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A new species of the genus *Achalinus* from southwestern Guangdong Province, China (Squamata: Xenodermatidae)

JIAN WANG[1], YAO LI[1], ZHAO-CHI ZENG[1], ZHI-TONG LYU[1], YIK-HEI SUNG[2], YOU-YU LI[3], CHAO-YU LIN[4] & YING-YONG WANG[1]

[1] State Key Laboratory of Biocontrol / The Museum of Biology, School of Life Sciences, Sun Yat-sen University, Guangzhou 510275, P.R.China.
[2] Division of Ecology and Biodiversity, School of Biological Sciences, The University of Hong Kong, Pokfulam, Hong Kong SAR, P.R. China
[3] Guangdong Yunkaishan National Nature Reserve, Maoming 525300, P.R. China
[4] Department of Zoology, Graduate School of Science, Kyoto University, Kyoto 606-8502, Japan
Corresponding author: YING-YONG WANG (wangyy@mail.sysu.edu.cn)

Abstract

A new species of xenodermatid snake, *Achalinus yunkaiensis* **J. Wang, Y. Li & Y.Y. Wang**, **sp. nov.** was described based on a series of specimens collected from Dawuling Forestry Station located in the Yunkai Mountains of western Guangdong Province. It can be distinguished from known congeners by a significant genetic divergence at the mitochondrial CO1 gene fragment examined (*p*-distance ≥ 12.0%) and the following combination of characters: (1) dorsal scales strongly keeled, in 23 rows throughout the body, the most outer row on each side smooth and significantly enlarged; (2) tail relatively shorter, TaL/TL ratio 18.5–20.0%; (3) maxillary teeth 20–22; (4) length of suture between internasals subequal to that between the prefrontals; (5) nasal divided into two sections by nasal cleft, posterior one half as long as anterior; (6) loreal elongated, nearly twice as wide as high; (7) supralabials six; (8) infralabials six; (9) temporals 2+2+3 (rarely 2+2+4), the two anterior temporals in contact with eye; (10) ventrals 151–162, subcaudals 49–56 arranged in single row, not paired; (11) cloacal entire; (12) uniform brown (in adults) or black (in juveniles) above, tinged weakly iridescent, with a longitudinal dark-colored vertebral line; (13) light brown (in adults) or greyish white (in juveniles) beneath; and (14) dorsum with a longitudinal dark brown vertebral stripe from posterior margin of parietals to tail tip. Currently, 13 species are known in the genus *Achalinus*, with seven from mainland China.

Key words: *Achalinus yunkaiensis* **sp. nov.**, China, morphology, molecular analyses, taxonomy

Introduction

The odd-scaled snakes (also called burrowing snakes) in the genus *Achalinus* Peters, 1869 within the family Xenodermidae currently contains 12 species and are distributed from Japan west to China, and extended to northern Vietnam (Goris & Maeda 2004; Karsen *et al.* 1986; Koshikawa 1982; Ota & Toyama 1989; Smith 1943; Van Denburgh 1912; Wang *et al.* 2017; Ziegler 2002; Zhao *et al.* 1998; Zhao 2006; Ziegler *et al*. 2019). Among them, *A. ater* Bourret 1937 was recorded from northern Vietnam, and Guangxi and Guizhou of China, *A. hainanus* Huang 1975 only from Hainan Island of China, *A. jinggangensis* (Zong & Ma 1983) is restricted to Mt. Jinggang of Jiangxi and neighboring Taoyuandong Nature Reserve of Hunan, southeastern China, *A. meiguensis* Hu & Zhao 1966 from Sichuan and Yunnan of China, *A. rufescens* Boulenger 1888, and *A. spinalis* Peters 1869 from Japan westwards to China and northern Vietnam, *A. wernei* Van Denburgh, 1912 is endemic to Japan, *A. formosanus* Boulenger, 1908 is only known from the southern Ryukyu islands of Japan and the Taiwan Island of China, *A. niger* Maki 1931 is endemic to Taiwan Island, *A. emilyae* Ziegler, Nguyen, Nguyen, Pham, Nguyen, Pham, Schingen, Nguyen & Le, 2019, *A. juliani* Ziegler, Nguyen, Nguyen, Pham, Nguyen, Pham, Schingen, Nguyen & Le, 2019 and *A. timi* Ziegler, Nguyen, Nguyen, Pham, Nguyen, Pham, Schingen, Nguyen & Le, 2019 are only known from northern Vietnam.

In recent herpetological surveys in southern China, a number of xenodermatid snake specimens were collected

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from Dawuling Forestry Station and adjacent Xianrendong Scenic Area located in the Yunkai Mountains in southwestern Guangdong Province. According to the diagnostic characters for the genus *Achalinus* as indicated by Smith (1943) and Zhao *et al.* (1998), these specimens could be assigned to the genus *Achalinus* the following characters: (1) maxillary teeth 20–22, small, equal; (2) mandibular teeth equal; (3) head not or only scarcely distinct from neck; (4) eye small or moderate, with round or vertically subelliptical pupil; (5) nostril in the anterior part of a large concave nasal, divided by a vertical suture; (6) the loreal extending from the nasal to the eye; (7) body slender, cylindrical; (8) dorsal scales in 23 rows, keeled; (9) subcaudals single. Further morphological examinations and molecular analyses revealed that these specimens represent a separately evolving lineage that can be distinguished from all recognized species, we herein describe these specimens as a new species.

Material and methods

Sampling. For molecular analyses, muscle tissues from 12 specimens of four species of the genus *Achalinus* were sequenced, including *A. ater*, *A. rufescens*, *A. spinalis* and an unknown species from Dawuling Forestry Station of Guangdong Province. Sequences of *A. meiguensis* and *A. niger* were downloaded from GenBank. Additionally, sequences of *Fimbrios klossi* Smith, 1921 and *Parafimbrios lao* Teynié, David, Lottier, Le, Vidal & Nguyen, 2015 were downloaded from GenBank as the outgroups. Details are shown in Table 1.

