A new species of the genus *Sinomicrurus* (Serpentes: Elapidae) from China and Vietnam

DEAR EDITOR,

A new species of *Sinomicrurus* Slowinski, Boundy, and Lawson, 2001 is described herein based on a series of specimens. The new species, *Sinomicrurus peinani* **sp. nov.**, occurs in southern China and northern Vietnam. *Sinomicrurus peinani* **sp. nov.** is distinguished from its congeners by the following combination of characters: (1) 30–35 black crossbands on body and tail; (2) 13 dorsal scale rows throughout, all smooth; (3) white belly with black cross-bands or irregular spots; (4) broad white transverse bar on top of head with inverted V-shaped anterior margin, white bar wider than anterior black bar; and (5) frontal V-like, 1.3 times as long as wide. In addition, new occurrences of *S. houi* in Guangxi, China, and Vietnam are discussed.

Sinomicrurus Slowinski, Boundy, and Lawson, is a group of small to medium-sized Asian coral snakes (Slowinski et al., 2001; Zhao, 2006). They are widely distributed in eastern and southeastern Asia, ranging from Nepal in the west to Japan in the east and from Hunan, China, in the north to Taiwan, China, in the south (Zhao, 2006).

Previously, members of the genus *Sinomicrurus* were contained within *Calliophis* Gray (Slowinski et al., 2001; Zhao et al., 1998). However, phylogenetic analyses based on morphological characters and cytochrome *b* sequences suggested that the Asian coral snakes fell into three major clades (Slowinski et al., 2001). Thus, Slowinski et al. (2001) erected a new genus *Sinomicrurus* to accommodate the northern tropical/subtropical mainland species, including *C. hatori* (Takahashi, 1930), *C. japonicas* (Günther, 1868), *C. kelloggi* (Pope, 1928), *C. macclellandi* (Reinhardt, 1844), and *C. sauteri* (Steindachner, 1913). Recently, Peng et al. (2018) described a new species from Hainan Island, China. Thus, six species are recognized presently within this genus.

In recent decades, a series of field surveys have been

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conducted in southern China (Guangxi) and Vietnam, involving the collection of several *Sinomicrurus* coral snakes. Further study based on morphological comparisons and genetic data indicated they were different from all putative species of this genus. Thus, we evaluated these specimens as a new species, which is described herein.

Twenty-one specimens of *Sinomicrurus* collected from China were morphologically examined (Supplementary Appendix S1). Scalation, color pattern, and body proportions were recorded and measured. Measurements of body and tail lengths were taken with a ruler to the nearest 1 mm; other measurements were taken with a slide caliper to the nearest 0.1 mm. Meristic characters were recorded on both sides. Ventral scales were counted following Dowling (1951). For comparison, characters of other *Sinomicrurus* species were taken from previous publications (Peng et al., 2018; Pope, 1935; Stejneger, 1907; Zhao, 2006; Zhao et al., 1998).

Abbreviations used for measurements and meristic characters are as follows: SVL (snout-vent length): distance between tip of snout and cloaca; TaL (tail length): distance between cloaca and tip of tail; DS: dorsal scales; VS: ventral scales; SC: subcaudal scales; IL: infralabials; SL: supralabials; Tem: temporals; Lo; Loreals; Poo: postoculars; Pro: preoculars; CB: number of cross-bands on body. All specimens are deposited in Yibin University (YBU), Sichuan, China

In total, 25 individuals representing three Chinese species and one unidentified taxon were sequenced and analyzed (Supplementary Appendix S2). Genomic DNA was extracted from liver samples preserved in 85% alcohol using Tissue DNA kits (Omega Bio-Tek, Inc., USA). A partial sequence for the mitochondrial cytochrome c oxidase I (COI) was amplified

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using the primer chmf/chmr and cycling parameters provided in Che et al. (2012). The double-stranded products were sequenced by a commercial company (Genewiz, China), with the sequences then edited manually using SeqMan in Lasergene v15.1 (DNASTAR Inc., USA) (GenBank accession Nos.: MN 685872–MN685896). An additional nine sequences representing S. sauteri, S. hatori, S. macclellandi, and S. kelloggi were retried from GenBank and added to the dataset for subsequent analyses (Supplementary Appendix S2). Micrurus nigrocinctus and M. lemniscatus were chosen as outgroups based on previous work (Pyron et al., 2013).

