Zootaxa 4950 (3): 528–546
https://www.mapress.com/j/zt/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.4950.3.6
http://zoobank.org/urn:lsid:zoobank.org:pub:B59987CB-7394-4A8E-841B-D090F65A7F84

Two new species and a new country record of the genus *Achalinus* (Reptilia: Squamata: Xenodermidae) from China

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Abstract

Combining the results from morphological and molecular analyses, we explore the taxonomy of the genus *Achalinus* from Southwest China. As a result, we describe two new species, *A. panzhihuaensis* **sp. nov.** and *A. yangdatongi* **sp. nov.** from southern Sichuan and southern Yunnan provinces, respectively, and we record a new country record, *A. emilyae*, from Guangxi Zhuang A. R.. The mitochondrial genealogy suggests that *A. panzhihuaensis* **sp. nov.** is sister to *A. meiguensis*, while *A. yangdatongi* **sp. nov.** clusters with the sister species *A. juliani* and *A. ater*. Both new species show considerable genetic divergence from their recognized congeners (uncorrected *p*-distance > 6.2 % in *COI* gene). Furthermore, both new species can be diagnosed from closely related congeners by a combination of pholidosis characters. With our discovery, we provide a revised key to the 13 species from China and discuss some of the remaining issues regarding the taxonomy of the genus in China.

Key words: cryptic diversity, fossorial, morphology, phylogenetics, Serpentes, taxonomy

Introduction

Being one of the most diverse genera in the family Xenodermidae, the genus *Achalinus* Peters, 1869 is a group of nocturnal, fossorial snakes that are distributed in Eastern and Southeastern Asia, including Japan, southern China, and northern Vietnam (Wang *et al.* 2019; Ziegler *et al.* 2019). Currently it contains 16 recognized species, 10 of which are found in China (Li *et al.* 2020; Zhao 2006; Wang *et al.* 2019, 2020).

Despite the wide distribution range of *Achalinus* from Japan to northern Vietnam, there are still many gaps throughout its range and un-surveyed regions across its distribution based on current data, particularly in China (Zhao *et al*. 1998; Zhao 2006; Yang & Rao, 2008). For example, although *Achalinus* species have been recorded from southern Sichuan Province (Zhao 2006), no records of any congeners have been reported from Panzhihua, despite the presence of continuous and suitable habitats. Similarly, although multiple species have been recorded from Vietnam on the border between Vietnam and China (Ziegler *et al.* 2019), no congener has been reported from China (southern Yunnan Province) either (Yang & Rao 2008). As studies have suggested the presence of unrecognized diversity within *Achalinus*, it is possible that these un-surveyed regions harbor additional, undocumented diversity of the genus (Ziegler *et al*. 2019).

 Accepted by V. Deepak: 16 Feb. 2021; published: 30 Mar. 2021

Furthermore, the currently recognized endemic species from northern Vietnam may also be present on the other side of the border in southern China. Recently, Ziegler *et al.* (2019) described three new species of *Achalinus* from northern Vietnam, close to the Chinese border, including *A. emilyae* Ziegler, Nguyen, Pham, Nguyen, Pham, Schingen, Nguyen & Le, *A. juliani* Ziegler, Nguyen, Pham, Nguyen, Pham, Schingen, Nguyen & Le and *A. timi* Ziegler, Nguyen, Pham, Nguyen, Pham, Schingen, Nguyen & Le (Ziegler *et al*. 2019). Previous studies have already demonstrated that species thought to be endemic to Vietnam were later found to have much wider distribution across the national borders (Ota *et al.* 2000; Bain *et al*. 2009; Wang *et al.* 2017; Chen *et al.* 2017, 2018, 2020; Ren *et al.* 2018), thus, it is very likely that these *Achalinus* occur in the nearby provinces in China.

During recent herpetological surveys, we collected specimens of the genus *Achalinus* from southern Sichuan, southern Yunnan provinces and southern Guangxi Zhuang A. R. of China (Fig. 1). After morphological comparisons and genetic analyses, we found the newly collected specimens from Yunnan and Sichuan are morphologically and phylogenetically distinct from presently recognized congeners, hence we describe them as two new species. The specimen from Guangxi Zhuang A. R. is aligned with *A. emyliae* in morphological characters as well as phylogenetic analyses, and we record this species from China for the first time and provide a description of the Chinese specimen. Additionally, we discuss diversity of the genus *Achalinus* and present an updated key to the genus.

Material and methods

Sampling. A single specimen of *Achalinus* was collected from Sichuan, Yunnan and Guangxi Provinces in China, respectively, which represents two putative new species and putative *A. emilyae* (Fig. 1). After euthanasia, liver tissues were taken and preserved in 95% ethanol, and specimens were fixed in 10% buffered formalin solution and then transferred to 70% ethanol after two days. In addition, two snake sheds were collected near the locality of the Southern Yunnan specimen and stored in 95% ethanol and their DNA were used for species. All three newly collected specimens were deposited in Kunming Institute of Zoology, Chinese Academy of Sciences (KIZ). Morphological data of recognized species, were obtained from the following literatures (Boulenger 1888; Van Denburgh 1912; Bourret 1937; Hu & Zhao 1966; Koshikawa 1982; Zong & Ma 1983; Ota & Toyama 1989; Zhao *et al.* 1998; Zhao 2006; Ziegler *et al.* 2019; Wang *et al.* 2019; Li *et al*. 2020).

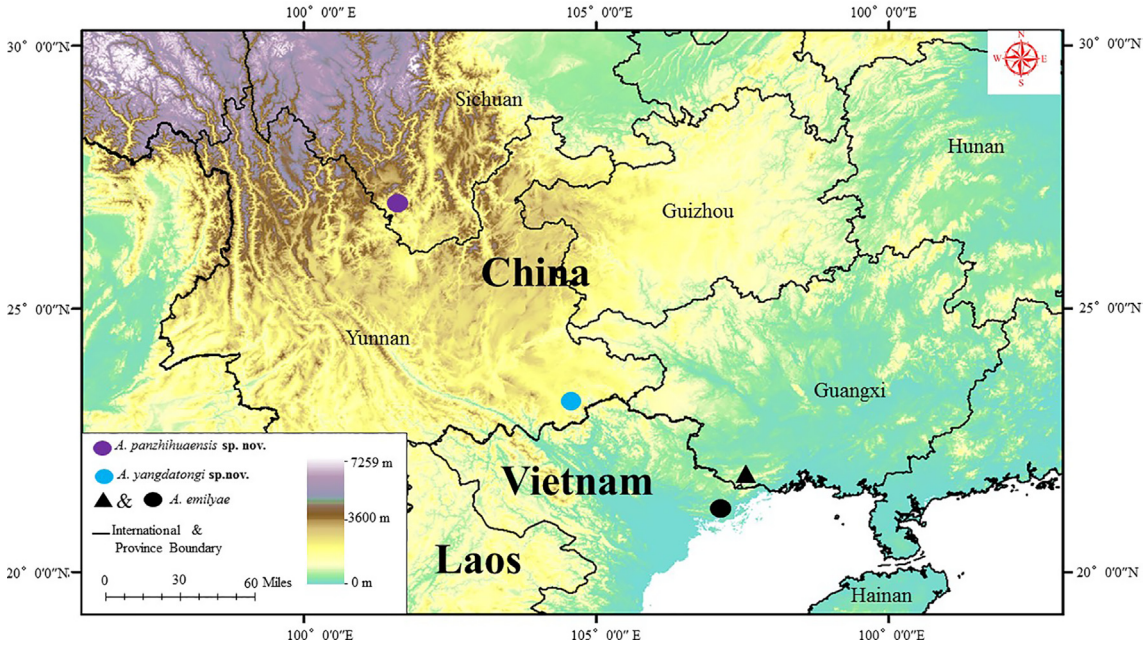

FIGURE 1. Distribution of selected species of the genus *Achalinus* in southern China and Vietnam, including the type locality (indicated by circle) of *A. panzhihuaensis* **sp. nov.** in Sichuan (purple), of *A. yangdatongi* **sp. nov.** in Yunnan (blue), and *A. emilyae* in Vietnam and China (black). The black triangle indicates the new locality of *A. emilyae* in Guangxi, China.

