

Discovery of a New Species of Enigmatic Odd-Scaled Snake (Serpentes: Xenodermidae: *Achalinus*) from Ha Giang Province, Vietnam

Aryeh H. Miller^{1,2}, Hayden R. Davis^{3,4}, Anh Mai Luong⁵, Quyen Hanh Do⁵, Cuong The Pham⁵, Thomas Ziegler^{6,7}, Justin L. Lee¹, Kevin de Queiroz¹, R. Graham Reynolds², and Truong Quang Nguyen^{5,8}

***Achalinus*, commonly known as odd-scaled snakes due to their unique scutellation, are a fascinating group of xenodermid snakes distributed throughout east Asia. Currently, northern Vietnam is known to host six species of *Achalinus*, three of which are considered endemic. During recent herpetofaunal surveys conducted in the Bac Me District of Ha Giang Province, we collected a single specimen of *Achalinus* in a lowland region surrounded by secondary forest on karst. We conducted Bayesian and maximum likelihood phylogenetic analyses using multiple mitochondrial protein-coding loci and a ribosomal subunit to ascertain the phylogenetic position of the Ha Giang specimen among currently recognized odd-scaled snake species. The results of those analyses along with morphological differences support this specimen as representing a new species, which we describe as *Achalinus zugorum*, new species. We provide detailed morphological descriptions and comparisons of the new species as well as brief comments on the biogeography and conservation of *Achalinus* in northern Vietnam.**

THE family Xenodermidae (nomenclature following Savage, 2015) is a Late Cretaceous diverging group (stem age) of caenophidian snakes (Burrill et al., 2019) composed of five poorly known genera—*Achalinus*, *Fimbrios*, *Parafimbrios*, *Stoliczka*, and *Xenodermus*. Odd-scaled snakes (*Achalinus*) are an aptly named group of nocturnal xenodermid snakes distributed throughout Japan, China, Taiwan, and Vietnam, characterized by a unique non-imbricate scale organization where the dorsal scales form a dissociated and non-overlapping pattern. The secretive nature of snakes in the genus *Achalinus* (Yamasaki and Mori, 2017) could perhaps explain why the group is so rarely detected and thus consequently why studies concerning ecology and natural history of *Achalinus* are almost non-existent.

Recently, an increasing number of species descriptions (Wang et al., 2019; Ziegler et al., 2019) has brought further attention to *Achalinus*, suggesting that diversity within odd-scaled snakes was highly underestimated. Currently, minimal genetic data (e.g., single locus mitochondrial DNA [mtDNA]) exist for only 10 of the 13 recognized species of *Achalinus*—*A. ater*, *A. emilyae*, *A. formosanus*, *A. hainanus*, *A. jinggangensis*, *A. juliani*, *A. meiguensis*, *A. niger*, *A. rufescens*, *A. spinalis*, *A. timi*, *A. wernerii*, and *A. yunkaiensis*. Furthermore, our cumulative understanding of odd-scaled snake distributions is rather

poor owing to infrequent observations. The bulk of diversity is contained in mainland China (*A. ater*, *A. jinggangensis*, *A. meiguensis*, *A. rufescens*, *A. spinalis*, and *A. yunkaiensis*) with three of those species also ranging into northern Vietnam (*A. ater*, *A. rufescens*, and *A. spinalis*). Of the 13 species currently recognized, six are known from Vietnam, including three endemic species recently described from provinces in the northern portion of the country (Ziegler et al., 2019). The recent detection of novel species of *Achalinus* in Bac Giang, Cao Bang, Quang Ninh, and Son La Provinces (Ziegler et al., 2019) further underscores the likelihood of undiscovered species elsewhere in northern Vietnam.

Bordering Cao Bang immediately to the west is Ha Giang Province, a province of similar size with a diverse but still poorly characterized herpetofaunal assemblage (Bain and Nguyen, 2004; Ziegler et al., 2014; Pham et al., 2017). Ha Giang lies east of the Red River and is characterized by precipitous limestone formations coupled with rolling mountains and valleys that dominate the subtropical landscape. The province is drenched in approximately 2,400 mm of rainfall annually, with as much as 515.6 mm falling during July (Institute of Geography, 1989). Ha Giang falls into the ‘South China’ subunit of the Indo-Malayan Realm, a biogeographic area which conjoins southern China and northeastern Vietnam (MacKinnon, 1997). In Ha Giang,

¹ Department of Vertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, D.C. 20013; Email: (AHM) aryehmiller@wustl.edu; (JLL) justinlee@verizon.net; and (KdQ) deQueirozK@si.edu. Send reprint requests to AHM.

² Department of Biology, University of North Carolina Asheville, One University Heights, Asheville, North Carolina 28804; Email: (RGR) greynold@unca.edu.

³ Department of Biology and Center for Biodiversity and Ecosystem Stewardship, Villanova University, 800 Lancaster Avenue, Villanova, Pennsylvania 19085.

⁴ Department of Biology and Burke Museum of Natural History and Culture, University of Washington, Seattle, Washington 98195; Email: hrdavis1@uw.edu.

⁵ Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Road, Hanoi, Vietnam; Email: (AML) luongmaianhsinhk62b@gmail.com; (QHD) quyendo.k59@gmail.com; (CTP) cuongiebr@gmail.com; and (TQN) nqt2@yahoo.com.

⁶ AG Zoologischer Garten Köln, Riehler Strasse 173, D-50735 Cologne, Germany; Email: ziegler@koelnerzoo.de.

⁷ Institute of Zoology, University of Cologne, Zùlpicher Street 47b, D-50674 Cologne, Germany.

⁸ Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Road, Hanoi, Vietnam. Submitted: 5 April 2020. Accepted: 30 May 2020. Associate Editor: B. L. Stuart.

© 2020 by the American Society of Ichthyologists and Herpetologists DOI: 10.1643/CH2020060 Published online: 7 December 2020

Table 1. Loci, primers, corresponding primer reference, and best-fit nucleotide substitution models used in this study.

Gene	Primers	Reference	Selected model
Cytochrome b (<i>Cytb</i>)	L14910 and H16064	Burbrink et al. (2000)	TPM2+F+G4
Cytochrome oxidase subunit 1 (<i>COI</i>)	COI-ReptBCF and COI-ReptBCR	Castañeda and de Queiroz (2011)	TIM2+F+G4
16S rRNA (<i>16S</i>)	16SL2 and 16SH2	Schubart et al. (2000, 2002)	TIM2+F+G4

this area contains tropical semi-evergreen rainforest, sub-tropical lowland moist forest, and limestone forest—however, the lowlands of this region are fairly populated (94 person/km²; General Statistics Office of Vietnam, 2011), thus much of the primary lowland forest has been cleared and converted for agricultural purposes (MacKinnon, 1997; Bain and Nguyen, 2004). Consequently, much of the intact forest is restricted to more montane regions (Bain and Nguyen, 2004).

Prior surveys of the region (most recently reviewed by Pham et al., 2017) brought the total number of recognized reptile species from Ha Giang to 57, although the province has been most recently highlighted in the literature for its increasingly known amphibian diversity (Ohler et al., 2011; Nishikawa et al., 2013; McLeod et al., 2015). To better characterize herpetofaunal diversity within the region and northeastern Indochina more broadly, we conducted several rapid inventory surveys in Bac Me District, which is in the southeastern portion of Ha Giang Province. During the course of the surveys, we collected a single xenodermid snake matching the diagnosis of *Achalinus* (Smith, 1943). Subsequent comparisons of morphology and mtDNA sequence data with currently recognized odd-scaled snake species led us to describe this specimen as representing a previously unknown species.