We reconstructed phylogenetic relationships using Bayesian inference (BI) and Maximum likelihood (ML) methods. Prior to analyses, the optimal evolutionary models for each codon position were selected using PartitionFinder under Bayesian Information Criterion (BIC) (Lanfear et al., 2012). BI analysis was performed using MrBayes v3.2.2 (Ronguist et al., 2012). All searches were performed with two independent runs, with each initiating a random tree. Each run consisted of four Markov chains (three heated and one cold chain) estimated for 5×106 million generations and sampled every 2 000 generations, with 25% of initial samples discarded as burn-in. Convergence was assessed by examining effective sample sizes (ESS) (ESS>200) and likelihood plots through time in TRACER v1.4 (Rambaut & Drummond, 2007). The resulting trees were combined to calculate posterior probabilities (PP) for each node in a 50% majority-rule consensus tree. The ML trees were constructed using RaxML v7.2.6 (Stamatakis, 2008) with the GTRCAT model under the same partitioning scheme as Bayesian analysis. Branch support was assessed using 1 000 non-parametric bootstrap (BS) topological replicates. Pairwise distances (P-distances) between in-group taxa were calculated in Mega 6.0 (Kumar et al., 2008; Tamura et al., 2013).

Taxonomy

Sinomicrurus peinani sp. nov. (Figure 1A-D; Table 1)

Holotype: YBU 16086, adult female, from Cangwu County (N23.65°, E111.56°), Guangxi, China, elevation of ~30 m a.s.l.; collected on 05 June 2016.

Paratypes (three specimens): YBU 16054, female; YBU 16066, male; YBU 16067, female. Same locality and date as holotype.

Diagnosis: All examined specimens possessed a small to medium-sized body, varying from 368 mm to 620 mm, as well as: (1) 30–35 black cross-bands on body and tail; (2) 13 dorsal scale rows throughout, all smooth; (3) white belly with black speckles or bands; (4) broad white transverse bar on top of head with inverted V-shaped anterior margin, white bar wider than anterior black bar; and (5) frontal V-like, 1.3 times as long as wide.

Description of holotype: Adult female. Total length 550 mm, tail length 45 mm. Body rather slender; head short, broad, and rounded with obtuse muzzle. Rostral trapezoidal, 1.5 times as broad as deep. Internasals large and square, slightly wider

than long, in contact with each other and with rostral. Prefrontals pentagonal, length equal to width. Frontal V-shaped, nearly 1.3 times as long as wide. Supraoculars large, twice as long as broad, shorter than frontals. Parietals large, 1.6 times as long as broad. Single loreal, large; one preocular, longer than wide; two postoculars, one below other. Two temporals, one behind other, in contact with supralabials. Seven supralabials on each side, third and fourth bordering eye. Six infralabials, second smallest, first four touching chinshield: two pairs of chin-shields. in contact with each other.

Dorsal scales in 13 rows throughout, all smooth. Ventrals 219+4, cloaca scale divided. Subcaudals 28, paired; dorsal tail scale rows reduced from six to four at sixth subcaudal. Tail short and tip pointed.

Dorsal surface brownish, with 27+3 regular, narrow, black transverse bands edged with yellow, each band in contact with cross-band on belly, forming closed ring. Quadrangular spots or irregular short bands between rings on belly. Black rings occasionally not reaching abdomen, forming half rings. Head black above with very broad white transverse bar behind eyes. Anterior margin inverted V-shaped white band, extending to prefrontals; posterior margin nearly straight, exceeding end of frontals; white bar wider than anterior black bar, yellowish white below, with 47+5 cross-bars or quadrangular spots.

Ecological notes: The specimens were found in meadowland in bamboo forest (Figure 1A). An individual of *Achalinus* sp. was found in the stomach of *Sinomicrurus peinani* **sp. nov**. No data on diet or reproduction are available.

Etymology: The species is named after Professor Pei-Nan Yu, a distinguished doctor in China, in recognition of his great contribution to the treatment of snakebite. We suggest the following common names for this species: "广西华珊瑚蛇" (Chinese) and Guangxi coral snake (English).

Description of paratypes: The three paratypes are similar to the holotype in body color and pattern. A summary of the morphological and meristic data of the three paratypes is given in Table 1.