Morphological data. Measurement and scale counting methods follow Zhao *et al*. (1998). The snout-vent length (SVL) and tail length (TaL) were measured after preservation using a measuring tape (accuracy 1 mm), while the head length (HL) was taken with a digital caliper to the nearest 0.1 mm. Sex of the specimen was determined by dissection of hemipenis after preservation. ToL: total length (SVL+TaL); Lor: loreal scale count; HiL: height of loreal scale; LeL: length of loreal scale; INT: internasal scale count; PRF: prefrontals scale count; LSBP: length of suture between the prefrontals; LSBI: length of suture between internasals; LSBP/LSBI: ratio length of suture between the prefrontals/length of suture between internasals; In addition to the morphometric characters, the following characters were also examined (abbreviations before colon): PRO: preoculars scale count; PTO: postoculars scale count; SPO: supraoculars scale count; SL: supralabials scale count; IL: infralabials scale count; TMP: temporals scale count; Ven: ventral scales count; SC: subcaudals scale count; DSR: dorsal scale row; MT: maxillary teeth counts.

For DSR, it was counted at one head length behind head (DSRH), at mid-body (DSRM), and at one head length before vent (DSRV), respectively. For SL, scale count was given in "A–B–C" format, where A is the number of anterior most SL that does not enter the orbit, B is number of SL that enters the orbit, and C is the number of remaining SL that does not enter and posterior to the orbit. For IL, scale counts were given in "A(B)" format, where A is the number of total IL, and B is the number of IL that are in contact with the anterior chin shield. For TMP, scale count was given in "A+B" format, where A and B are the number of anterior and posterior temporal scales respectively. MT was counted by number of teeth or sockets on right upper maxilla via a dissecting microscope.

Genetic data. DNA genomes were extracted from tissues and snake sheds using standard phenol-cholroform extraction (Sambrook *et al.* 1989). A fragment of cytochrome c oxidase subunit 1 (*COI*) was amplified using the primer pairs Chmf4 (5'-TYT CWA CWA AYC AYA AAG AYA TCG G-3') and Chmr4 (5'-ACY TCR GGR TGR CCR AAR AAT CA-3') (Che *et al.* 2012) through PCR. Amplifications were performed in a 25 reaction volume with the following cycling conditions: an initial denaturing step at 94°C for 5 min, 35 cycles of denaturing at 94°C for 45s, annealing at 46°C for 30s and extending at 72°C for 1 min, and a final extending step of 72°C for 10 min. All the PCR products were sequenced using an ABI 3730 automated sequencer. As results, five new *COI* sequences were generated and deposited on GenBank (GenBank numbers MW664861–MW664865, Table 1).

Phylogenetic analyses. In addition to the newly generated sequences, 29 sequences of 13 recognized species of *Achalinus* and three outgroup taxa (*Fimbrios klossi* Smith 1921*, Parafimbrios vietnamensis* Ziegler, Ngo, Pham, Nguyen, Le & Nguyen 2018 and *Parafimbrios lao* Teynie, David, Lottier, Le, Vidal & Nguyen 2015) were downloaded from GenBank. The final alignment consisted of 551bp, with 212 variable positions and 195 parsimony informative sites. Alignments were conducted using MEGA7.0 (Kumar *et al.* 2016) with default parameters and the alignment being checked and manually revised, and the uncorrected pairwise genetic distances among congeners were also calculated using MEGA7.0.

Phylogenetic trees were constructed using both Maximum Likelihood (ML) and Bayesian Inference (BI) analyses. For both analyses, genetic data were partitioned by codon positions. Partitioned ML analyses were performed with RAxML version 8.2.4 (Stamatakis 2014) on the CIPRES web server (Miller *et al*. 2010) under a GTR+GAMMA model. Nodal support was determined based on 1,000 bootstrap replicates. BI analyses were performed using MrBayes v.3.2.1 (Ronquist *et al*. 2012). The best-fit model was determined for each codon position using the Bayesian Information Criterion (BIC) computed with jModelTest 2 (Darriba *et al*. 2012). Two independent runs were conducted for 10 million generations, sampling every 1000, with four independent chains and a burn-in of 25%. Convergence was assessed by confirming that all parameters had reached stationarity and had satisfactory effective sample sizes (> 200) using Tracer v.1.6. (Rambaut *et al*. 2014).

Results

Morphological results. The three specimens are morphologically consistent with the recognized species of the genus *Achalinus* by having the following characters: (1) body slender, cylindrical; (2) head not or only scarcely distinct from neck; (3) subcaudals unpaired; (4) eye small or moderate, with round or vertically subelliptical pupil; (5) loreal contacting orbit; (6) postocular absent or a small one; and (7) middorsal scale rows 21–27 (Smith 1943; Hu & Zhao 1966; Zhao *et al*. 1998).

TABLE 1. Localities, voucher information, and Genbank accession numbers for all samples used in this study, "–" indicates the snake sheds with no voucher specimens.

ID	Species name	Locality	**Voucher**	***COI***	**Reference**
1	*Achalinus ater*	China: Huaping Nature Reserve, Guangxi	SYS r000852	MN380334	Wang *et al.* 2019
2	*Achalinus emilyae*	Vietnam: Dong Son–Ky Thuong Nature Reserve, Hoanh Bo District, Quang Ninh	IEBR 4465	MK330857	Ziegler *et al.* 2019
3	*Achalinus emilyae*	China: Dongzhong, Fangchenggang, Guangxi	KIZ 022248	MW664861	this study
4	*Achalinus formosanus*	China: Taiwan	RN2002	KU529452	–
5	*Achalinus formosanus*	China: Taiwan	RN2003	KU529453	–
6	*Achalinus formosanus*	China: Taiwan	RN2004	KU529454	–
7	*Achalinus juliani*	Vietnam: forest of Duc Quang Commune Ha Lang District, Cao Bang	IEBR A.2018.8	MK330854	Ziegler *et al.* 2019
8	*Achalinus juliani*	Vietnam: Phia Oac–Phia Den National Park Nguyen Binh District, Cao Bang	IEBR A.2018.9	MK330855	Ziegler *et al.* 2019
9	*Achalinus meiguensis*	China: Qianfoshan Nature Reserve, Sichuan	–	FJ424614	–
10	*Achalinus meiguensis*	–	CHS898	MK064943	–
11	*Achalinus niger*	China: Taiwan	RN0667	KU529434	–
12	*Achalinus niger*	China: Taiwan	RN0647	KU529433	–
13	*Achalinus panzhihuaensis* **sp. nov**.	China: Hongbao, Yanbian, Sichuan	KIZ 040189	MW664862	this study
14	*Achalinus pingbianensis*	China: Honghe, Yunnan	YBU 18273	MT365521	Li *et al.* 2020
15	*Achalinus rufescens*	China: Heishiding Nature Reserve, Guangdong	SYS r001527	MN380335	Wang *et al.* 2019
16	*Achalinus rufescens*	China: Guangzhou, Guangdong	SYS r001795	MN380337	Wang *et al.* 2019
17	*Achalinus rufescens*	China: Shimentai Nature Reserve, Guangdong	SYS r001689	MN380336	Wang *et al.* 2019
18	*Achalinus rufescens*	China: Guangzhou, Guangdong	SYS r001796	MN380338	Wang *et al.* 2019
19	*Achalinus rufescens*	China: Hongkong	SYS r001866	MN380339	Wang *et al.* 2019
20	*Achalinus spinalis*	China: Mt. Badagong, Hunan	SYS r001327	MN380340	Wang *et al.* 2019
21	*Achalinus timi*	Vietnam: Copia Nature Reserve, Thuan Chau District, Son La	IEBR A.2018.10	MK330856	Ziegler *et al.* 2019