MATERIALS AND METHODS

We collected specimens in the field by hand during biodiversity surveys led by Smithsonian Institution National Museum of Natural History (NMNH) and Institute of Ecology and Biological Resources (IEBR) scientists in Bac Me District, Ha Giang Province, Vietnam. We euthanized specimens following University of North Carolina Asheville Institutional Animal Care and Use Committee (IACUC) protocol (2019-04). We extracted liver samples from euthanized animals and stored them in DMSO/EDTA buffer (as modified by Mulcahy et al., 2016), while we fixed whole voucher specimens in 10% formalin, and subsequently transferred them to 70% ethanol for long-term preservation. We collected and exported specimens and tissue samples to the NMNH with permission and a Memorandum of Understanding (MOU) arranged between the IEBR and the NMNH. We deposited tissue samples in the NMNH Biorepository and stored them at −80°C. We performed extractions of genomic DNA from all specimens on an AutoGen prep 965 (2011 AutoGen, Inc.) using standard phenol manufacturer protocols. We eluted genomic DNA in 100 µl of AutoGen R9 re-suspension buffer. We conducted polymerase chain reactions (PCRs) for the mtDNA loci cytochrome-oxidase I (*COI*), cytochrome-oxidase b (*Cytb*), and 16S rRNA (*16S*) using primers listed in Table 1. We performed PCRs in 96-well plates, in 10 µl reactions, following protocols ‘3.6 PCR Methods: Amplification’ and ‘3.8 PCR Purifications: EXOSAP-IT’ in Weigt et al. (2012) with annealing temperatures between 46–54°C. We performed sequence reactions in 96-well plates with both forward and

reverse PCR primers using BigDye® Terminator v3.1 Cycle Sequencing Kits in 0.25 × 10 µl reactions and then ran them on an Automated ABI3730 Sequencer (2011 Life Technologies). We then edited raw trace files in Geneious v10.2.4 (Biomatters Ltd.), aligned and edited complementary strands, and then inspected protein-coding sequences for translation. All novel sequences were manually edited and aligned with GenBank sequences (Supplementary Table 2; see Data Accessibility) in Geneious using the MUSCLE v7.308 plugin (Edgar, 2004) with default settings and subsequently inspected for translation for *COI* and *Cytb*. We deposited novel mtDNA sequences for *16S*, *Cytb*, and *COI* for *Achalinus* under the GenBank accession numbers MT503100, MT513238, and MT502775.

We calculated Tamura-Nei genetic distances using MEGA7 (Tamura and Nei, 1993; Kumar et al., 2016). We performed maximum likelihood (ML) phylogenetic analyses for the *COI* dataset using IQ-TREE v2.0 (Minh et al., 2020), deploying the Ultrafast Bootstrap (1,000 replicates; Minh et al., 2013) and ModelFinder options (Kalyaanamoorthy et al., 2017), although we did not use the invariant sites (+I) parameter if a gamma (+G) parameter was already present within a selected model (because the I parameter overlaps a G rate category). We estimated a Bayesian topology using BEAST2 v2.5.2 (Bouckaert et al., 2019). We performed evolutionary model averaging with bModelTest (Bouckaert and Drummond, 2017). We applied a relaxed log-normal clock and birth-death model as the molecular clock and tree priors, respectively; all other priors were set to default values. We performed two independent MCMC runs, each consisting of 50 million generations. We subsequently combined sampling parameters from both MCMC runs using LogCombiner. We then assessed appropriate MCMC mixing by calculating effective sample size (ESS) values for each parameter using the program Tracer v. 1.7.1 (Rambaut et al., 2018), with ESS values greater than 200 indicating adequate sampling of the posterior distribution. If sampled trees achieved convergence from both runs, we then combined them and discarded the first 25% of sampled trees as burn-in. Finally, we used TreeAnnotator to construct a maximum clade credibility (MCC) tree. We considered nodes with a posterior probability of 0.95 or above as highly supported.

To explore evolutionary scenarios where lineage diversification occurred in a non-bifurcating (‘non-tree-like’) manner and thus following more reticulate patterns, we implemented the NeighborNet algorithm (Bryant and Moulton, 2004) in SplitsTree v. 4.15.1 (Huson and Bryant, 2006) to visualize phylogenetic network relationships. To infer support of the network, we applied 1,000 bootstrap replicates.

We also collected morphological data from the single Ha Giang specimen and compared its characteristics to those of previously described species of *Achalinus* (Van Denburgh, 1912; Bourret, 1935, 1937; Pope, 1935; Smith, 1943; Hu et al., 1975; Karsen et al., 1986; Ota and Toyama, 1989; Inger et al., 1990; Ziegler, 2002; Goris and Maeda 2004; Zhao, 2006;

Das, 2010; Wang et al., 2019; Ziegler et al., 2019). All morphological data were taken by a single observer (JLL).

We obtained the body measurements snout–vent length (SVL), tail length (TailL) and total length (TotalL) using a flexible ruler. We took the following head and scale measurements using Mitutoyo® Digital Calipers to the nearest 0.1 mm: Head Length, measured from the posterior margin of the parietal scale to the tip of the rostral scale (HeadL); Head Width, measured from the widest point between the head (HeadW); Snout Length, measured from the anterior point of the eye to the tip of the rostral scale (SnL); Snout Width, measured as a straight-line distance between the median of both nostrils (SnW); Eye Diameter, measured horizontally from the posterior and the anterior margins of the eye (EyeD); Naris–Eye distance, measured from the anterior point of the eye to the medial point of the nostril (NarEye); Interorbital Distance, the straight-line distance between both eyes at the border of the supraoculars (InterorbD); Internasal Suture Length, the length of the suture between the internasal scales (InternasalSL); Prefrontal Suture Length, the length of the suture between the prefrontal scales (PrefrontalSL); Frontal Length, the maximum length of the frontal scale (FrontalL); Frontal Width, the maximum width of the frontal scale (FrontalW); Parietal Suture Length, the length of the suture between the parietals (ParietalSL); Loreal Length, the maximum horizontal length of the loreal scale (LorealL); Anterior Nasal Height, the maximum vertical length of the anterior portion of the nasal scale (NasalAH); and Posterior Nasal Height, the maximum vertical length of the posterior portion of the nasal scale (NasalPH). In addition, we calculated the following body and head ratios based on the aforementioned measurements: TailL/TotalL, HeadL/W, SnL/HeadL, EyeD/SnL, EyeD/HeadL, FrontalW/InterorbD, InternasalSL/PrefrontalSL, FrontalL/W, ParietalSL/FrontalL. We counted dorsal scales anteriorly at one head length behind the head, at midbody halfway between the terminus of the head and the vent, and posteriorly at one head length anterior to the cloacal plate (given as anterior–midbody–posterior in the description); we counted ventral scales according to Dowling (1951); the tail tip was not included in the number of subcaudal scales; counts for head scales are given in left/right order. Hemipenial terminology follows Dowling and Savage (1960). Institutional abbreviations follow Sabaj (2020) unless otherwise noted.

RESULTS

The *16S* and *Cytb* alignments (Supplementary Data; see Data Accessibility) were 500 bp (5 outgroup, 19 ingroup) and 587 bp in length (4 outgroup, 16 ingroup), respectively. We conducted ML phylogenetic inference on the *Cytb* and *16S* datasets (Supplementary Data; see Data Accessibility), but ultimately refrained from including them owing to poor taxon sampling, which prevented us from drawing any meaningful conclusions concerning the phylogenetic relationships among species of *Achalinus* and the affinities of the Ha Giang specimen.

The *COI* alignment (Supplementary Data; see Data Accessibility) was 654 bp in length and included a total of 46 individuals (8 outgroup, 38 ingroup) and included representatives of 10 of 13 (*A. ater*, *A. emilyae*, *A. formosanus*, *A. juliani*,

A. meiguensis, *A. niger*, *A. rufescens*, *A. spinalis*, *A. timi*, *A. yunkaiensis*) currently recognized species of *Achalinus*.

The *COI* Bayesian (Fig. 1) and ML (Fig. 2A) phylogenetic reconstructions were largely concordant concerning the relationships among species within the major clades of *Achalinus*, with the monophyly of odd-scaled snakes well supported (posterior probability [PP] = 0.94; Ultrafast Bootstrap [UFBoot] = 92), although the positions of the major clades in the ML and Bayesian topologies differed as follows: the ML analysis placed *A. meiguensis* sister to all other odd-scaled snakes (UFBoot = 94), whereas the Bayesian reconstruction placed this species (PP = 0.59) as sister to a clade composed of *A. niger*, *A. formosanus formosanus*, *A. sp. A*, *A. spinalis*, and *A. yunkaiensis*, which together were sister to a clade composed of all northern Vietnamese species and *A. rufescens*. Both analyses yielded strong support for the sister relationships of *A. emilyae* + *A. rufescens sensu stricto* (PP = 1.00; UFBoot = 100) and *A. ater* + *A. juliani* (PP = 1.00; UFBoot = 100), but only moderate support for *A. yunkaiensis* + *A. spinalis* (PP = 0.77; UFBoot = 64) and *A. niger* + *A. formosanus* (PP = 0.76; UFBoot = 96).