TABLE 1. Localities, voucher information, and Genbank accession numbers for all specimens used in this study.

ID	Species name	Locality	Voucher	Genbank Number
				CO1 gene
1	*Achalinus yunkaiensis* **sp. nov.**	China: Dawuling Forestry Station, Guangdong	SYS r001443	MN380329
2	*Achalinus yunkaiensis* **sp. nov.**	China: Dawuling Forestry Station, Guangdong	SYS r001502	MN380330
3	*Achalinus yunkaiensis* **sp. nov.**	China: Dawuling Forestry Station, Guangdong	SYS r001503	MN380331
4	*Achalinus yunkaiensis* **sp. nov.**	China: Dawuling Forestry Station, Guangdong	SYS r001902	MN380332
5	*Achalinus yunkaiensis* **sp. nov.**	China: Dawuling Forestry Station, Guangdong	SYS r001903	MN380333
6	*Achalinus ater*	China: Huaping Nature Reserve, Guangxi	SYS r000852	MN380334
7	*Achalinus meiguensis*	China: Qianfoshan Nature Reserv, Sichuan	/	FJ424614
8	*Achalinus niger*	China: Taiwan Island	RN0647	KU529433
9	*Achalinus niger*	China: Taiwan Island	RN0667	KU529434
10	*Achalinus rufescens*	China: Heishiding Nature Reserve, Guangdong	SYS r001527	MN380335
11	*Achalinus rufescens*	China: Shimentai Nature Reserve, Guangdong	SYS r001689	MN380336
12	*Achalinus rufescens*	China: Guangzhou, Guangdong	SYS r001795	MN380337
13	*Achalinus rufescens*	China: Guangzhou, Guangdong	SYS r001796	MN380338
14	*Achalinus rufescens*	China: Hongkong	SYS r001866 (Topotype)	MN380339
15	*Achalinus spinalis*	China: Mt. Badagong, Hunan	SYS r001327	MN380340
16	*Fimbrios klossi*	Vietnam: Gia La	IEBR A.2013.56	KP410745
17	*Parafimbrios lao*	Laos: Louangphabang	MNHN 2013.1002	KP410746

All specimens were fixed in 10% buffered formalin and then transferred to 70% ethanol for preservation; samples were preserved in 95% ethanol for molecular studies. All specimens and samples were deposited in the Museum of Biology, Sun Yet-sen University (**SYS**).

DNA Extraction, PCR and sequencing. DNA was extracted from muscle tissue using a TIANamp Genomic DNA Kit primarily following manufacturers' instructions. The partial mitochondrial cytochrome c oxidase 1 gene (CO1) was sequenced. Fragments of the CO1 gene was amplified using primer pairs Dglco (5'-GGTCAACAAAT-CATAAAGAYATYGG-3'), and Dghco (5'-TAAACTTCAGGGTGACCAAARAAYCA-3') (Meyer *et al*. 2005). PCR amplifications were performed in a 20 µl reaction volume with the following cycling conditions: an initial denaturing step at 95°C for four min; 35 cycles of denaturing at 95°C for 30s, annealing at 48°C for 30s and extending at 72°C for one min, and a final extending step of 72°C for 10 min. PCR products were purified with spin columns. The purified products were sequenced with both forward and reverse primers using Big Dye Terminator Cycle Sequencing Kit according to the guidelines of the manufacturer. The products were sequenced on an ABI Prism 3730 automated DNA sequencer in Shanghai Majorbio Bio-pharm Technology Co., Ltd. All sequences were deposited in GenBank.

Phylogenetic analyses. Sequences were first aligned using Clustal X 2.0 (Thompson *et al.* 1997) with default parameters. The alignments were checked visually in MEGA 6 (Tamura *et al.* 2013). The length of the alignments was 624 base pairs (bp), including 227 bp variable sites and 178 bp parsimony-informative sites. The best nucleotide substitution models were tested in jModelTest 2.1.2 (Darriba *et al.* 2012) with Akaike information criteria, and the GTR+G model was the best model. Phylogenetic trees were inferred using maximum likelihood (ML) implemented in RaxmlGUI 1.3 (Silvestro & Michalak 2012), and Bayesian inference (BI) in MrBayes 3.2.4 (Ronquist *et al.* 2012). For ML analysis, a majority rule consensus tree was calculated with 1,000 bootstrap replicates. For BI analysis, two independent runs with four Markov Chain Monte Carlo simulations were performed for two million iterations and sampled every 1,000 generations. The first 25% of samples were discarded as burn-in. Convergence of the Markov Chain Monte Carlo simulations was assessed by checking the average standard deviation of split frequencies between two runs using Tracer v.1.4 (http://tree.bio.ed.ac.uk/software/tracer/). We also calculated uncorrected pairwise genetic distances (*p*-distance) in MEGA 6 (Tamura *et al.* 2013).

Morphometrics. Measurements follow Zhao *et al.* (1998), and were taken with digital calipers to the nearest 0.1 mm. Abbreviations in this study are as follows: total length (**TL**); snout-vent length from tip of snout to anterior margin of the cloaca (**SVL**); tail length from posterior margin of cloaca to tip of tail (**TaL**); length of loreal (**LeL**); high of loreal (**HiL**); length of anterior section of nasal (**LaSN**); length of posterior section of nasal (**LpSN**);length of suture between the prefrontals (**LSBP)**; length of suture between internasals (**LSBI**); preoculars (**PrO**); postoculars (**PtO**); supraoculars (**SpO**); supralabials (**SPL**); infralabials (**IFL**); anterior temporals (**aTMP**); middle temporals (**mTMP**); posterior temporals (**pTMP**); ventral scales (**V**); subcaudals (**SC**). Dorsal scale rows (**DSR**) were counted at one head length behind head, at midbody, and at one head length before vent respectively. Maxillary teeth counts (**MT**) were determined by subequal teeth or sockets on right upper maxilla counted via a microscope. Bilateral scale counts were given as left/right. Sex was determined by dissection or by the presence/absence of everted hemipenes.