.....continued on the next page

TABLE 1. (Continued)

ID	Species name	Locality	**Voucher**	***COI***	**Reference**
22	*Achalinus tranganensis*	Vietnam: near Tran Temple, Ninh Binh	VNUF R.2018.21	MW023086	Luu *et al.* 2020
23	*Achalinus yangdatongi* **sp. nov.**	China: Wenshan Nature Reserve, Yunnan	–	MW664863	this study
24	*Achalinus yangdatongi* **sp. nov.**	China: Wenshan Nature Reserve, Yunnan	–	MW664864	this study
25	*Achalinus yangdatongi* **sp. nov.**	China: Wenshan Nature Reserve, Yunnan	KIZ 034327	MW664865	this study
26	*Achalinus yunkaiensis*	China: Dawuling Forestry Station, Guangdong	SYS r001443	MN380329	Wang *et al*. 2019
27	*Achalinus yunkaiensis*	China: Dawuling Forestry Station, Guangdong	SYS r001503	MN380331	Wang *et al*. 2019
28	*Achalinus yunkaiensis*	China: Dawuling Forestry Station, Guangdong	SYS r001502	MN380330	Wang *et al*. 2019
29	*Achalinus yunkaiensis*	China: Dawuling Forestry Station, Guangdong	SYS r001902	MN380332	Wang *et al*. 2019
30	*Achalinus yunkaiensis*	China: Dawuling Forestry Station, Guangdong	SYS r001903	MN380333	Wang *et al*. 2019
31	*Achalinus zugorum*	Vietnam: Lùng Ca`ng Village, Bac Me District, Ha Giang	IEBR 4698	MT502775	Miller *et al.* 2020
32	*Fimbrios klossi*	Vietnam: Gia Lai	IEBR A.2013.56	KP410745	Teynie *et al*. 2015
33	*Parafimbrios lao*	Laos: Louangphabang	MNHN 2013.1002	KP410746	Teynie *et al*. 2015
34	*Parafimbrios vietnamensis*	Vietnam: Lai Chau	IEBR A.2018.7	MH884515	Ziegler *et al*. 2018

The newly collected specimens from Yunnan and Sichuan can be easily diagnosed from each other and from all recognized congeners by a suit of morphological characters (detail to see comparisons below). For the specimen from Guangxi, it aligns with the diagnosis of *A. emilyae* (see description of Guangxi specimen below) (Table 2. and 4.).

Genetic results. Both ML and BI analyses yield near-identical topologies except for the seven poorly supported nodes (Fig. 2). *Achalinus* was recovered as a monophyletic with strong support from both analyses (ML 100/BPP 1.00), and all newly collected specimens and sheds are nested within the genus (Bayesian posterior probability 1.00/ML bootstrap support 100, here in given in this order). The specimen from Panzhihua, Sichuan (KIZ 040189) was recovered as the sister species of *A. meiguensis* Hu & Zhao (1.00/95). Specimen (KIZ 034327) and snake shed samples from Wenshan, Yunnan are recovered as the strongly supported sister species *A. juliani* and *A. ater* Bourret (1.00/86). The specimen from Fangchenggang, Guangxi (KIZ 022248), is recovered as the sister species to the holotype of *A. emilyae* (IEBR 4465) (1.00/100).

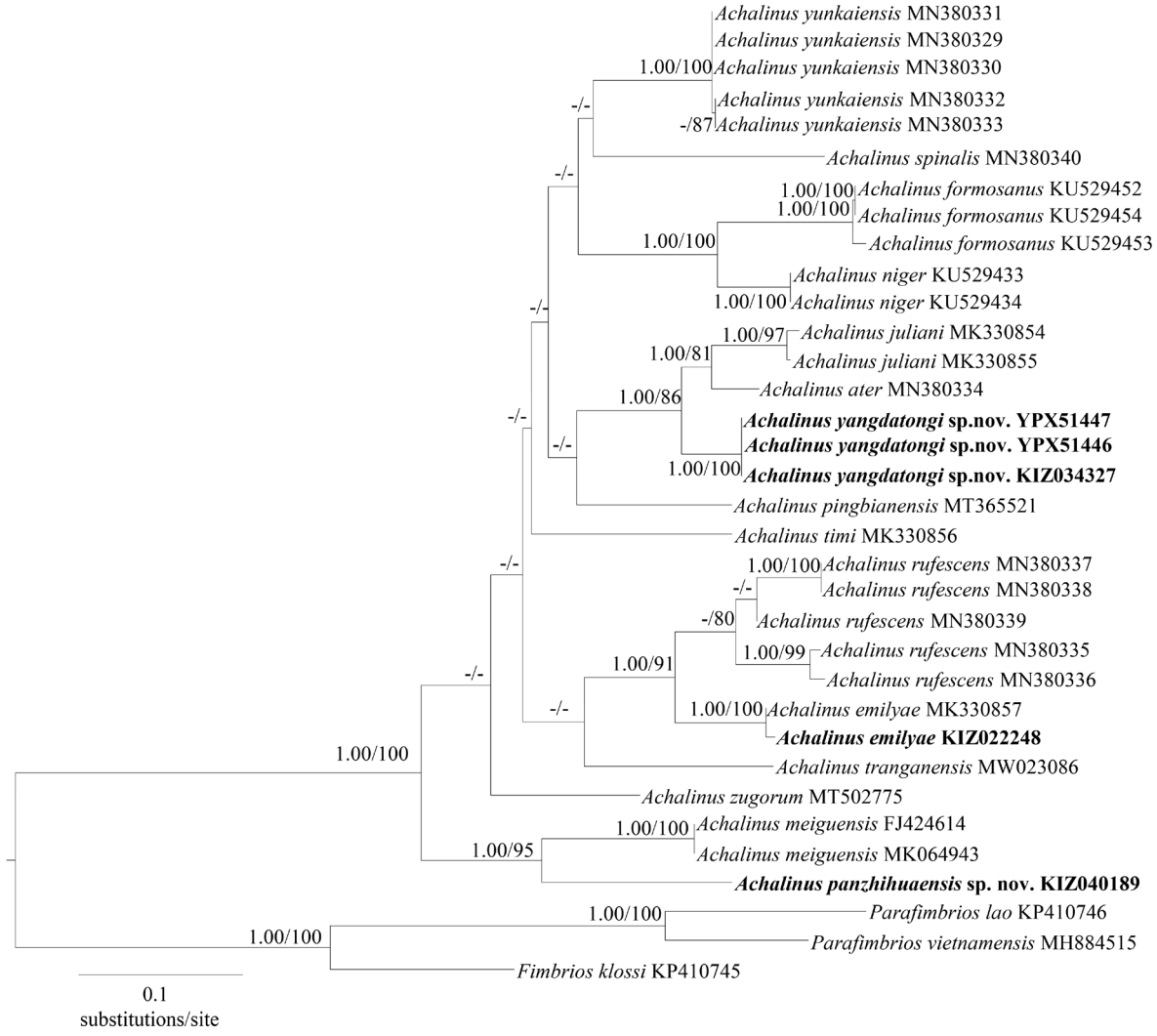

FIGURE 2. Phylogenetic analyses using maximum-likelihood based on 551bp of *COI* fragment. Nodal supports from BI analyses are shown on the topology from ML analyses. Numbers before slashes indicate Bayesian posterior probabilities (>95% retained) and numbers after slashes are bootstrap support for maximum likelihood analyses (>75 retained). Dashes indicate differential topology between BI and ML analyses. New sequences are indicated in bold.

TABLE 2. Morphometric and pholidosis characters of the holotypes of *A. panzhihuaensis* **sp. nov.**, *A. yangdatongi* **sp. nov.**, and the Chinese specimen of *A. emilyae*. Abbreviations are presented in method.