The phylogenetic affinity and placement of *Achalinus* sp. A, a clade of two specimens from Anhui Province, China, remain unclear, although this clade likely represents an undescribed species (Li et al., 2020). One specimen in this clade, HS14023, identified as *A. rufescens* in GenBank, is likely misidentified, as suggested by substantial divergence from the topotypic sample of *A. rufescens* (SYS r001866). Both reconstructions found the Taiwanese endemic *A. niger* to be paraphyletic with respect to the other Taiwan denizen, *A. formosanus formosanus*.

The phylogenetic position of the Ha Giang specimen was consistent, although poorly supported (PP = 0.76; UFBoot = 56), as sister to a single specimen of *A. timi* collected southwest of Ha Giang Province in Thuan Chau District, Son La Province (Ziegler et al., 2019). *Achalinus timi* and the Ha Giang specimen were sister to a clade composed of *A. ater*, *A. emilyae*, *A. juliani*, and *A. rufescens*, but with poor support (PP = 0.71; UFBoot = 49).

As in other recent studies (Wang et al., 2019; Ziegler et al., 2019), the deeper nodes of the phylogeny of *Achalinus* were poorly supported with low posterior probability and UFBoot values (PP < 0.95; UFBoot < 95). Genetic structuring among samples analyzed via the SplitsTree analysis resulted in a well-supported phylogenetic network (Fig. 2B), where the Ha Giang specimen formed a distinct lineage separate from other lineages of *Achalinus*, including *A. timi*.

At the *COI* locus, the Ha Giang specimen exhibits minimally a 12% sequence divergence from all sampled *Achalinus* and maximally 16.6% (Table 2; Supplementary Table 3; see Data Accessibility). The Ha Giang specimen exhibited similar levels of divergence in the other protein-coding locus sampled, *Cytb*, with a minimal divergence of 12.7% and maximum divergence of 20.7% relative to other species of *Achalinus* (Supplementary Table 4; see Data Accessibility). The large ribosomal subunit (*16S*) exhibited expectedly lower pairwise distances (Supplementary Table 5; see Data Accessibility) between taxa (protein-coding locus versus ribosomal subunit). For one specimen of *Achalinus rufescens sensu lato* (MVZ 224137), the *16S* sequence was just over 1% divergent from the Ha Giang specimen, compared to the 12.7% divergence at the *Cytb* locus. In the *16S* reconstruction, the MVZ specimen was placed sister to the

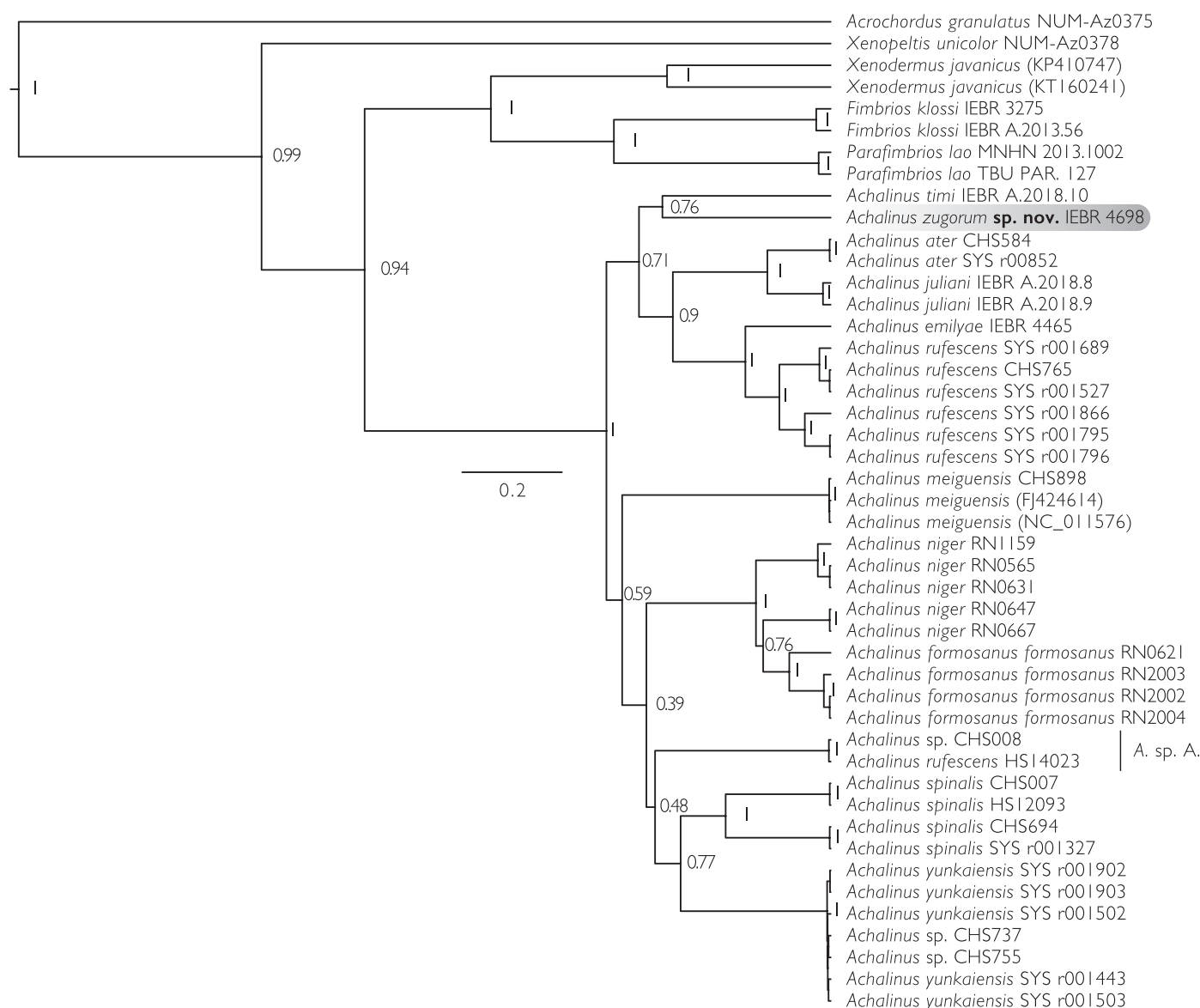


Fig. 1. Ultrametric Bayesian phylogeny inferred using the *COI* dataset with bModelTest (Bouckaert and Drummond, 2017) in BEAST2. Posterior probabilities (PP) are provided at relevant nodes. Scale bar indicates substitutions per site. Voucher museum catalog numbers are listed adjacent to each specimen, with GenBank accessions in parentheses if no voucher numbers could be located. See Data Accessibility for tree file.

Ha Giang specimen (UFBoot = 82), which together were within a clade comprising a number of samples of *A. rufescens*. By contrast, the *Cytb* reconstruction placed the MVZ specimen sister to a sample of *A. rufescens* (UFBoot = 100) from Guangdong, China, and the Ha Giang specimen sister to a specimen of *A. ater* from Guangxi, China with poor support (UFBoot = 71). No *COI* sequences were available for the MVZ specimen, thus we were unable to compare these individuals at this locus.

The discordance in sequence divergence between *16S* and *Cytb* was greater than expected given previously noted mutation rate differences between ribosomal and protein-coding mitochondrial loci (Mueller, 2006). Regardless, the presence of topotypic *A. rufescens* (Hong Kong) in the *COI* phylogenies (SYS r001866) clearly indicates that the Ha Giang specimen is not conspecific with *A. rufescens sensu stricto* and rather that the MVZ specimen is possibly misidentified.

Furthermore, the Ha Giang specimen is morphologically distinct from all other species of *Achalinus* in Asia. Its sister species, *A. timi*, is the most similar species in terms of scutellation, but the coloration of the Ha Giang specimen along with the number of dorsal scales at midbody and number of subcaudals serve to robustly differentiate them (see Comparisons for more details).