While comparing the unknown species with the wide-spread species *Achalinus rufecens*, we rely on its original description, our newly collected topotype specimen and those specimens which are clustered with the topotype specimen in the phylogenetic trees in this study. Comparative morphological data of recognized *Achalinus* species were obtained from examination of museum specimens (see Appendix 1) and from the literatures: *A. ater* (Bourret 1937), *A. emilyae* (Ziegler *et al*. 2019), *A. formosanus* (Ota & Toyama 1989; Zhao 2006; Zhao *et al.* 1998), *A. hainanus* (Koshikawa 1982), *A. jinggangensis* (Zong & Ma 1983; Zhao 2006; Zhao *et al.* 1998), *A. juliani* (Ziegler *et al*. 2019), *A. meiguensis* (Hu & Zhao, 1966), *A. niger* (Zhao 2006; Zhao *et al.* 1998), *A. rufecens* (Boulenger 1888), *A. spinalis* (Zhao 2006; Zhao *et al.* 1998), *A. timi* (Ziegler *et al*. 2019), and *A. werneri* (Van Denburgh 1912).

Results

The Maximum likelihood (ML) and Bayesian inference (BI) analyses resulted in essentially identical topologies were integrated in Figure 1 and the *p*-distances were given in the Table 2. The two analyses resulted in essentially identical topologies (Figure 1). The specimens from Dawuling Forestry Station were clustered in a clade with high

strong node support values (1.00 in BI and 100% in ML) and small genetic differences (*p*-distances 0–0.2%), and nested into the genus *Achalinus* composed by *A. spinalis, A. niger* and *A. meiguensis*, *A. rufecens* and *A. ater* with deep genetic divergences (*p*-distances 12.0–15.2%, see Table 2). Given that the *Achalinus* population from Dawuling Forestry Station possess significant morphological differencesfrom all known congeners of the genus *Achalinus* and molecular differences from five species of the genus included in the molecular analyses (see Table 2), it is described as a new species below.

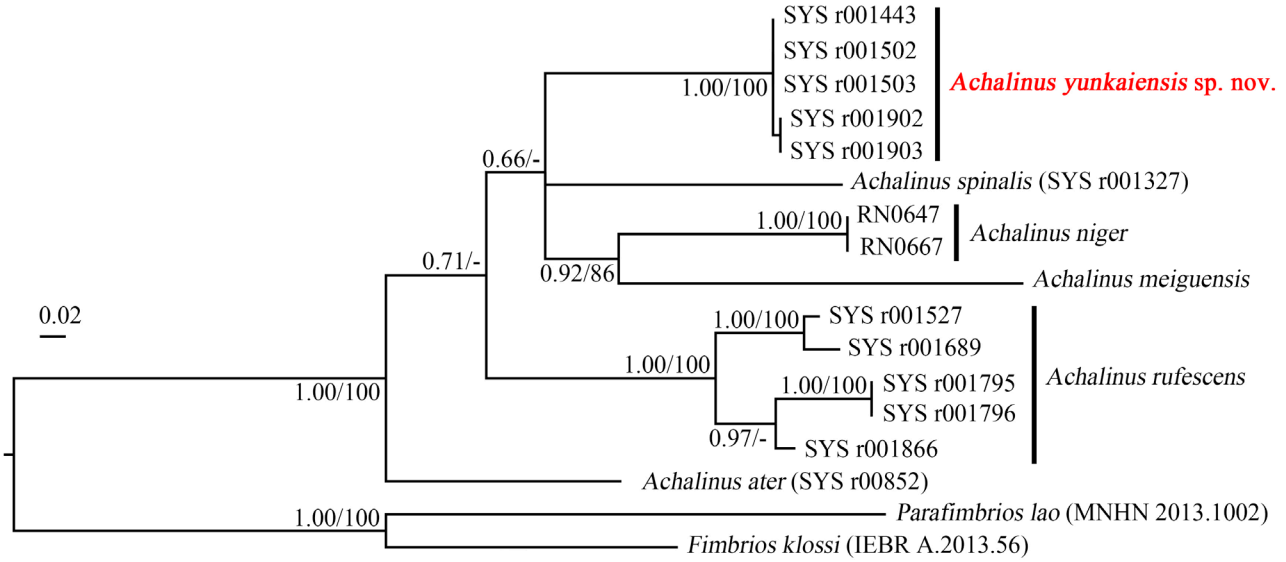

FIGURE 1. Bayesian inference and maximum-likelihood phylogenies. Numbers before slashes indicate Bayesian posterior probabilities (>0.50 retained) and numbers after slashes are bootstrap support for maximum likelihood (1000 replicates) analyses (>50 retained). The symbol "-" represents bootstrap value below 50%.

TABLE 2. Uncorrected *p*-distances among the *Achalinus* species and outgroups based on partial mitochondria CO1 gene.