Species	*A. panzhihuaensis* **sp. nov.**	*A. yangdatongi* **sp. nov.**	*A. emilyae*
Voucher	KIZ 040189	KIZ 034327	KIZ 022248
Sex	male	male	female
ToL (mm)	257	397	453
SVL (mm)	194	293	361
TaL (mm)	63	104	92
TaL/ToL	24.6%	26.2%	20.3%
MT	28	19	28
Lor	1	1	1
LeL (mm)	1.2	1.4	1.6
HiL (mm)	0.8	0.8	1.3
LeL/HiL	150.0%	175.0%	123.1%
INT	NO	yes	yes
LSBP	2.1	1.3	0.8
LSBI	–	1.9	2.1
LSBP/LSBI	–	68.4%	38.1%
PrO	NO	NO	NO
PtO	A small one	NO	NO
SpO	1	1	1
SPL	3–2–1	3–2–1	3–2–1
IFL	6(3)	6(3)	5(3)
TMP	2+2+3	2+2+3	2+2+3
Ven	160	161	157
Prec	single	single	single
SC	73	82	65
DSR	23–23–19	23–23–19	23–23–23

TABLE 3. Uncorrected *p*-distances among the species of *Achalinus* based on partial mitochondria *COI* gene. Mean uncorrected intraspecific *p*-distances for each species are shown in the diagonal in bold.

ID	*Achalinus* Species	1	2	3	4	5	6	7
1	*A. ater*	–						
2	*A. emilyae*	11.3–11.8	**0.5**					
3	*A. formosanus*	13.6	14.0–14.5	**0–0.9**				
4	*A. juliani*	5.8–6.4	12.9–14.0	11.4–12.3	**0.9**			
5	*A. meiguensis*	14.8	15.1–15.2	15.8–16.3	16.2–16.3	**0.0**		
6	*A. niger*	13.1	12.3–12.9	8.9–9.4	11.2–12.7	13.8	**0.0**	
7	*A. panzhihuaensis* **sp. nov.**	16.2	16.5–16.9	16.2	15.4	11.3	14.3	–
8	*A. pingbianensis*	17.1	16.0–16.6	18.8	13.4–15.1	22.6	14.7	19.4
9	*A. rufescens*	10.1–12.5	7.4–10.3	13.1–14.2	10.9–13.2	16.7–18.0	12.5–14.0	15.6–16.3
10	*A. spinalis*	14.5	14.5–15.1	14.5	14.0–14.2	15.6	14.2	15.8
11	*A. tranganensis*	12.3	12.3–12.9	17.2	14.0	15.8	14.7	16.3
12	*A. timi*	13.1	12.7–13.2	13.6	13.8–14.3	16.0	11.6	15.4
13	*A. yangdatongi* **sp. nov.**	6.2	12.7–13.1	14.5	7.1–7.3	16.7	13.6	15.4
14	*A. yunkaiensis*	12.3–12.5	13.1–13.4	12.3–12.5	11.8–12.9	15.4–15.6	12.2–12.3	15.6–16.8
15	*A. zugorum*	13.2	12.7–13.2	13.8	13.2–13.8	14.7	13.1	15.2

Continued.

ID	*Achalinus* Species	8	9	10	11	12	13	14	15
1	*A. ater*								
2	*A. emilyae*								
3	*A. formosanus*								
4	*A. juliani*								
5	*A. meiguensis*								
6	*A. niger*								
7	*A. panzhihuaensis* **sp. nov.**								
8	*A. pingbianensis*	–							
9	*A. rufescens*	16.0–19.0	**0–7.6**						
10	*A. spinalis*	16.8	12.5–14.2	–					
11	*A. tranganensis*	13.4	11.4–12.5	15.2	–				
12	*A. timi*	14.5	14.0–14.2	14.2	13.8	–			
13	*A. yangdatongi* **sp. nov.**	13.4–13.7	11.4–12.3	14.2	12.7	13.1	**0.0**		
14	*A. yunkaiensis*	13.7–14.0	12.2–14.5	12.0–12.2	13.8–14.0	13.6–13.8	12.0–12.2	**0.0–0.2**	
15	*A. zugorum*	10.9	12.2–14.2	13.6	11.8	13.6	12.2	10.5–10.7	–

The uncorrected pair-wise sequence divergence between the specimen from Panzhihua and the other recognized congeners ranges from 11.3% (with *A. meiguensis*) to 16.8 % (with *A. yunkaiensis* Wang, Li & Wang). The vouchered sample and snake sheds from Wenshan are genetically identical to each other, and the uncorrected pair-wise sequence divergence between these sample and their congeners ranges from 6.2 % (with *A. ater*) to 16.7% (with *A. meiguensis*). The genetic distance between the specimens from Panzhihua and Wenshan is 15.4 %, and that between the Guangxi specimen and the holotype of *A. emilyae* is 0.5% (Table 3).

Therefore, combining the morphological comparison and phylogenetic data, we confirm that the specimen from Panzhihua, Sichuan Province and Wenshan, Yunnan Province each represents a distinct taxon that has not been described. Here we describe each of them as a new species. For the sample from Guangxi, we consider it to be *A. emliyae*, which represents a new national record of China.

Taxonomic accounts

Achalinus panzhihuaensis sp. nov.

(Figs. 3 and 4)

Holotype. KIZ 040189, adult male, collected by Benfu Miao and Kai Wang on 10 May 2018 from Hongbao Village (27.00°N, 101.53°E), Yanbian County, Panzhihua, Sichuan Province, China.

Diagnosis. *Achalinus panzhihuaensis* **sp. nov.** can be distinguished from recognized congeners by a combination of the following characters: (1) TaL/ToL 24.6% in the single male; (2) two nasal scales in contact with each other behind the rostral; (3) internasal absent; (4) loreal rectangular; (5) supralabials 6; (6) postocular single and small; (7) temporals 2+2+3, anterior pair elongated, upper one smaller, only uppermost in contact with eye; (8) infralabials 6; (9) mental in contact with first pair of chin shields, fully separating first pair of infralabials; (10) dorsal scales 23–23–19 rows; (11) ventrals 160; (12) subcaudals 73, unpaired; (13) precloacal scale entire; (14) maxillary teeth 28; and (15) all scales iridescent with metallic luster, brown dorsally, with single indigo-colored vertebral line.

Description of holotype. Body size small, total length 257 mm (SVL 194 mm, TaL 63 mm); tail long, 24.6% total length; body slender, cylindrical in cross section. Head slightly distinct from neck, HL 7.8 mm; eye small, pupil vertically subelliptic. Rostral small, triangular, invisible from above; nasal divided, each half in contact with each other; internasal absent; prefrontals paired, suture length 2.1 mm; frontal pentagonal, slightly wider than long,

pointed posteriorly; single pair of parietals; loreal pentagonal, tip pointing anteriorly, longer (LeL: 1.2 mm) than high (HiL: 0.8 mm), LeL/HiL 150.0%; supraocular single, in contact with loreal, prefrontals, frontal, parietals, and superior anterior temporals. Temporals in three groups, 2+2+3; superior one of anterior most pair triangular, small, inferior one much larger, elongated, in contact with fourth and fifth supralabials and parietal; the middle pair, superior one parallelogram, small, inferior one much larger, elongated, in contact with sixth supralabials and three posterior temporals; superior most one of last trios biggest, size gradually decreases inferiorly; supralabials six, first one smallest, fourth and fifth in contact with eyes, sixth longest. Mental arc-shaped, in contact with first pair of chin shields; three pairs of chin shields, first pair in fan-shaped, remaining ones of second and third pairs in unequilateral-quadrilateral shape. Infralabials six, first pair not in contact with each other, first three in contact with anterior-most pair of chin shields, third and fourth infralabials in contact with middle pair.

FIGURE 3. An unvouchered topotypic individual of *A. panzhihuaensis* **sp. nov.** in life. Photo by Benfu Miao.

Dorsal scales elliptical, 23–23–19 rows, medial 6–11 rows distinctly keeled, remaining outer rows smooth. Ventrals 160, rounded laterally; subcaudals 73, unpaired; precloacal entire.