Following the General Lineage Species Concept (de Queiroz, 1998), the Ha Giang specimen represents an independently evolving lineage and merits recognition as a distinct species. This conclusion is based on support from both maximum likelihood and Bayesian mtDNA phylogenetic reconstructions, SplitsTree network analysis, substantial sequence divergence at multiple mtDNA loci, and diagnostic morphological characters (see below). In addition, the discovery of *Achalinus* in Lũng Càng Village represents the first record of odd-scaled snakes in Ha Giang Province (Fig. 3). The Ha Giang specimen is the seventh species of *Achalinus*

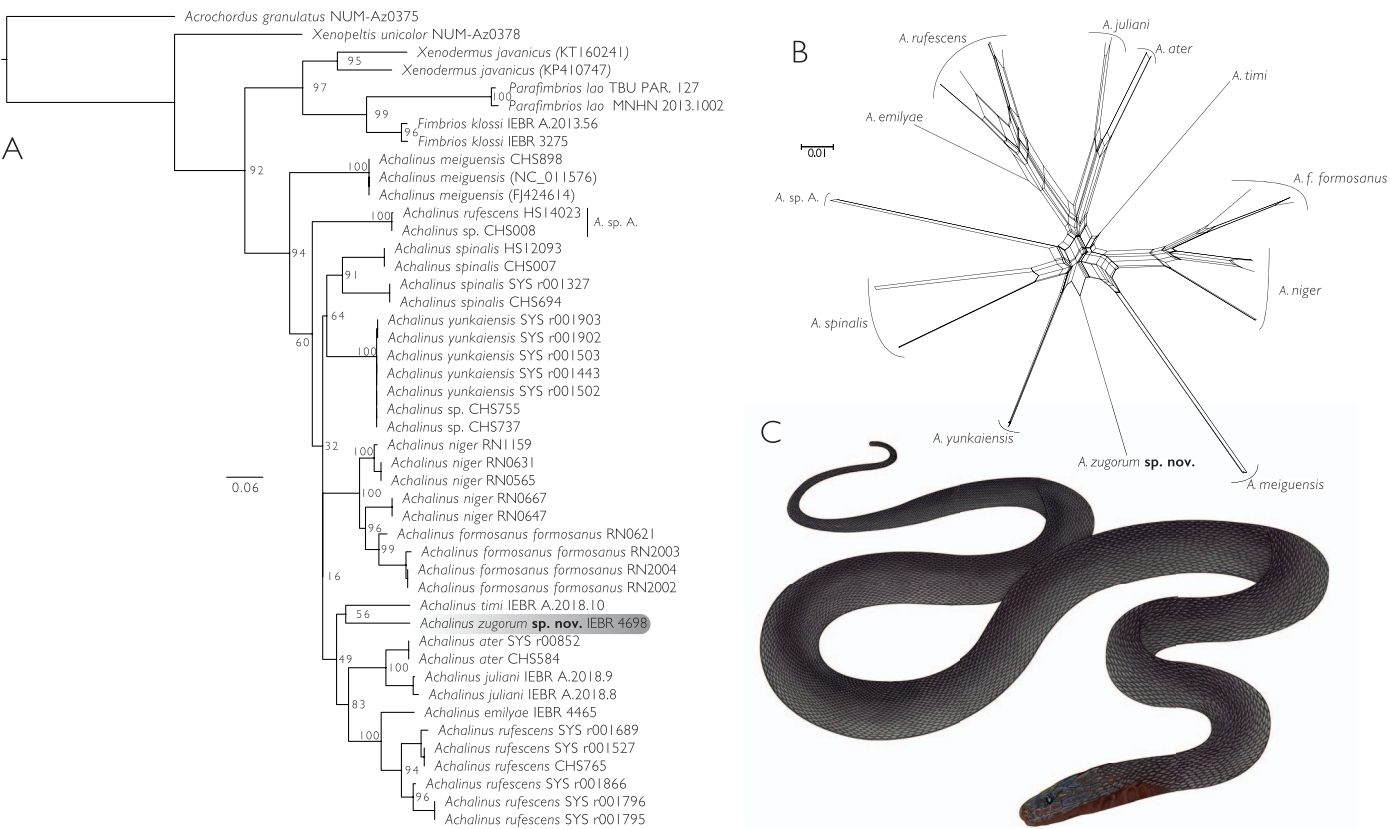


Fig. 2. (A) Maximum likelihood phylogeny inferred using the *COI* dataset in IQ-TREE. Ultrafast bootstrap values (UFBoot) are provided at relevant nodes. Voucher museum catalog numbers are listed adjacent to each specimen, with GenBank accessions in parentheses if no voucher number could be located. (B) SplitsTree network inferred with the *COI* dataset. (C) Drawing of *Acalinus zugorum*, new species, illustrated by Sydney Sieh-Takata. Scale bars indicate substitutions per site. See Data Accessibility for tree file.

known from Vietnam, potentially the fourth endemic species from Vietnam, and the 14th species of odd-scaled snake recognized to date.

***Acalinus zugorum*, new species**

urn:lsid:zoobank.org:act:1769A394-CC3C-47D3-B42E-33D2444DF3B4

Vietnamese Common Name: Rắn xe điều zug

English Common Name: Zugs' Odd-Scaled Snake

Figures 4, 5; Supplementary Figure 1; Supplementary Table 1

Holotype.—IEBR 4698 (field series tag: USNM-FS 280214), adult male (Figs. 4, 5), Lũng Càng Village, Minh Ngọc Commune, Bac Me District, Ha Giang Province, Vietnam, 22.716220°N, 105.189278°E (WGS 84), 228 m elevation, Aryeh H. Miller, Hayden R. Davis, Anh M. Luong, and Quyen H. Do, 19 June 2019 (at night between 2000–2100 hrs).

Diagnosis.—*Acalinus zugorum* differs from all other species of *Acalinus* by the following combination of morphological characteristics (Supplementary Table 1; see Data Accessibility): dorsal scales elliptical; one dorsal scale directly above

Table 2. Pairwise mean Tamura-Nei genetic distances (Tamura and Nei, 1993) for *COI* among operational taxonomic units (OTUs) sampled. Bolded values represent mean within-group genetic distances with NA values indicating that only one sample exists.

OTU	1	2	3	4	5	6	7	8	9	10	11	12
1. <i>A. meiguensis</i>	0											
2. <i>A. sp. A</i>	18.6	0.2										
3. <i>A. ater</i>	17.0	15.7	0									
4. <i>A. juliani</i>	18.7	16.3	7.3	1.1								
5. <i>A. emilyae</i>	17.0	17.5	12.9	13.8	NA							
6. <i>A. rufescens</i>	19.9	16.6	13.9	13.5	9.8	4.9						
7. <i>A. timi</i>	18.3	17.9	15.0	16.3	14.9	16.7	NA					
8. <i>A. spinalis</i>	17.1	15.4	17.0	16.1	15.2	15.6	15.7	7.8				
9. <i>A. niger</i>	14.7	17.1	14.3	13.5	14.0	14.4	14.0	13.8	5.0			
10. <i>A. f. formosanus</i>	17.1	17.8	15.8	14.3	15.4	15.6	15.3	15.5	8.1	3.0		
11. <i>A. zugorum</i>	16.6	16.1	14.7	14.9	13.8	15.3	15.6	15.2	14.9	15.0	NA	
12. <i>A. yunkaiensis</i>	17.4	15.2	14.5	14.5	14.8	14.9	16.2	12.9	13.0	13.8	12.0	0.1