Distribution: This species is currently known from China (Cangwu, Guangxi) and Vietnam (Cao Bằng and Vinh Phuc). The speciemens from Vietnam were unavailable for examination, but molecular phylogeney indicated that they should be conspecific with those from Cangwu, Guangxi, China (Supplementary Appendix S3).

Phylogenetic position: Both BI and ML analyses recovered a consistent topology, with slight disagreement in support values in some nodes (Figure 1C). In the BI tree, all individuals of Sinomicrurus formed a monophyly with high support (1.00 PP and 100% BS). Within Sinomicrurus, the two species (S. sauteri and S. hatori) endemic to Taiwan, China formed the basal lineage. Sinomicrurus kelloggi and the recently described S. houi Peng, Wang, Ding, Zhu, Luo, Yang, Huang, Lv, & Huang, 2017, formed a highly supported lineage (1.00 PP and 100% BS). All putative individuals of S. macclellandi formed a monophyly with high support indices (1.00 PP and 97% BS), which was sister to a well-supported monophyly

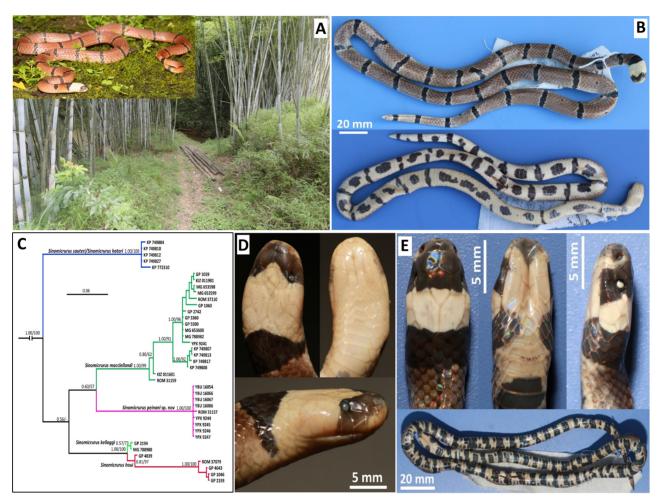


Figure 1 Holotype and phylogenetic position of Sinomicrurus peinani sp. nov.

A: General view of holotype (YBU 16086) in life and habitat of new species Sinomicrurus peinani sp. nov. B: Dorsal and ventral views of holotype (YBU 16086) in preservative. C: Bayesian 50% majority-rule consensus tree inferred from COI. Posterior probabilities and bootstrap support values for clades are shown adjacent to nodes to which they refer. D: Dorsal, ventral, and lateral views of head of holotype (YBU 16086) in preservative. E: Dorsal, ventral, and lateral views of head and ventral view of belly of Sinomicrurus macclellandi (YBU 14127) from Sichuan Province, China.

Table 1 Morphological characters of the type specimens of Sinomcrurus peinani sp. nov.

Specimens	Sex	SVL (mm)	TaL (mm)	DS	VS	SC	IL	SL	Tem	Lo	Poo	Pro	СВ
YBU16086	F	550	45	13-13-13	210	28	7/7	6/6	1+1/1+1	0	2/2	1/1	27+3
YBU16054	F	575	45	13-13-13	208	27	7/7	6/6	1+1/1+1	0	2/2	1/1	26+3
YBU16066	M	335	33	13-13-13	217	28	7/7	6/6	1+1/1+1	0	2/2	1/1	31+4
YBU16067	F	500	49	13-13-13	225	28	7/7	6/6	1+1/1+1	0	2/2	1/1	32+4

For abbreviations, see text. F: Female; M: Male.

consisting of several individuals from Wuzhou and Guangxi, China and from Vietnam with poor support (0.60 PP and 57% BS). However, unexpectedly, all samples of S. m. swinhoei (Denburgh) were nested within the nominated subspecies (Figure 1C). In addition, two specimens from Guangxi, China and Vietnam formed a well-supported clade (97% BS) with all samples of S. houi from Hainan, China.

Uncorrected P-distances between in-aroup each taxa/lineage of Sinomicrurus ranged from 1.3% (between S. sauteri and S. hatori) to 14.6% (between S. houi and S. macclellandi) (Supplementary Appendix S4).

Based on a combination of morphological comparisons and molecular phylogenies, we deemed these specimens to represent an undescribed species, which we described

herein.