Coloration: In life, all scales are weakly iridescent with metallic luster. Dorsum is purplish brown. The vertebral and three paravertebral rows of dorsal scales are darker indigo, which form a darker longitudinal vertebral stripe extending from the posterior margin of the parietals to the tip of tail. Ventral surface of the body is greyish white, and the subcaudal region is purplish brown.

In preservative, all scales are still iridescent. Coloration becomes darker after preservation. The dorsum becomes dark grey, and the vertebral stripe turns black. The ventral surface of the body becomes greyish brown, and the ventral tail is dark greyish brown.

Comparisons. *A. panzhihuaensis* **sp. nov.** is most similar to its sister species *A. meiguensis*, in which both species have divided nasal scales in contact with each other, no internasal, a single postocular, 6 supralabials, 6 infralabials, mental in contact with first pair of chin shields, and fully separated first pair of infralabials. However, the new species can be diagnosed readily from *A. meiguensis* by having more subcaudals (SC 73 vs. 39–60), more ventrals in male (VEN 160 vs. 146–155), and more DSRM (23 vs. 19–21) (Table 4).

Achalinus panzhihuaensis **sp. nov.** can be easily distinguished from *A. ater*, *A. emilyae*, *A. formosanus* Bou-

lenger, *A. hainanus* Huang, *A. jinggangensis* Zong & Ma, *A. juliani*, *A. niger* Mahi, *A. pingbianensis* Li, Yu, Wu, Liao, Tang, Liu & Guo, *A. rufescens* Boulenger, *A. spinalis* Peters, *A. tranganensis* Luu, Ziegler, Ha, Lo, Hoang, Ngo, Le, Tran & Nguyen, *A. timi*, *A. yunkaiensis*, *A. werneri* Van Denburgh and *A. zugorum* Miller, Davis, Luong, Do, Pham, Ziegler, Lee, De Queiroz, Reynolds & Nguyen, by having divided nasal scales in contact each other behind the rostral (vs. separated), mental in contact with the first pair of chin shields (vs. separated), first pair of infralabials separated from each other (vs. in contact), as well as an absence of internasal (vs. present), and by the presence of a small postocular (vs. absent). Furthermore, the new species differs from *A. jinggangensis*, *A. pingbianensis*, *A. timi* and *A. formosanus* by having loreal separated from prefrontal (vs. fused); and from *A. emilyae*, *A. hainanus* and *A. rufecens* by having more infralabials (6 vs. 5).

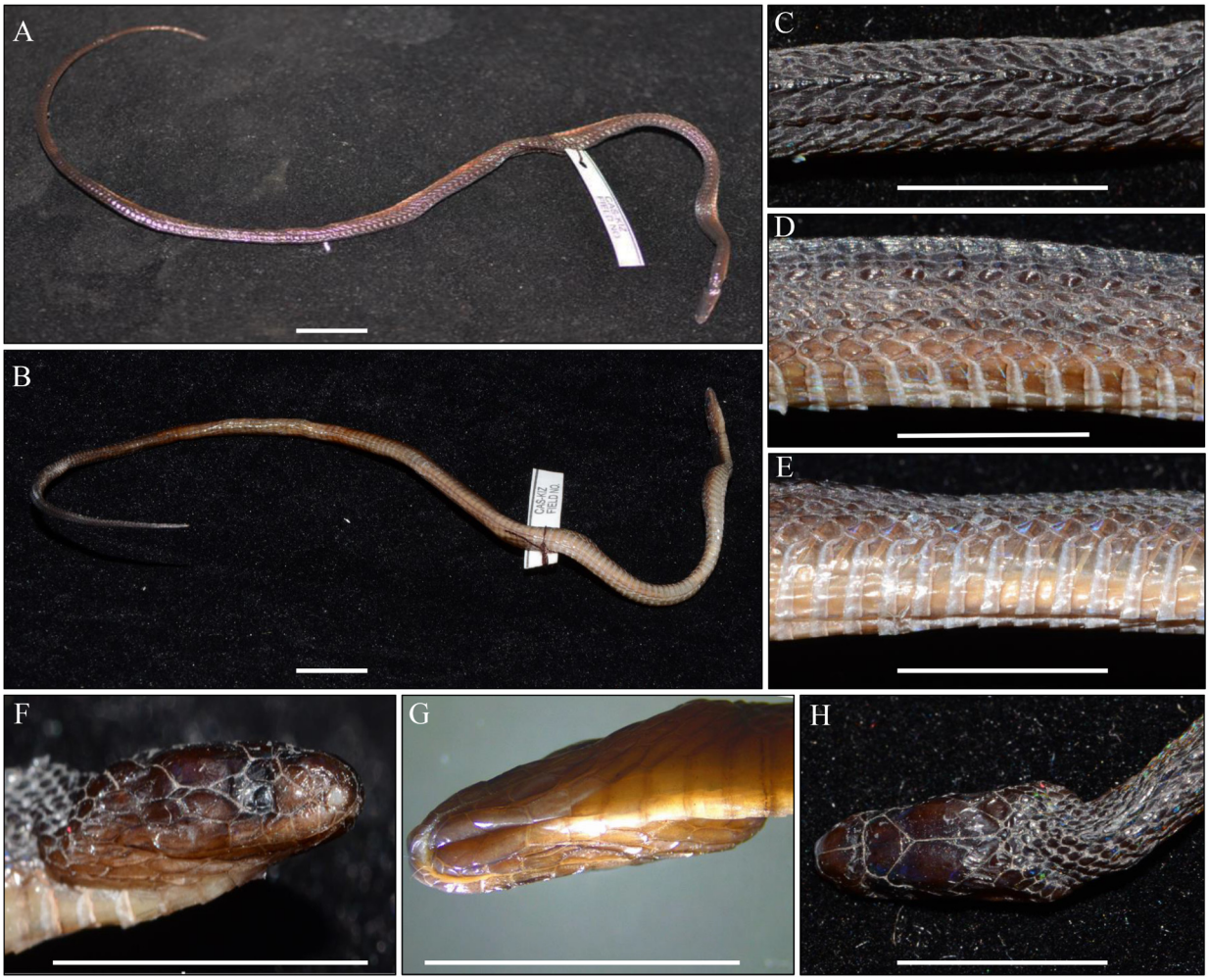

FIGURE 4. Holotype of of *A. panzhihuaensis* **sp. nov.** (KIZ 040189) in preservation, showing (A) dorsal review, (B) ventral review; (C) dorsal mid-body close-up, (D) lateral mid-body body close-up; (E) ventral mid-body close-up, (F) lateral right head view, (G) ventral head and (H) dorsal head. Scale bar = 10 mm. Photos by Shaobing Hou.

Natural history and distribution. The holotype was found on a montane road at night. The surrounding habitat was of secondary forest of evergreen broadleaf forest with shrubs and vines (Fig. 5). According to locals, road-killed individuals are somewhat common in the summer. At the type locality, the species is sympatric with *Diploderma swild* Wang, Wu, Jiang, Chen, Miao, Siler, Che, 2019, *Lycodon* cf. *gongshan* Vogel, Luo, 2011, *Hebius yanbianensis* Liu, Zhong, Wang, Liu, Guo, 2018, *Ptyas nigromarginata* (Blyth, 1854), *Megophrys platyparietus* (Yang, Rao, 1997), and *Odorrana* sp.. The new species is currently only known from the type locality in Panzhihua, Sichuan Province, China (Fig. 1).

Etymology. The specific epithet "*panzhihuaensis*" is named after the type locality of the new species, Panzhihua, Sichuan Province, China. We propose "Panzhihua Odd-scaled Snake" as its common English name and "攀枝花脊蛇" (Pinyin: Pan Zhi Hua Ji She) as its Chinese common name.

FIGURE 5. Habitat of *A. panzhihuaensis* **sp. nov.** (KIZ 040189) at Hongbao Village, Yanbian County, Panzhihua, Sichuan Province, China. Photo by BenFu Miao.