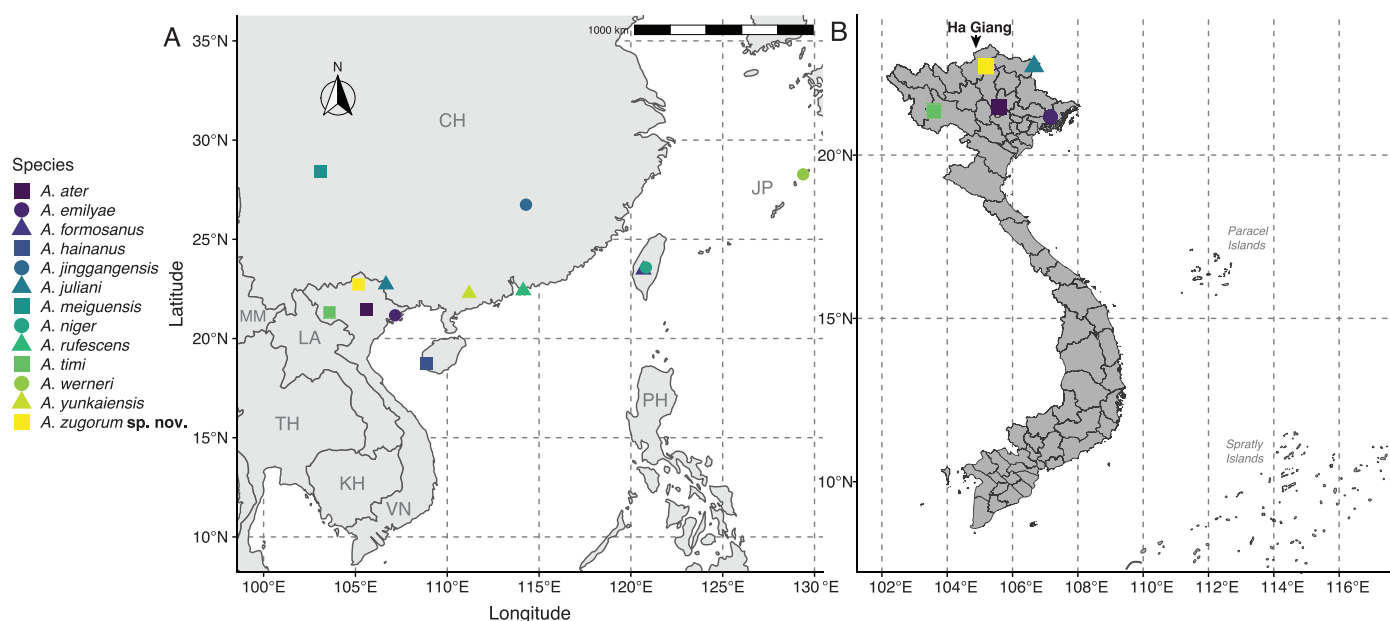


Fig. 3. (A) Map of all type localities for odd-scaled snakes (*Achalinus*) and (B) province-level map of Vietnam with type localities in that country. Black arrow indicates location of Ha Giang Province. All type localities of recognized *Achalinus* are included with the exception of that for *A. spinalis*, which is geographically imprecise (“angeblich aus Japan” = allegedly from Japan; Peters, 1869). Countries are labeled with ISO alpha-2 codes.

each ventral scale; absence of everted labial scales; preocular absent (fused with prefrontal or loreal scale); maxillary teeth 28, all teeth curved and equal in size; internasal suture distinctly longer than that between the prefrontal suture; internasals not fused to prefrontals; loreal fused with prefrontals; seven infralabials; mental very narrow, separated from anterior chin shields; two elongated anterior temporals, in contact with eye, and two posterior temporals; dorsal scales in 25–23–23 rows, keeled; ventrals 173; subcaudals 70, unpaired; a maximum known total length of 458 mm, TailL/TotalL ratio of 0.23; dorsum purple to jet black in life and in preservative, lower and posterior portions of head pink-gray, ventral surface iridescent and uniform black with margins of ventral scales pink-gray.

Description of the holotype.—Adult male in excellent condition, one midline incision for liver tissue extraction (Fig. 4). Hemipenes partially everted. SVL 353 mm, TailL 105 mm, TotalL 458 mm, HeadL 10.9 mm, HeadW 6.0 mm, SnL 3.5 mm, SnW 2.0 mm, EyeD 1.2 mm, NarEye 2.7, InterorbD 3.7, InternasalSL length 1.9 mm, PrefrontalSL 1.3 mm, ParietalSL 5.0 mm, FrontalL 2.5 mm, FrontalW 2.8 mm, LorealL 2.0 mm, NasalAH 0.5 mm, NasalPH 0.7 mm. TailL/TotalL 0.23, HeadL/W 0.55, SnL/HeadL 0.32, EyeD/SnL 0.35, EyeD/HeadL 0.11, FrontalW/InterorbD 0.78, InternasalSL/PrefrontalSL 1.42, FrontalL/W 0.87, ParietalSL/FrontalL 2.0.

Body moderately elongate and subcylindrical, head oblong and slightly distinct from neck dorsally covered with large head scales (Fig. 5). Scales across dorsal portion of head distinctly striated; eyes small, round with vertically subelliptical pupils; nostrils large compared to nasal scale and elliptical. Tail elongate (slightly over one-fifth of total body length), tapering to round blunt tip. Rostral scale small, subpentagonal in frontal view, barely visible from above. Posterior scale suture of rostral with internasals “gull-wing” shaped (broad obtuse angle). Mental scale much smaller and

narrower than rostral, subrectangular. Internasals paired, rounded. Suture between internasals 1.4x longer than prefrontal suture; prefrontals paired, subrectangular, 1.7x wider than long. Frontal scale shield-shaped with weak crease along midline, 0.9x longer than wide; anterior angle formed by suture of frontal bordering prefrontals straight (180°); posterior angle formed by the sutures producing the posterior vertex of the frontal a weak obtuse angle (100°). Supraoculars rectangular, 2.1x longer than wide. Supraoculars shorter than frontal. Parietals paired, weakly subtrapezoidal, 2.5x longer than wide. Parietal suture 2.0x longer than frontal; anterior parietal angle formed by the sutures between the parietal+frontal and the suture between the supraocular+parietal moderately obtuse (112°) with the lateral ray of the angle pointing posterolaterally. Parietals bordered by one elongate nuchal (second row of posterior temporal) scale on each side; nuchals blocked from contacting at midline by one small scale, each scale in contact with first row of posterior temporals. Loreal scale fused with prefrontal scale; nasal scale divided below nostril, posterior portion of nasal larger than anterior. No preocular or postocular. Supralabials 6/6, first very small, contacting mental, afterwards gradually increasing in size. Supralabials 1–3 contacting nasal, 3–4 contacting fused loreal-prefrontal, 4–5 contacting orbit, 4th in narrow contact, 5th in broad contact, 6th supralabial largest, 2.6x as long as 5th supralabial, broadly subrectangular. Infralabials 7/7, first pair in contact, blocking mental from contacting anterior pair of genials; infralabials 1–3 in contact with anterior pair of genials; 1st infralabial smallest, increasing in size until 6th infralabial, which is the longest; the 7th infralabial is significantly smaller than the 6th infralabial; both scales are elongate and contact the 6th supralabial, and is thus considered an infralabial based on this characteristic. Genial scales wider than long, subrectangular and asymmetrical. Posterior genials longer than