ID	*Achalinus* Species	1−5	6	7	8−9	10−14	15	16	17
1−5	*A. yunkaiensis* **sp. nov.**	0−0.2							
6	*A. ater*	12.8−13.0	−						
7	*A. meiguensis*	15.1−15.2	14.9	−					
8–9	*A. niger*	12.2−12.3	13.5	13.0	0				
10–14	*A. rufescens*	12.7−13.8	11.9−12.5	16.5−17.6	12.7−13.6	0−7.7			
15	*A. spinalis*	12.0−12.2	15.2	15.2	13.5	12.5−13.5	−		
16	*Fimbrios klossi*	19.4−19.6	19.2	19.6	18.3	18.6−20.5	20.5	−	
17	*Parafimbrios lao*	20.0−20.2	20.2	22.1	21.2	19.9−21.3	20.4	15.7	−

Achalinus yunkaiensis J. Wang, Y. Li & Y.Y. Wang, sp. nov.

Figure 2 & 3A, B

Holotype. SYS r001903 (Figure 2), adult male, collected by Jian Wang and Honghui Chen on 10 April 2018 from Dawuling Forestry Station (22.27580°N, 111.19524°E; 1,500 m a.s.l.), Maoming City, Guangdong Province, China.

Paratypes. Adult male, SYS r001443 (Figure 3, A1−A3), collected by Jian Wang and Zhao-Chi Zeng on 16 April 2016; juvenile males, SYS r001502, 1503 (Figure 3, B1−B3), collected by Jian Wang, Can-Rong Lin, Zhao-Chi Zeng and Chun-Peng Guo on 27 June 2016; and a single adult female, SYS r001902, collected by Jian Wang and Hong-Hui Chen on 10 April 2018; all from the same locality as the holotype.

Diagnosis. (1) dorsal scales strongly keeled, in 23 rows throughout the body, the most outer rows on both sides smooth and significantly enlarged; (2) tail relatively shorter, TaL/TL ratio 18.5–20.0%; (3) maxillary teeth 20–22; (4) length of suture between internasals subequal to that between the prefrontals; (5) nasal divided into two sections by nasal cleft, posterior one nearly half as long as anterior, LaSN/LpSN 0.4–0.5; (6) loreal elongated, nearly twice as wide as high, HiL/LeL 0.5–0.6; (7) supralabials six, the fourth and fifth ones widely in contact with eye; (8) infralabials six, the first three (rarely the first four) in contact with the first pair of chin shields; (9) temporals 2+2+3 (rarely 2+2+4), the two anterior temporals in contact with eye; (10) ventrals 151–162, subcaudals 49–56 arranged in single row, not paired; (11) cloacal entire; (12) uniform brown (in adults) or black (in juveniles) above, tinged weakly iridescent, with a longitudinal dark-colored vertebral line; (13) light brown (in adults) or greyish white (in juveniles) beneath; and (14) dorsum with a longitudinal dark brown vertebral stripe from posterior margin of parietals to tail tip.

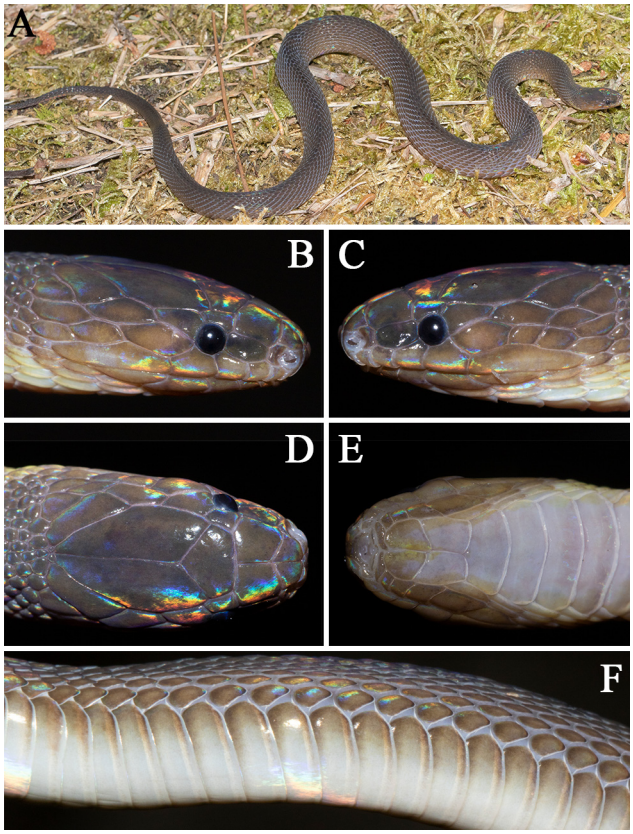

FIGURE 2. General aspect in life of the adult male holotype of *Achalinus yunkaiensis* **sp. nov.**, SYS r001903.

Comparisons. Coloration of adults of *Achalinus yunkaiensis* **sp. nov.** is most similar to that of *A. rufescens* (Figure 3), and the new species can be distinguished from the examined specimens of *A. rufescens* by less maxillary teeth, MT 20–22 (vs. 23–25 in *A. rufescens*), more ventrals in a single female, V 156 (vs. V 132–140 in females of *A. rufescens*), more infralabials, IFL 6 (vs. IFL 5 in *A. rufescens*), length of suture between internasals subequal to that between the prefrontals (vs. distinctly longer than that between prefrontals in *A. rufescens*), nasal section behind nasal cleft nearly half as long as those before nasal cleft, LaSN/LpSN 0.4–0.5 (vs. nearly subequal in *A. rufescens*, LaSN/LpSN 0.9–1.2); loreal nearly twice as wide as high, HiL/LeL 0.5–0.6 (vs. nearly subequal in *A. rufescens*, HiL/LeL 0.8–1.0), moreover, the new species can be distinguished from the holotype of *A. rufescens* by SPL 6, the 4^{th}–5^{th} in contact with eye (vs. SPL 5, the 3^{rd}–4^{th} in contact with eye), IFL 6 (vs. IFL 5), both of the two anterior temporals in contact with eye (vs. only the upper on in contact with eye), V 151–162 (vs. V136), SC 56–59 (vs. SC 82); details see Table 4.