***Achalinus yangdatongi* sp. nov.**
(Figs. 6 and 7)

Holotype. KIZ 034327, adult male, collected by Kai Xu on 15 April 2018 from Xiaoqiaogou (23.361°N, 104.686°E; 1609 m a.s.l.), Xichou County, Wenshan, Yunnan Province, China.

Diagnosis. *Achalinus yangdatongi* **sp. nov.** can be distinguished from recognized species of *Achalinus* by a combination of the following characters: (1) TaL/ToL 26.2% in the male; (2) suture between internasals distinctly longer than that between prefrontals; (3) internasal present; (4) loreal present; (5) supralabials 6; (6) temporals 2+2+3, anterior two temporals in contact with eye; (7) infralabials 6; (8) first pair of infralabials in contact with each other behind mental; (9) dorsal body scales in 23–23–19 rows; (10) scales behind head irregular in shape, smooth; (11) ventrals 161; (12) subcaudals 82, unpaired; (13) precloacal scale entire; (14) maxillary teeth 19; and (15) body surface black above and beneath, iridescent.

Description of holotype. Total length 397 mm (SVL 293 mm, TaL 104 mm); tail long, TaL/ToL 26.2%; body slender, cylindrical; head slightly distinct from neck, HL 11.6 mm; eye small, pupil vertically subelliptic. Rostral small, triangular, slightly visible from above; suture between internasals (1.9 mm) longer than that between prefrontals (1.3 mm); nostril in anterior part of nasal; frontal pentagonal, slightly broader than long, pointed backwards, much shorter than parietals; single pair of parietals; loreal rectangle, wider (LeL: 1.4 mm) than the height (HiL: 0.8 mm); single supraocular, in contact with loreal, prefrontals, frontal, parietals and superior anterior temporals; temporals 2+2+4, two anterior temporals all pentagonal and in contact with eye, superioanterior temporals in contact with parietal, inferioanterior temporal in contact with fourth and fifth supralabials; middle temporals elongated, inferior middle temporal in contact with sixth supralabials; four posterior temporals, superioposterior temporals biggest, inferioposterior temporal smallest; supralabials 6, first smallest, fourth and fifth entering orbit, sixth longest;

mental in arc shape, separated from anterior chin shields; infralabials 6, first pair of infralabials in contact with each other behind mental; two pairs of chin shields, anterior one semicircle-shape, posterior pair in unequilateral quadrilateral shape; first three infralabials in contact with anterior chin shields; third and fourth infralabials in contact with posterior chin shields.

All scales with metallic luster, weakly iridescent; scales behind head irregular in shape, smooth without keeled; dorsal scale rows 23–23–19, scales lanceolate and strongly keeled; ventrals 161; subcaudals 82, unpaired; precloacal entire.

Coloration: In preservative, the dorsal surface of the body is black, the anterior portion of the coloration of underside of head is dark brown, and the posterior portion and throat is light brown. The color becomes darken gradually from the throat posteriorly until it becomes black, except the free margin of each ventral scale, which is grayish white.

Comparisons. *A. yangdatongi* **sp. nov.** is most similar to *A. ater*, in which both species have a suture between internasals distinctly longer than that between prefrontals, equal number of supralabials and infralabials (both 6), anterior temporals in contact with eyes, first pair of infralabials in contact with each other behind the mental, dorsal scales in 23–23–19 rows, as well as by the presence of internasal and loreal scales. However, the new species can be diagnosed from *A. ater* by having more subcaudals (SC 82 vs. 47–70), a comparatively longer tail (TaL/ToL 26.2% vs. 19.0%–22.0%), and different coloration of ventral head (the anterior portion dark brown, posterior portion and throat light brown vs. uniformly yellowish-white).

Achalinus yangdatongi **sp. nov.** differs from *A. juliani* by having fewer ventrals (161 vs. 173–179), fewer DSRH (23 vs. 25), and a distinct coloration (black on both dorsal and ventral surfaces vs. greyish brown dorsally, greyish cream ventrally).

Achalinus yangdatongi **sp. nov.** differs from *A. tranganensis* by having fewer ventrals (161 vs. 171), by having dorsal scale rows 23–23–19, smooth without keeled (vs. dorsal scales in 25–23–23 rows, keeled), by having temporals 2+2+4 (vs. 2+3).

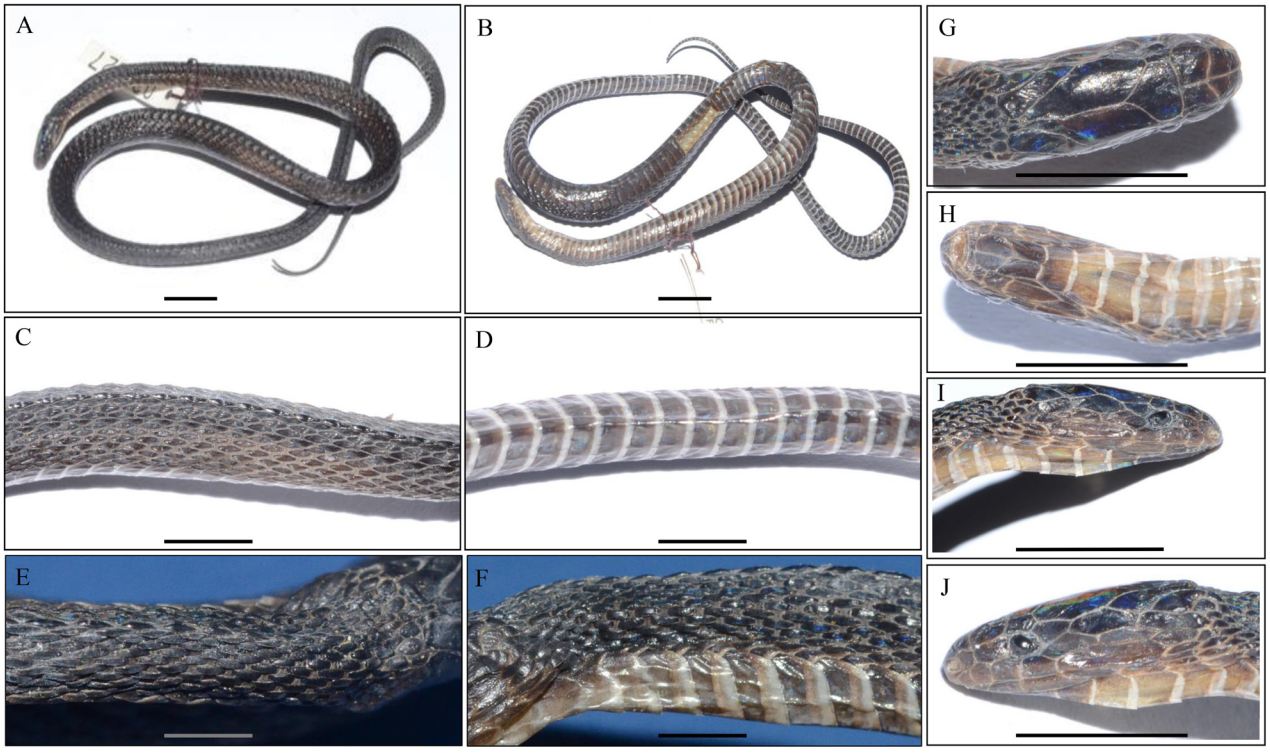

FIGURE 6. Holotype of *A. yangdatongi* **sp. nov.** (KIZ 034327) in preservation, showing (A) dorsal review, (B) ventral review; (C) dorsal mid-body closeup, (D) ventral mid-body closeup, (E) anterior dorsal body closeup, (F) lateral anterior body closeup; (G) dorsal head, (H) ventral head, (I) lateral right head view and (J) lateral left head view. Scale bar = 10 mm. Photos by Shaobing Hou.