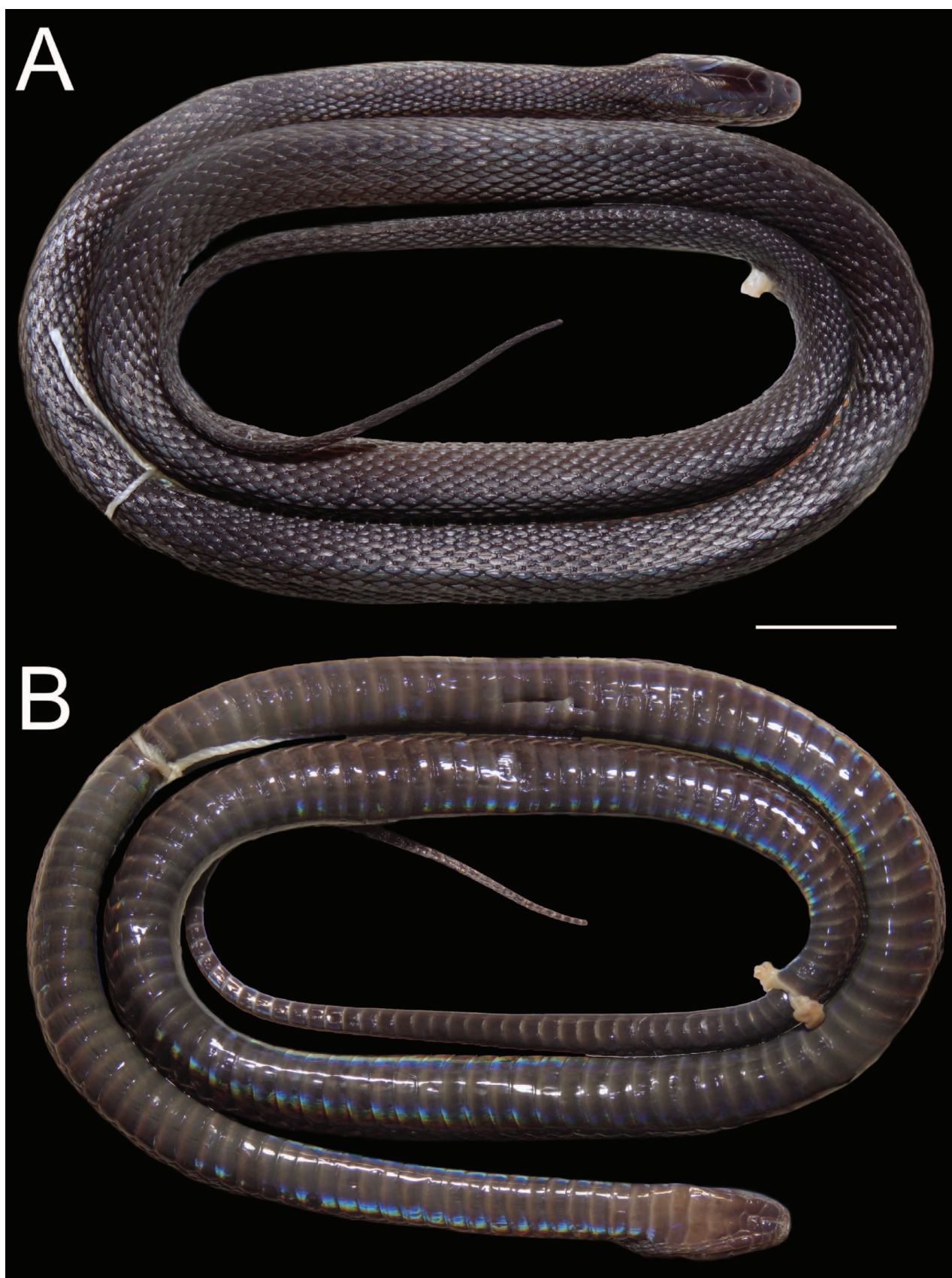


Fig. 4. (A) Dorsal and (B) ventral views of the holotype of *Achalinus zugorum*, new species (IEBR 4698), after preservation. White bar = 10 mm.

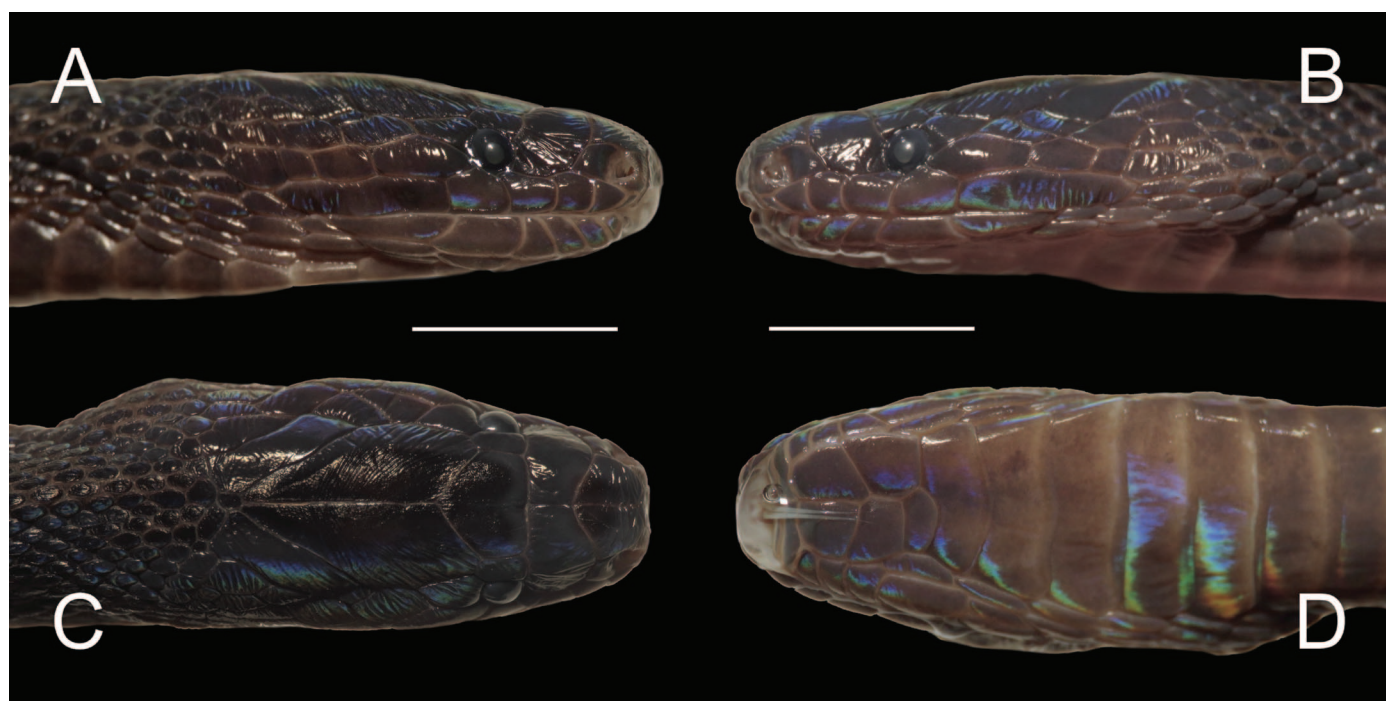


Fig. 5. (A, B) Lateral, (C) dorsal, and (D) ventral views of the head of the holotype (IEBR 4698) of *Achalinus zugorum*, new species, after preservation. Each white bar = 5 mm.

anterior genials, right posterior genial divided into anterior and posterior scales.

Dorsal scale rows 25–23–23, all dorsal scales elliptical and distinctly keeled posterior to nape, outermost dorsal scale row slightly enlarged from innermost rows. Ventrals smooth, border with dorsal scales a broad obtuse angle. 173 ventrals with no preentrals; cloacal plate single; subcaudals 70, unpaired.

Maxillary teeth 28, anterior teeth equal in size, all curved. Mandibular and palatine teeth all equally sized and similar in shape to those of maxilla.

Hemipenes small, partially everted organ extending 5–6 subcaudal scales in length, retracted back to 2–3 subcaudal scales after preservation. Organ bilobed, semicapitate based on partial eversion; shape uniform but inflated on the sulcate side near point of lobular bifurcation. The *sulcus spermaticus* is forked, at centrifugal position, dividing at point of bifurcation and extending across the lobes towards the apex. Sulcus lips elevated, naked, creating large channel. The lobes of each hemipenis have not been fully prepared but are mostly naked except for a few small horizontal flounces; on the left organ, a few spinous calyces are present on the lobes; the base of the organ is somewhat flounced, without other ornamentation.

Coloration in preservative.—After formalin fixation and subsequent preservation in 70% ethanol for six months, dorsum uniform dark purple throughout entire body; dorsal portion of head same as dorsum, interstitial skin of dorsal and head scales violet-brown; medial portion of labial scales around mouth pinkish-gray; ventral region of snout dark purple, strongly iridescent; dark violet-gray along gular region; ventral ground color of body dark purple, margins of ventral scales pinkish-gray, also strongly iridescent.

Coloration in life.—The color pattern of the holotype in life is similar to the coloration after preservation. The dorsum is blackish and dark purple; interstitial skin between the dorsal scales dark gray; head same color as dorsum; underside of gular region dark brown, margins of labial scales dark brown; venter uniform jet black with the outer margins of each ventral scale dark gray, strong iridescence also present.

Etymology.—The specific epithet, a plural noun in the genitive case, honors George R. Zug and Patricia B. Zug. George is commemorated for his contributions to herpetology (reviewed by Mitchell, 2018), especially within Southeast Asia, and for his mentorship to two of the authors (AHM, JLL) and many young scientists before them. We also celebrate Patricia for her contributions to herpetology, as well as her ever endearing and compassionate spirit which continues to inspire the authors.

Distribution and natural history.—The holotype of *Achalinus zugorum* was collected on a small gravel road running through Lũng Càng Village, Minh Ngọc Commune, Bac Me District, Ha Giang Province, Vietnam at an elevation of 228 meters above sea level. So far, the species is known only from the type locality and no specimens have been collected since its discovery. Nothing is known about the ecology, behavior, diet, or preferred microhabitat of this species, though it is presumably a semi-fossorial species that was drawn out by recent heavy rainfall prior to collection. The habitat surrounding its collection location consisted primarily of secondary forest with medium to small hardwoods on karst. The mean annual temperature in Ha Giang Province is 23.3°C, with a dry season typically extending from October to March and the rainy season lasting April until September (Le et al., 2007).