Coloration of juveniles of *Achalinus yunkaiensis* **sp. nov.** is most similar to that of *A. spinalis* and *A. ater*, and the new species can be distinguished by the coloration of adult individuals, dorsum brown (vs. dorsum black in *A. spinalis* and *A. ater*) (Figure 3); venter light brown in adults and greyish white in juveniles (vs. venter black brown in *A. spinalis* and *A. ater*); presence of a longitudinal mid-dorsal stripe (vs. absence in *A. spinalis* and *A. ater*); length of suture between internasals subequal to that between the prefrontals (vs. shorter in *A. spinalis* and *A. ater*).

In having 23 mid-dorsal scale rows and presence of internasals, *Achalinus yunkaiensis* **sp. nov.** can be easily distinguished by *A. fomosanus* (mid-dorsal scale rows 27, lacking internasals), *A. hainanus* (lacking internasals), *A. meiguensis* (mid-dorsal scale rows 19–21, lacking internasals), and *A. niger* (mid-dorsal scale rows 27). *A. yunkaiensis* **sp. nov.** further differs from *A. jinggangensis* by having a single loreal, length of suture between internasals equal to that between the prefrontals (vs. loreal absent, length of suture between internasals distinctly longer than that between prefrontals in *A. jinggangensis*).

TABLE 3. Measurements, scale counts and body proportions of *Achalinus yunkaiensis* **sp. nov.**.

Voucher	SYS r 001443	SYS r 001502	SYS r 1503	SYS r 001903	SYS r 001902
Sex	♂	♂juv.	♂juv.	♂	♀
TL	417.6	258.4	232	343	448.1
SVL	358.5	206.8	43.3	279.7	386.3
TaL	59.1	51.6	188.7	63.3	61.8 (broken)
TaL/TL	broken tail	20.0%	18.7%	18.5%	broken tail
MT	21	21	21	20	22
SPL	6	6	6	6	6
SPL-Loreal	3rd–4th	3rd–4th	3rd–4th	3rd–4th	3rd–4th
IFL	6	6	6	6	6
IFL-1st chin	1st–3rd	1st–3rd	1st–3rd	1st–3rd	1st–3rd/1 st–4th
Loreal	1	1	1	1	1
HiL	1.3	0.9	0.8	1.0	1.2
LeL	2.2	1.4	1.3	1.8	2.2
HiL/LeL	0.6	0.6	0.6	0.6	0.5
LaSN	0.6	0.3	0.3	0.5	0.6
LpSN	1.4	0.8	0.7	1.0	1.2
LaSN/ LpSN	0.4	0.4	0.4	0.5	0.5
SpO	1	1	1	1	1
TMP	2+2+3/2+2+4	2+2+3/2+2+3	2+2+3/2+2+3	2+2+3/2+2+3	2+2+3/2+2+3
Elongate aTMP	2nd	2nd	2nd	2nd	2nd
Elongate mTMP	1st	1st	1st	1st	1st
Elongate pTMP	1st	1st	1st	1st	1st
DSR	23–23–23	23–23–23	23–23–23	23–23–23	23–23–23
V	155	151	162	151	156
SC	42 (broken)	56	50	49	38 (broken)

Achalinus yunkaiensis **sp. nov.** can be easily distinguished from *A. emilyae*, *A. juliani* and *A. timi*, which are currently recorded from northern Vietnam, as followed: having 23 mid-dorsal scale rows (vs. mid-dorsal scale rows 25 in *A. timi*); presence of a single loreal (vs. loreal absent in *A. timi*); length of suture between internasals equal to that between the prefrontals (vs. length of suture between internasals distinctly longer than that between prefrontals in *A. emilyae*, *A. juliani* and *A. timi*); less maxillary teeth, MT 20–22 (vs. MT 27–28 in *A. emilyae*, MT 28 in *A. juliani*, and MT 27 in *A. timi*); infralabials six (vs. IFL 5 in *A. emilyae*).

Achalinus yunkaiensis **sp. nov.** differs from *A. werneri* by the relatively shorter tail length, TaL/TL ratio 18.5–20.0% (vs. TaL/TL ratio 25.0–30.0% in *A. werneri*); temporals 2+2+3 (seldom 2+2+4) (vs. temporals 2+2 in *A. werneri*); subcaudals 49–56 (vs. subcaudals 67–98 in *A. werneri*).

Description of holotype. An adult male with a total length of 343 mm (SVL 279.7 mm and TaL 63.3 mm); tail relatively shorter, TaL/TL ratio 18.5%; body slender, cylindrical; head slightly distinct from neck; eye small, with vertically oval pupil; left maxillary teeth 22, equally sized and curved; rostral small, triangular, slightly visible from above; length of the suture between the internasals subequal to that between the prefrontals; nostril in the anterior

part of the nasal, posterior margin of nostril with a distinct nostril cleft, the posterior section of nasal vertically rectangular, posterior section nearly half as long as the anterior part, LaSN/LpSN 0.4; a single pentagonal frontal, slightly broader than long, pointed backwards, much shorter than the parietals; each parietal bordered by an elongated nuchal; nuchals separated from each other behind parietals by two small scales; second pair of nuchals about half the size of first pair; a single loreal, distinctly wider than high, extending from the nasal to the eye, HiL/LeL 0.6; a single supraocular, elongate, two times wider than high; two anterior temporals, elongated, upper one smaller, wide in contact with eye, the lower one narrowly in contact with eye; two elongated middle temporals, the upper much larger, the lower one in contact with the sixth supralabial; three elongate posterior temporals, the most upper one the largest; supralabials six, the first smallest, fourth and fifth widely in contact with the eye, sixth longest; third and fourth in broad contact with the loreal; one mental, followed by six infralabials; first pair of infralabials in contact with each other behind the mental; first three infralabials on left and first four on right in contact with the first pair of chin shields; posterior pair of chin shields smaller, length of the suture between the first pair equal to that between the second pair, laterally in broad contact with the fourth infralabial on left, in broad contact with the fourth infralabial, and in narrow contact with the fifth infralabial on right.