Achalinus yangdatongi **sp. nov.** differs from *A. emilyae* and *A. rufescens* by having more infralabials (6 vs. 5 in both *A. emilyae* and *A. rufescens*), distinct body coloration (black on dorsal body and belly vs. dorsum iridescent pale yellowish brown in *A. emilyae*, and uniform pale reddish or reddish-brown dark grey dorsally in *A. rufescens*). Furthermore, the new species differ from *A. rufescens* by having more ventrals in males (161 vs. 131–137) (Table 4).

Achalinus yangdatongi **sp. nov.** differs from *A. niger*, *A. werneri*, *A. yunkaiensis*, and *A. spinalis* by having suture between the internasals distinctly longer than that between the prefrontals (vs. less than or subequal to), a comparatively longer tail (TaL/ToL 26.2% vs. 15.0%–18.0% in *A. niger*, 15.0%–25.0% in *A. spinalis*, and 18.0%–20.0% in *A. yunkaiensis*), fewer subcaudals in male (SC 82 vs. 89–98 in *A. werneri*); from *A. formosanus*, *A. jinggangensis*, *A. pingbianensis*, *A. timi* and *A. zugorum* by presence of loreal scale (vs. absence), fewer dorsal scale rows (23–23–19 vs. 25–25–23 in *A. timi*, and 27–27–25 in *A. formosanus*), and more subcaudals (SC 82 vs. 51–64 in *A. jinggangensis*, 56 in *A. pingbianensis* and 70 in *A. zugorum*); from *A. meiguensis and A. panzhihuaensis* **sp. nov.** by presence of internasal (vs. absent), absence of postocular (vs. present), as well as by having different state of nasal scales (separated vs. in contact each other behind the rostral), mental separated from anterior chin shields (vs. in contact), and first pair of infralabials in contact with each other (vs. separated); and from *A. hainanus* by having different temporal formula (2–2–3 vs. 1–2–3), more subcaudals (SC 82 vs. 67–69), and more infralabials (6 vs. 5).

Natural history and distribution. The holotype was found on a paved road near a reservoir on a drizzly night. The nearby habitat is characterized by secondary forests and abandoned farmlands (Fig. 7). At the type locality, this species is sympatric with *Trimerodytes percarinatus* (Boulenger, 1899), *Protobothrops mucrosquamatus* (Cantor, 1839), and *Pareas* sp. With the holotype and the genetically identified snake sheds, *A. yangdatongi* **sp. nov.** is only known from its type locality at Xiaoqiaogou, Xichou county, Wenshan Prefecture, Yunnan Province, China (Fig. 1).

Etymology. The species name, *yangdatongi*, is a patronym honoring the Chinese herpetologist, Dr. Da-Tong Yang. We name the new species after Dr. Yang in recognition of his great contributions to the herpetological research in Southwestern China, particularly in Yunnan Province where the new species is found. We suggest "Yang's Odd-scaled Snake" as its common English name, and "杨氏脊蛇" (Pinyin: Yang Shi Ji She) as its Chinese common name.

FIGURE 7. Habitat of *A. yangdatongi* **sp. nov.** (KIZ 034327), Xiaoqiaogou, Xichou County, Wenshan, Yunnan Province, China. Photo by Shaobing Hou.

***Achalinus emilyae* Ziegler, Nguyen, Pham, Nguyen, Pham, Van Schingen, Nguyen & Le, 2019**
(Figs. 8 and 9)

Chinese Name. We suggest "越北脊蛇" (Pinyin: Yue Bei Ji She) as its Chinese common name.

Specimen examined. Single adult female (KIZ 022248), road-killed individual collected by Zhiyong Yuan and Jinmin Chen from Dongzhong (21.719° N, 107.583°E), Fangchenggang County, Guangxi Zhuang A. R., China, on 2 September 2012 (Fig. 1).

Description. Total length 453 mm (SVL 361 mm, TaL 92 mm, TaL/ToL 20.3%); body slender, cylindrical; head slightly distinct from neck, dorsally covered with large shields; eye small, with pupil vertically subelliptic. Rostral small, triangular, slightly visible from above; frontal pentagonal, slightly broader than long, pointed backwards, much shorter than parietals; parietal long, more than half length of head; nasal divided, nostril in anterior half; one loreal, wider than high, extending from nasal to eye; single supraocular, in contact with loreal, prefrontals, frontal, parietals, and superior anterior temporals; two anterior temporals, only uppermost in contact with eye; two elongated middle temporals, superior one much larger, inferior one in contact with sixth supralabial; three elongate posterior temporals, most superior one largest, separated from each other behind parietals by one small scale; supralabials six, first smallest, third and fourth in contact with loreal; fourth and fifth in contact with eye, sixth longest; mental in arc shape, separated from anterior chin shields, followed by five infralabials; first pair of infralabials in contact with each other; first three infralabials in contact with anterior chin shields; posterior chin shields smaller, laterally in contact with third and fourth infralabials.

Dorsal scales elliptical, keeled from neck region onwards; dorsal scale rows 23–23–23; ventrals 157 (potential preventrals included), rounded laterally; subcaudals 56, unpaired; precloacal entire.

Coloration. In life, the dorsal body surfaces of the snake are greyish brown with a dark greyish brown vertebral stripe along the body. The ethanol-preserved specimen is greyish brown above, venter is greyish cream, with the ventral surface of the tail being somewhat darker, and the gular region somewhat paler. Infralabials and chin shields light greyish brown.

FIGURE 8. Adult female of *A. emilyae* (KIZ 022248) from Dongzhong, Fangchenggang, Guangxi, China, showing (A) dorsal view, (B) ventral view, (C) dorsal mid-body closeup, (D) ventral mid-body closeup; (E) dorsal head, (F) ventral head, (G) lateral right head view and (H) lateral left head view. Scale bar = 10 mm. Photos by Shaobing Hou.

Comments. The Guangxi specimen matches with most of the diagnosis of *A. emilyae*, including having (1) TaL/ToL 20.3%; (2) suture between internasals distinctly longer than that between the prefrontals; (3) internasal

present; (4) loreal present, wider than high, extending from nasal to eye; (5) supralabials 6; (6) infralabials 5; (7) first pair of infralabials in contact with each other; (8) first three infralabials in contact with anterior chin shields; (9) mental separated from anterior chin shields; (10) temporals 2+2, only the superioanterior one in contact with eye; (11) ventrals 157 in female; (12) subcaudals unpaired; (13) dorsal scale rows 23–23–23; (14) maxillary teeth 28; and (15) dorsum iridescent pale yellowish brown with a dark longitudinal vertebral stripe. The only deviation from the diagnosis of the type series is the number of subcaudal scale (65 for Guangxi specimen vs. 63 for the female paratype).

Natural history. The specimen was a road-kill, and its head was found swallowed by a road-killed *Bungarus fasciatus* (Fig. 9). The nearby habitat consists of secondary forest of broadleaf evergreen forest mixed with shrubs and vines (Provided by Jin-Min Chen, who collected this specimen in the wild). At the type locality, the species is sympatric with *Boiga multomaculata* (Boie, 1827), *Hypsiscopus plumbea* (Boie, 1827), *Pareas margaritophorus* (Jan, 1866), *Ptyas dhumnades* (Cantor, 1842) and *Ptyas multicinctus* (Roux, 1907).

Distribution. Currently *A. emilyae* is only known from southern China and northern Vietnam. In China, this species is known from a single locality in Guangxi Zhuang A. R. (Fig. 1).

FIGURE 9. Road-kill *Achalinus emilyae* (KIZ 022248) and its predator *Bungarus fasciatus* from Dongzhong, Fangchenggang, Guangxi, China. Both snakes were road-killed during the predation process. Photo by Zhiyong Yuan.

Discussion

Being a fossorial and range-restricted endemic, specimens of the genus *Achalinus* are scarce in natural history collections. In addition to the lack of detailed information in the original descriptions for most species, misidentifications over congeners are common in the literature. Species with wide distributions likely represent species complexes. For example, *A. rufescens* as is currently has a wide distribution across southern China and northern Vietnam, while available genetic data shows considerable genetic divergences among populations (maximum *p*-distance=7.6%), suggesting it is composed of more than one species. Future studies would clarify the taxonomy of *Achalinus* in southern China, particularly for species with wide distributions.