Comparisons.—We compare *Achalinus zugorum* to all other known species of snakes in the genus *Achalinus* and all other genera of xenodermid snakes known to inhabit Southeast Asia. Detailed comparisons between *A. zugorum* and other *Achalinus* are given in Supplementary Table 1 (see Data Accessibility). *Achalinus zugorum* is identified as a member of the genus *Achalinus* and can be differentiated from all other snakes in the family Xenodermidae inhabiting Southeast Asia by the following characters: dorsal scales elliptical (vs. cycloid in *Fimbrios* and *Parafimbrios*, raised dorsal scales present in *Xenodermus*); one dorsal scale directly above each ventral scale (vs. two scales above each ventral in *Parafimbrios* and *Xenodermus*); absence of distinctly raised and everted supralabial scale edges (vs. present in *Fimbrios* and *Parafimbrios*); 28 maxillary teeth equal in size (vs. more than 30 teeth in *Fimbrios*, less than 20 teeth in *Stoliczka* and *Xenodermus*, teeth gradually enlarging posteriorly in *Parafimbrios*); dorsal scales in 25–23–23 rows (vs. greater than 25 in *Fimbrios*, *Parafimbrios*, *Stoliczka*, and *Xenodermus*) and additional characteristics (Jerdon, 1870; de Rooij, 1917; Smith, 1921, 1943; Teynié et al., 2015; Ziegler et al., 2018). *Achalinus zugorum* can be differentiated from all other members of *Achalinus* except for *A. formosanus chigirai*, *A. jinggangensis*, and *A. timi* by the combination of the following morphological characters: loreal scale fused with the prefrontal; internasal scale not fused with prefrontal scale; internasal suture longer than prefrontal suture; 25 dorsal scale rows anteriorly; 173 ventral scales; 28 maxillary teeth; six supralabials; seven infralabials; and a uniform jet-black dorsal color pattern in life (Supplementary Table 1; see Data Accessibility).

Achalinus zugorum is differentiated from *A. formosanus chigirai* by having a TailL/TotalL ratio of 0.23 (vs. 0.32); 28 maxillary teeth (vs. 14); 23 dorsal scale rows at midbody and posteriorly (vs. 25–27 at midbody, 25 posteriorly); 173 ventrals (vs. 161–167 in males); 70 subcaudals (vs. 96–97 in males); 7 infralabials (vs. 5–6); and by having a dark purple or blackish dorsal coloration (vs. dark gray dorsal color pattern with darker longitudinal line along middorsum). *Achalinus zugorum* is differentiated from *A. jinggangensis* by having a TailL/TotalL ratio of 0.23 (vs. 0.17–0.22); 28 maxillary teeth (vs. 22); 173 ventrals (vs. 156–164, sex not given); 70 subcaudals (vs. 51–64, sex not given); and 7 infralabials (vs. 6). Lastly, *A. zugorum* is most similar morphologically to *A. timi*, its possible sister species, but is differentiated by having a TailL/TotalL ratio of 0.23 (vs. 0.22); 28 maxillary teeth (vs. 27); two anterior temporals contacting the eye (vs. only one contacting the eye); 23 dorsal scale rows at midbody (vs. 25); 173 ventrals with no prefrontals (vs. 171 ventrals in males with one prefrontal); 70 subcaudals (vs. 72 in males); 7 infralabials (vs. 6); and a uniform jet black dorsal color pattern in life (vs. reddish to grayish brown dorsal color pattern, with wide portion of the vertebral region being distinctly darker). Despite the morphological differences noted above, we are unable to characterize intraspecific variation as these data are derived from a single specimen—consequently, we expect some degree of meristic and mensural variation given an ample population sample.

Conservation status.—As only a single specimen of *Achalinus zugorum* is thus far known, it is impossible to infer demographic parameters. However, Ha Giang Province, like much of Vietnam, is hurtling towards an increasingly

ecologically fragmented and destabilized community as deforestation resulting from logging, agricultural expansion, and residential development, as well as limestone quarrying, continue to threaten the regional biota. The surrounding lowland habitat beyond the secondary forest at the type and only known locality has been heavily deforested and converted for agricultural use, thus the immediate area is seriously threatened. However, it would be inappropriate to assert that this species is a microendemic occurring only at its type locality, as many species of *Achalinus* are known to occur across broader geographic areas, but their secretive habits make estimating population sizes and distributions exceedingly difficult.

Odd-scaled snakes appear vulnerable to desiccation (Pope and Granger, 1929; Ota, 2014)—a likely consequence of their non-imbricate scutellation where more interstitial skin is exposed—thus habitat modification (such as agricultural activities) could force odd-scaled snakes to drier forest edge habitats (where *A. zugorum* was encountered), which would represent a formidable threat to this group. Populations of *A. formosanus chigirai* are in decline for this reason exactly (Ota, 2014; Zhou et al., 2017). Pope and Granger (1929) stated upon collection of *A. spinalis* in Fujian Province, China that the specimens were “extremely delicate” and that it “dries and hardens in a most unusual way”, further signifying the importance of moist, humid forest habitats for this group.

Currently available data are inadequate to make a sound decision concerning the distribution, abundance, and population status of this species, thus we are unable to assess extinction risk. We recommend classifying *Achalinus zugorum* as Data Deficient (DD) under the International Union for the Conservation of Nature (IUCN) Red List of Threatened Species criteria (IUCN, 2020). The Data Deficient categorization is based on the paucity of data to make an appropriate classification in another Red List category, but with sufficient concern to suggest that the species is facing threats owing to development and habitat modification near the type locality.

DISCUSSION

Achalinus zugorum joins a number of recently described species of *Achalinus* from northern Vietnam (Ziegler et al., 2019), adding to the growing view that odd-scaled snakes are a moderately more diverse group than previously appreciated. In addition to their systematics, very little is known about the natural history, biology, ecology, and functional morphology of *Achalinus* in comparison to other caenophidian snakes in Southeast Asia. The unique dentition of *Achalinus*, consisting of equally sized, curved maxillary teeth with medial or lingual ridges (Zaher et al., 2019), is a morphological trait that usually occurs in caenophidian snakes consuming soft-bodied prey (Vaeth et al., 1985; Hosoi et al., 2007; Ray et al., 2012). Indeed, the only study on the diet of *Achalinus* (Yamasaki and Mori, 2017) showed that one species, *A. spinalis*, seems to specialize on a diet of megascolecid earthworms. Another intriguing characteristic of the clade is the lack of a mental groove, a characteristic shared with slug- and snail-eating snakes in the family Pareidae and members of the genus *Dipsas* (Dipsadinae) which improves jaw rigidity when feeding (Peters, 1960; Guo et al., 2011).

In addition to the distinct dentition of odd-scaled snakes, Underwood (2002) reported that the retinas in *Achalinus*

have only one type of photoreceptor—rods—and that cones, which are typically associated with photopic (bright-light) vision (Kojima et al., 1992; Bhattacharyya et al., 2017), have been lost. This character state leads us to infer that *Achalinus* would have a fairly low visual acuity and prefer dim-light environments over bright-light environments, thus suggesting crepuscular or nocturnal activity and/or a more secretive, subterranean or fossorial life history (although the retinal structure of *A. zugorum* has yet to be characterized). The form–function relationship of these traits along with other aspects of skull anatomy, morphology, and dentition in *Achalinus* remain largely unstudied.

The (incompletely sampled) Bayesian phylogeny divides *Achalinus* into two large clades—one composed of southern Chinese and northern Vietnamese species (*A. ater*, *A. emilyae*, *A. juliani*, *A. rufescens sensu stricto*, *A. timi*, and *A. zugorum*), and one composed of species from throughout China, Taiwan, and Hainan (*A. formosanus*, *A. meiguensis*, *A. niger*, *A. sp. A*, *A. spinalis*, and *A. yunkaiensis*). Since no molecular data (nor accessible tissues) exist for *A. hainanus*, *A. jinggangensis*, and *A. weneri*, we were unable to ascertain their phylogenetic positions relative to other species of *Achalinus*. Biogeographic affinities between Hainan and northern Vietnam (Liang et al., 2018) suggest that *A. hainanus* is likely closely related to the northern Vietnam species—a prediction also supported by the morphological similarity between *A. emilyae* and *A. hainanus* (e.g., shared characters of 23 anterior scale rows, 5 infralabials, similar ventral scale counts). *Achalinus weneri*, a species known only from the Ryukyu Islands, is presumably closely allied to the species on Taiwan to the south (*A. formosanus* and *A. niger*) given geographic proximity, as well as meristic similarities to species of *Achalinus* from Taiwan. *Achalinus jinggangensis* is known only from Jiangxi Province in southeast China—the little morphological data available for this species are uninformative for phylogenetic inference. Poor taxonomic sampling and a lack of genome-scale data combine to hinder our confident inference of the phylogenetic relationships of *Achalinus*.