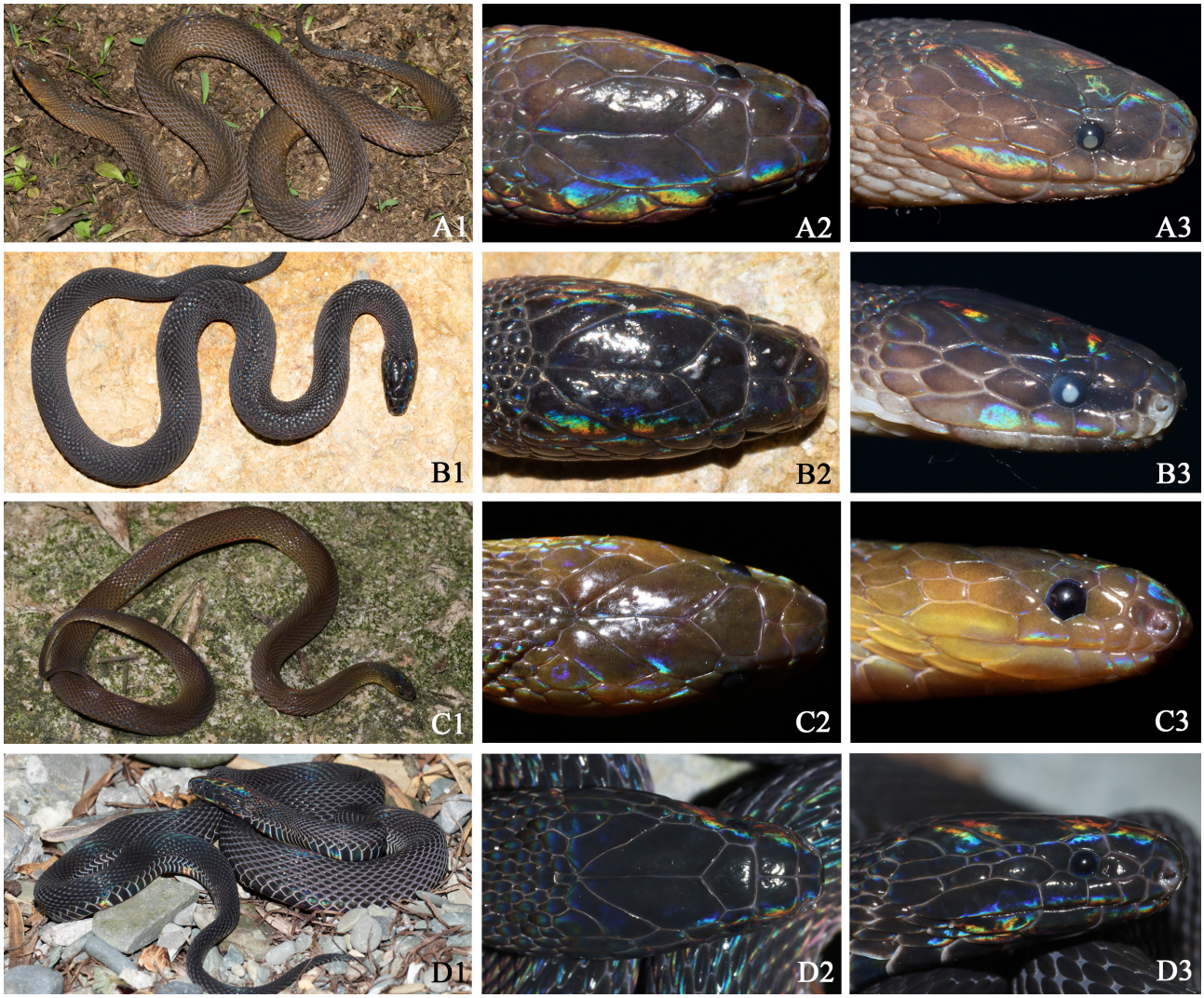

FIGURE 3. Coloration and head scalation differences among: **A.** adult *Achalinus yunkaiensis* **sp. nov.**, SYS r001443; **B.** juvenile *A. yunkaiensis* **sp. nov.**, SYS r001503; **C.** *A. rufescens*, SYS r001689 from Shimentai Nature Reserve, Guangdong Province; **D.** *A. spinalis*, SYS r001327 from Mt. Badagong, Sangzhi, Hunan Province.

Dorsal scales lanceolate and strongly keeled; dorsal in 23–23–23 scale rows, those of the most outer rows on both sides significantly enlarged and smooth; ventrals 151 (plus one preventral), distinctly rounded laterally; subcaudals 49, not paired; cloacal entire.

Coloration of holotype in life. All scales tinged weakly iridescent. Dorsum brown, the most inner three rows

of dorsal scale dark brown, forming a longitudinal vertebral line which starts from posterior margin of the parietals to tail tip. Margin of all scales greyish white. Coloration of supralabials and temporal region much lighter. Iris dark brown, pupil black. Venter light brown, coloration much lighter than that of supralabials and temporal region; ventral tail dark brown. The first pair of chin shields brown mottling with greyish white patches, the second pair greyish white with brown patches on sides. Ventral scales light brown with brown patches on both sides.

