believe the diversity of this genus is likely still not fully revealed, particularly in Indochina and southern China.

MATERIAL EXAMINED

Calamaria pavimentata (n = 3): KFBG 14507, SYS r 001725–1726, China, Guangxi, Nonggang Nature Reserve.

Calamaria septentrionalis (n = 3): KFBG 14506, China, Hainan, Bawangling Nature Reserve; KFBG 14547, China, Hong Kong; KFBG 14548, China, Guangdong, Baiyong Nature Reserve.

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