Multiple issues immediately arise concerning the status of *A. spinalis*, a species described from Japan with no precise type locality (Peters, 1869), but for which the only molecular samples available are from mainland China. First, two divergent clades (PP = 1.00; UFBoot = 91; intraspecific sequence divergence of 7.8% at the *COI* locus) of *A. spinalis* were inferred. Given that specimens in the two clades are from localities approximately 450 km apart, further sampling in the areas between these two populations is required to ascertain if this divergence is an artifact of isolation-by-distance or rather these populations represent different species.

The larger conundrum concerning the status of *A. spinalis* concerns the absence of samples from Japan. In the phylogenetic reconstructions, *A. spinalis* is (PP = 0.77; UFBoot = 64) sister to *A. yunkaiensis*, a species recently described from southwestern Guangdong Province, China (Wang et al., 2019). One would expect *A. spinalis sensu stricto* to be more closely allied with the Taiwanese species (*A. formosanus* and *A. niger*) owing to previously noted biogeographic relationships between snakes distributed throughout Taiwan, the Ryukyus, and the main islands of Japan (Kaito and Toda, 2016). This raises the question of whether the mainland Asia populations represent *A. spinalis sensu stricto*

or an undescribed morphologically similar continental species. Stejneger (1910) noted that *A. formosanus* “appears to be more nearly related to *A. spinalis* than to *A. rufescens*”, thus favoring the evolutionary alignment of *A. spinalis* with the Taiwanese species rather than its mainland counterpart (*A. rufescens*)—a conclusion also supported by our Bayesian analysis.

Similar to the archipelagic species, the evolutionary history of snakes in the genus *Achalinus* in northern Vietnam is still poorly characterized. The Red River, which diagonally bisects northern Vietnam, has been repeatedly demonstrated to serve as a significant biogeographic barrier, as exhibited by the many taxa that occur exclusively on only one side of the river (Bain and Hurley, 2011) or where observed historical cessations of gene flow and contemporary dispersal have been documented (Zhang et al., 2010). It is currently unclear if the lowland regions immediately surrounding the Red River act as a barrier to dispersal for snakes in the genus *Achalinus*, or if some areas do while others do not.

The Red River, which sits west of Ha Giang Province, divides *A. timi* and the newly discovered *A. zugorum*, yet these two species appear to be sisters (although further sampling and genomic data would elucidate if this is a well-supported relationship). It should be noted that various other species of *Achalinus* lie east of the Red River, including *A. juliani* from Cao Bang Province and *A. emilyae* from Quang Ninh Province (Ziegler et al., 2019). The Red River, along with northern Vietnam, has a complicated geological history, as well as a variable climatic history even as recently as the Pleistocene (Yuan et al., 2016).

As highlighted by historical demographic investigation into two species of frogs in the genus *Microhyla* on opposite sides of the Red River (Yuan et al., 2016), Pleistocene climatic cycles did not have uniform effects on the structuring and diversity of regional fauna in northern Vietnam. Since so little is known concerning the biology and ecology of *Achalinus*, it is hard to infer how changes in habitat type and habitat availability may have shaped contemporary diversity, distributions, and population structure. If snakes in *Achalinus* are indeed susceptible to desiccation, as noted by Pope and Granger (1929), then dramatic historical expansions of drier habitats, such as those that occurred in the late Miocene (Yuan et al., 2016), likely reduced the distribution of odd-scaled snakes and fragmented populations, possibly into wetter, more montane refugia.

It is plausible that *A. emilyae*, known only from Quang Ninh Province, which is adjacent to the Gulf of Tonkin, has experienced a substantial reduction in historical range as sea levels rose in the past 20,000 years and inundated much of Vietnam’s northeast coast (Voris, 2000). Our analyses support the sister relationship of *A. emilyae* and *A. rufescens sensu stricto* (PP = 1.00; UFBoot = 100), which raises important questions concerning the biogeographic history of *Achalinus* in northern Vietnam and southeastern China. While this result could be an artifact of poor sampling, this phylogenetic relationship calls for further investigation.

Many of the populations of *Achalinus* in southeastern China, such as those in Guangxi and Yunnan Provinces, likely reside under names (e.g., *A. spinalis*) that do not accurately reflect the lineage identity of those populations—a relic of previously underappreciated species diversity within the group. As sampling increases in southern China, populations of species of *Achalinus* currently thought to be

endemic to Vietnam will likely be discovered, thus expanding known geographic distributions for some species. The discovery of *Achalinus* in Ha Giang Province is far from surprising, as the genus had already been detected in multiple nearby provinces. The fundamental challenge in assessing diversity and distributions within this group stems from the rarity of detecting individuals during field surveys, as the secretive nature of odd-scaled snakes makes their finding largely serendipitous. In 20 years of herpetofaunal surveys in northern Vietnam, TQN has collected only six specimens of *Achalinus*.

The forests of northern Vietnam continue to yield discoveries for a vast array of biotic diversity, as demonstrated by the discovery of *A. zugorum*. The Ha Giang specimen provides additional insight into the phylogeny of *Achalinus* but remains a small piece of the larger evolutionary portrait that is the diversification and evolution of odd-scaled snakes, which remains nearly as enigmatic as the detectability of the snakes themselves in the field in northern Vietnam. To that end, ongoing study of *Achalinus* is critical for scientists to gain a better appreciation for the biology, ecology, and evolution of odd-scaled snakes. Doing so is necessary to develop and implement appropriate conservation and management strategies to assure the long-term survival of these enigmatic snakes in the face of existential threats.

DATA ACCESSIBILITY

We have deposited all trees, alignments, pairwise distance matrices, morphological data, specimen photographs, and supplementary tables in FigShare (<https://doi.org/10.6084/m9.figshare.c.5134586.v1>).

ACKNOWLEDGMENTS

We are greatly appreciative to The People's Committee of Bac Me and Vi Xuyen Districts, Department of Foreign Affairs and Director Ly Thi Lan for allowing us to conduct this field work under permit 262/SNgV-LS in Ha Giang Province, as well as Director of the IEBR, Prof. Dr. Nguyen Van Sinh, for helping facilitate the export of the scientific collection (Letter 575/STTNSV). We thank Tien Q. Phan (IEBR) for assistance in the field, and Daniel G. Mulcahy (NMNH) for support during portions of the laboratory work. We also appreciate the assistance of all collections management staff in the Division of Amphibians and Reptiles, National Museum of Natural History (NMNH). All portions of the laboratory work were conducted in and with the support of the Laboratories of Analytical Biology facilities of the NMNH. Field and lab work were funded by the Global Genome Initiative (NMNH) grant GGI-Rolling-2018-203 to AHM, RGR, and KdQ and by the Department of Vertebrate Zoology, NMNH, as well as the University of North Carolina Asheville. AHM and KdQ are grateful to Carole Baldwin, Sarah Loudin, Katharine Barker, Jonathan Coddington, Wendy Wiswall, Kirk Johnson, and the Smithsonian Institution's Office of the General Counsel for their assistance in facilitating the arrangement of an MOU between the NMNH and the IEBR. AHM thanks the Undergraduate Research Program at the University of North Carolina Asheville for additional financial support. HRD was partially funded by Aaron M. Bauer (Villanova University). Research of TQN was supported by the National Foundation for Science and Technology Development (NAFOSTED, Grant No. 106.05-2017.329). JLL thanks his family, the

University of Maryland College Park Scholars Life Sciences division, and the University of Maryland College of Computer, Mathematical, and Natural Sciences for financial and logistical support. We are thankful to Sydney Sieh-Takata, who graciously provided an illustration of the new species. Lastly, AHM gratefully thanks T. D. Miller and M. S. Fein for continued support.

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