Coloration of holotype in preservative. All scales tinged weakly iridescent. Coloration darkens from dorsum to venter, dorsum dark grey, longitudinal vertebral line black. Margin of all scales dark grey. Coloration of venter is fading, grey brown; ventral tail dark grey brown.

TABLE 4. Morphological data of the examined specimens of *Achalinus yunkaiensis* **sp. nov.** and *A. rufescens.*

Species	*Achalinus yunkaiensis* **sp. nov.**		*Achalinus rufescens*			
Sex	♂ (n=4)	♀ (n=1)	♂ (n=2)	♀ (n=3)	Sex unknown (Holotype)	♂ SYS r001866 (Topotype)
TaL/TL	18.5–20.0%	broken tail	19.1–22.1%	26.7–27.1%	27.6%	22.1%
MT	20–22		23		/	23
SPL	6		6		5	6
SPL-Loreal	4th–5th		4th–5th		3rd–4th	4th–5th
IFL	6		5		5	5
IFL-1st chin	1st–3rd (rarely 1st–4th)		1st–3rd		/	1st–3rd
Loreal	1		1		/	1
SpO	1		1		/	1
TMP	2+2+3 (rarely 2+2+4)		2+2+3 (rarely 1+2+2 or 2+2+4)		/	1+2+2
aTMP-Eye	2		1 or 2		1	1
Elongate aTMP	2nd		2nd (rarely 1st)		/	1
Elongate mTMP	1st		1st		/	1st
Elongate pTMP	1st		1st		/	1st
DSR	23–23–23		23–23–23		?–25–?	23–23–23
V	151–162	156	148–156	132–140	136	156
SC	56–59	broken tail	58–69	67–79	82	69
LSBI vs. LSBP	=		>		>	>
Shape of the posterior section of nasal	Vertical rectangular		subquadrate		/	subquadrate
LaSN/LpSN	0.4–0.5		0.9–1.2		/	1.0
Shape of loreal	narrow and elongate		subquadrate		/	subquadrate
HiL/LeL	0.5–0.6		0.8–1.0		/	0.9

Variations. Measurements, body proportions, teeth counts and scale counts were listed in Table 3. All paratypes are morphologically very similar to the holotype except that: (1) the adult male SYS r001443 possessed significantly larger body size, TL 417.6 mm, even with a broken tail (vs. TL 343 mm in the male holotype); (2) higher maxillary teeth counts in all paratypes, MT 21–22 (vs. MT 20 in the holotype); (3) the first four infralabials in contact with the first pair of chin shields on the right side in SYS r001902 (vs. the first three in contact with the first pair of chin shields on the other side of SYS r001902, in paratypes SYS r001443, 1502, 1503, and the holotype); (4) temporals 2+2+4 on the right side in SYS r001443 (vs. temporals 2+2+3 on the other side of SYS r001443, in paratypes SYS r001503, 1503, 1902, and the holotype); and (5) dorsum black and venter greyish white, both sides of each central scale black in both juvenile individuals SYS r001502, 1503 (vs. dorsum brown and venter light brown, both sides of each central scale brown in all three adult individuals SYS r001443, 1902, 1903) (Figure 3).

Etymology. The specific epithet, *yunkaiensis*, is in reference to the type locality, Dawuling Forestry Station and adjacent Xianrendong Scenic Area in Guangdong Province, China located in the Yunkai Mountains. For the

common name, we suggest "Yunkai Mountain's Odd-scaled Snake" or "Yunkai Mountain's Burrowing Snake", and Chinese name "Yun Kai Ji She (云开脊蛇)".

Distribution and habits. Currently, *Achalinus yunkaiensis* **sp. nov.** is only known from its type locality Dawuling Forestry Station in Maoming City and adjacent Xianrendong Scenic Area in Gaozhou City of Guangdong Province (Figure 4). The new species was found on leaf litters in well-preserved montane evergreen broadleaf forest (900–1,600 m a.s.l.).

Discussion

The discovery and description of *Achalinus yunkaiensis* **sp. nov.** brings the total species number of the genus *Achalinus* to 13, with nine species being recorded from China, seven of which from mainland China.

Due to the secretive life style and morphological similarities, congeners within the genus *Achalinus* are still imperfectly known. In addition, most of the original description of certain species only provided limited diagnostic characters, resulting in wrong species identification. Nowadays, the taxonomic methods based on combined molecular and morphological evidences greatly improves the accurate identifications, and a large number of cryptic species are constantly found and described (Ziegler *et al*. 2019). That is to say, subsequent expanded descriptions based on morphology may cause confusion in classification. Cryptic species may be "hidden" within known species, such as the widely distributed species *A. spinalis* and *A. rufescens* (Goris & Maeda 2004; Ziegler 2002; Zhao 2006; Zhao *et al.* 1998).