Our discovery of previously identified Vietnam endemic species *A. emilyae* further highlights the lack of knowledge on reptilian taxonomy and the underestimated biodiversity along the border areas in southern China. Future international collaborations are needed in order to obtain a better understanding of the biodiversity along the southern China border.

Based on our findings,we provide an updated key to currently recognized species of the genus *Achalinus* from China (morphological data taken from the following literature: Boulenger 1888; Bourret 1937; Hu & Zhao, 1966; Koshikawa 1982; Zong & Ma 1983; Ota & Toyama 1989; Zhao *et al.* 1998; Zhao 2006; Wang *et al*. 2019; Li *et al*. 2020).

TABLE 4. A morphological comparison of species of the genus *Achalinus*. Morphological data of unexamined congeners were taken from Van Denburgh (1912), Pope (1935), Bourret (1935, 1937), Smith (1943), Koshikawa (1982), Karsen *et al.* (1986), Ota & Toyama (1989), Zhao (1998), and Das (2010), Ziegler *et al.* (2019), Wang *et al.* (2019), Luu *et al.* (2020), Li *et al.* (2020) and Miller *et al.* (2020).

Species	ToL max	SVL max	TaL max	TaL/ToL	MT	INT	Lor	LSBP /LSBI
A. ater	401	–	70	19–22%	–	2	1	<1
A. emilyae	519	424	95	18–20%	27–28	2	1	<1
A. formosanus	860	720	140	16.3%	15	2	1	>1
A. hainanus	310	230	80	25.8–26.6%	–	2	1	=/<1
A. jinggangensis	460	360	80	17.4–21.7%	22	2	0	<1
A. juliani	413	304	109	22–37%	28	2	1	<1
A. meiguensis	555	474	81	14.2–23.8%	19	0	1	no
A. niger	730	620	110	15.1–17.9%	–	2	1	=/>1
A. panzhihuaensis **sp. nov.**	257	194	63	24.6%	28	0	1	no
A. pingbianensis	429	345	84	19.6%	–	2	0	=1
A. rufescens	420	347	73	17.0–26.9%	18–26	2	1	<1
A. spinalis	560	470	90	15–25%	16–20	2	1	=/>1
A. timi	178	140	38	21.3%	27	2	0	<1
A. tranganensis	448	334	114	25.4%	29	2	1	<1
A. werneri	550	–	–	25–30%	–	2	1	=1
A. yangdatongi **sp. nov.**	397	293	104	26.2%	19	2	1	<1
A. yunkaiensis	448	386	62	18.5–20.0%	20–22	2	1	<1
A. zugorum	458	353	105	22.9%	28	2	0	<1

TABLE 4. (Continued)

Species	PtO	SPL	IFL	TMP	Ven	SC	DSR
A. ater	NO	3–2–1	6(3)	2+2	160–170	47–70	23(21)–23–23
A. emilyae	NO	3–2–1	5(3)	2+2	157–166	60–63	23–23–23
A. formosanus	NO	3–2–1	6 or 7(3)	2+2	158–169	61–83	27–27–25
A. hainanus	NO	3–2–1	5(3)	1+2+3	165–168	67–69	23–23–23
A. jinggangensis	NO	3–2–1	6(4)	2(1)+2+3(4)	156–164	51–64	23–23–23
A. juliani	NO	3(4)-2-1	6(4)	2+2	173–179	77–91	25–23–23
A. meiguensis	A small one	3–2–1	6(3)	2(3)+2(3)	146–173	39–60	23(21)–21(19)–19
A. niger	NO	3–2–1	6(3)	2+2	169–185	52–72	25–25–23
A. panzhihuaensis **sp. nov.**	A small one	3–2–1	6(3)	2+2	160	73	23–23–19
A. pingbianensis	NO	3–2–1	6(3)	2+2(+3)	160	56	23–23–23
A. rufescens	NO	3–2–1	5 or 6(3)	2+2(3)	138–165	48–75	25–23–23
A. spinalis	NO	3(4)–2(1)–1	5 or 6(3)	2+2(3)	138–175	39–67	23(24, 25)–23(2, 24, 25)–23(22, 25)
A. timi	NO	3–2–1	6(3)	2+2	170	72	25–25–23
A. tranganensis	NO	3–2–1	6(3)	2+3	171	73+	25–23–23
A. werneri	NO	3–2–1	–	2+?	157–191	67–98	?–23–?
A. yangdatongi **sp. nov.**	NO	3–2–1	6(3)	2+2+3	161	82	23–23–19
A. yunkaiensis	NO	3–2–1	6(3 or 4)	2+2+3(4)	151–162	49–56	23–23–23
A. zugorum	NO	3–2–1	7(3)	2+2	173	70	25–23–23

Key to species of the genus *Achalinus* Peters, 1869

1a. Internasal absent . . . 2
1b. Internasal present . . . 3
2a. Ventrals 146–155 in male; subcaudals 39–60; DSRM 19–21 . . . *A. meiguensis*
2b. Ventrals 160 in male; subcaudals 73; DSRM 23 . . . *A. panzhihuaensis*
3a. Loreal absent . . . 4
3b. Loreal present . . . 6
4a. Dorsal scale rows 27–27–25 . . . *A. formosanus*
4b. Dorsal scale rows 23–23–23 . . . 5
5a. Suture between internasals longer than prefrontal suture . . . *A. jinggangensis*
5b. Length of suture between internasals subequal to that between prefrontals . . . *A.pingbianensis*
6a. Anterior temporals single; two superior posterior temporals in contact and overlap greatly posterior to parietal . . . *A. hainanus*
6b. Anterior temporals 2; two superior posterior temporals not in contact with each other posterior to parietal, or in contact but overlap slightly . . . 7
7a. Suture length between internasals less than or subequal to that between prefrontals . . . 8
7b. Suture length between the internasals much longer than that between prefrontals . . . 10
8a. Dorsal scale rows 25–25–23 . . . *A. niger*
8b. Middorsal scale rows 23 . . . 9
9a. Dorsal color brown in adults, ventral light brown; TaL/ToL 18–20% . . . *A. yunkaiensis*
9b. Dorsal color black, ventral black brown; TaL/ToL 15–25% . . . *A. spinalis*
10a. Infralabials 5 . . . 11
10b. Infralabials 6 . . . 12
11a. Ventrals 157–161 in females; subcaudals 63–65 . . . *A. emilyae*
11b. Ventrals 148–158 in females; subcaudals 54–61 . . . *A. rufescens*
12a. Subcaudals 47–70; TaL/ToL 19–22% . . . *A. ater*
12b. Subcaudals 82; TaL/ToL 26% . . . *A. yangdatongi*

Acknowledgments

We thank Kai Xu and Benfu Miao for their help in the field. This work was supported by the Second Tibetan Plateau Scientific Expedition and Research (STEP) program (2019QZKK0501) to PG, ZYY and JC; Strategic Priority Research Program of Chinese Academy of Sciences (CAS) (XDA19050303), Digitalization, development and application of biotic resource (202002AA100007), China's Biodiversity Observation Network (Sino-BON), Animal Branch of the Germplasm Bank of Wild Species, CAS (the Large Research Infrastructure Funding) to JC, NSF GRFP 2017216966 to KW, Young talent project of China Association for Science and Technology (2019–2021QNRC001) and Yunnan Fundamental Research Project (202001AW070016, 202005AC160046) to ZYY and NSFC 31900323 to JMC. Collections of all animals used for this present study obey the Wildlife Protection Act of China. Collection permits were issued by Kunming Institute of Zoology, Chinese Academy of Sciences (BBCJ-2014-001). IACUC protocols (IACUC R13-11) and relevant protocols of the Animal Care and Ethics Committee at the Kunming Institute of Zoology were followed for the proper treatments of animals in the field.

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