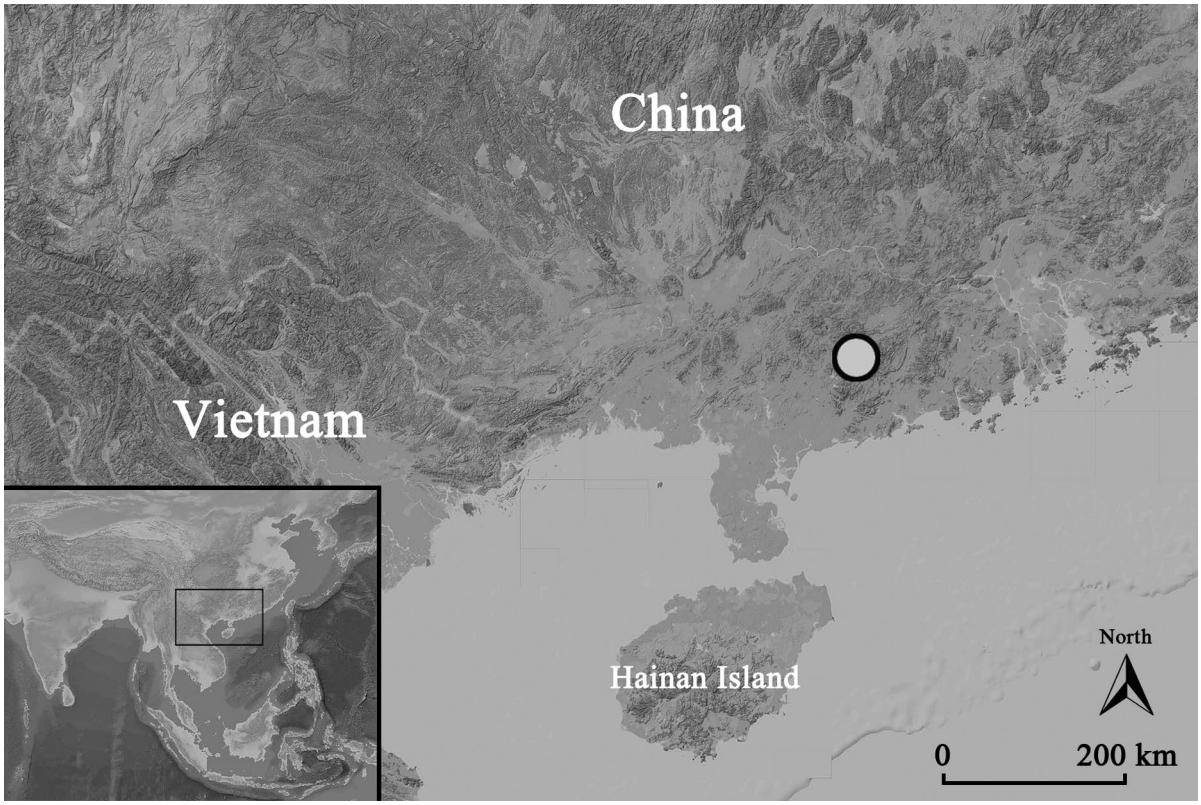

FIGURE 4. Map showing the type locality of *Achalinus yunkaiensis* **sp. nov.**, Dawuling Forestry Station and adjacent Xianrendong Scenic Area in southwestern Guangdong Province, China.

Although specimens SYS r001795, 1796 from Guangzhou City of Guangdong Province, the topotype SYS r001866 of *Achalinus refescens* from HongKong, SYS r001527 from Heishiding Nature Reserve of Guangdong Province and SYS r001689 from Shimentai Nature Reserve in Guangdong Province were clustered in a clade with high supporting value (1.00 in BI and 100% in ML) and a moderate genetic divergence (maximum *p*-distance=7.7%), the specimens were very similar and without significant differences in morphology, and morphologically matched the overall diagnoses of *A. rufescens* pointed by Zhao *et al.* (1998) by the following characters: (1)

length of suture between internasals distinctly longer than that between the prefrontals; (2) five infralabials, the first three in contact with the first pair of chin shields; (3) ventrals 136–165; and (4) dorsum brown with a dark vertical line at the central of back, venter beige. Herein, we treated these five specimens as *A. rufescens*. While for comparison of *A. yunkaiensis* **sp. nov.** with its most similar species, *A. rufecens*, we relyed on the data obtained from the original description, our newly collected topotype specimen and those specimens which are clustered with the topotype in the phylogenetic trees in this study.

Our field surveys uncovered a rich herpetodiversity in Dawuling Forestry Station located in the Yunkai Mountain of southwestern Guangdong Province, with some amphibians and reptiles being habitat specialists (Lyu *et al.* 2018; Wang *et al.* 2018a; Wang *et al.* 2018b). For example, *Amolops yunkaiensis* Lyu, Wang, Liu, Zeng & Wang, 2018, *Leptobrachella yunkaiensis* Wang, Li, Lyu & Wang, 2018, *Opisthotropis guangxiensis* Zhao, Jiang & Huang, 1978, and *O. maculosa* Stuart & Chuaynkern, 2007 are stream-adapted, *Gracixalus guangdongensis* Wang, Zeng, Lyu, Liu & Wang, 2018, *G. gracilipes* (Bourret, 1937), *Tylototriton asperrimus* Unterstein, 1930 and *Achalinus yunkaiensis* **sp. nov.** can only be found in well-preserved subtropical forest. However, such areas in Dawuling Forestry Station are under threat of degradation, particularly as a result of tourism, thus, conservation actions are urgently required.

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Appendix 1. Examined specimens.

Achalinus ater (n=1): China: Guangxi Province: Guilin: Huaping Nature Reserve: SYS r000852.

Achalinus jinggangensis (n=8): China: Jiangxi Province: Ji'an: Mt. Jinggang (type locality): SYS r000311, 0313, 0314, 0360, 0789; Hunan Province: Zhuzhou: Yanling: Taoyuandong Nature Reserve: SYS r000715, 0829, 0830.

Achalinus rufescens (n=5): China: Hongkong (type locality): SYS r001866; Guangdong Province: Yingde City: Shimentai Nature Reserve: SYS r001689; Zhaoqing City: Heishiding Nature Reserve: SYS r001527; Guangzhou City: SYS r001795, 1796.

Achalinus spinalis (n=7): China: Guizhou Province: Qiannan: Maolan Nature Reserve: SYS r000273; Bijie: Qixingguan: Zhaozishan Nature Reserve: SYS r002038, 2039; Liupanshui: SYS r002076; Sichuan Province: Baoxing: SYS r001608; Hunan Province: Zhangjiajie: Sangzhi: Mt. Badagong: SYS r001327; Huaihua: Hongjiang: SYS r002085.