Remarks

DNA barcoding based on *COI* is widely applied for rapid and unambiguous identification of species and can be used to screen for potentially cryptic species (Che et al., 2012; Liu et al., 2015). Our results indicated that the genetic distances between the new species and its congeners were much higher than that between other congeners (Supplementary Appendix S4), as well as that between other snakes (Liu et al., 2015), indicating that *Sinomicrurus peinani* **sp. nov.** should sever as a specific rank.

In addition to the large genetic distances between the new species and its congeners, *Sinomicrurus peinani* **sp. nov.** can also be differentiated by various phenotypic characters. The new species differs from *S. sauteri*, *S. hatori*, and *S. japonicus* by black longitudinal stripes on body absent (vs. present) (Günther, 1868; Steindachner, 1913; Takahashi, 1930). *Sinomicrurus peinani* **sp. nov.** is distinct from *S. houi* and *S. kelloggi* by 13 rows of dorsal scales throughout (vs. 15) and broad white band on top of head (vs. two narrow white bands) (Peng et al., 2018; Pope, 1928; Smith, 1943).

Sinomicrurus macclellandi currently contains four subspecies (Vogel, 2006). Besides the nominate form, which is mainly found in mainland China (Zhao, 2006), S. m. swinhoei (van Denburgh, 1912) is endemic to Taiwan, China. S. m. iwasakii (Maki, 1935) is endemic to Japan, and S. m. univirgatus (Günther, 1868) occurs in India and Nepal. Sinomicrurus peinani sp. nov. differs from S. m. univirgatus by its different body pattern, with the latter having black vertebral strip and transverse bars restricted to sides of body, or absent altogether (Günther, 1868). Sinomicrurus peinani sp. nov. can be distinguished from the other three subspecies by broad white transverse band with inverted V-shaped anterior margin on top of head (vs. two white bands with straight margin) and V-shaped frontal (vs. spindle-shaped frontal), small and not closely arranged cross-bands and spots (Figure 1D) (vs. more closely arranged black cross-bands and spots on belly, such that abdomen appears dark black, Figure 1E) (Reinhardt, 1844; Maki, 1935; van Denburgh, 1912; Zhao, 2006). Sinomicrurus peinani sp. nov. can be distinguished from S. macclellandi by white band on top of head wider than anterior black band vs. white band generally as wide as anterior black band. Historically, several other names have been allied to S. macclellandi, e.g., Calliophis macclellandi gorei Wall, 1910 and Calliophis macclellandi concolor Wall, 1925, with the first described from India and the latter described from Myanmar. Nevertheless, the new species differs from both by having black transverse bars on body (vs. without) (Wall, 1910, 1925).

Sinomicrurus houi Peng, Wang, Ding, Zhu, Luo, Yang, Huang, Lv, & Huang was originally described only from Hainan, China and was thus regarded as endemic to that area (Peng et al., 2018). However, our molecular phylogenetic results suggested that several closely situated specimens

from Guangxi, China and Vietnam formed a monophyly with *S. houi* from Hainan, China indicating that *S. houi* may occur on the Asian mainland and may not be endemic to Hainan, China alone. Unexpectedly, the *S. m. swinhoei* samples were nested within those from mainland China (*S. m. macclellandi*), indicating that the intraspecific relationship and taxonomy of *S. macclellandi* need to be further studied.

NOMENCLATURAL ACTS REGISTRATION

The electronic version of this article in portable document format represents a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new names contained in the electronic version are effectively published under that Code from the electronic edition alone (see Articles 8.5–8.6 of the Code). This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information can be viewed through any standard web browser by appending the LSID to the prefixhttp://zoobank.org/.

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SCIENTIFIC FIELD SURVEY PERMISSION INFORMATION

This field survey was approved by the Department of Forestry of Guangxi Zhuang Autonomous Region, China and Vietnam Adiministration of Forestry (982/TCLN-BTTN).

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

Q.L., P.G., and J.C. designed the study; P.G., J.W.Y., P.W., S.N.N., and R.W.M. collected specimens; P.G. collected morphological data; Q.L. and S.B.H. collected genetic data; Q.L. performed molecular and phylogenetic analyses and prepared the manuscript; P.G. and J.C. revised the manuscript. All authors read and approved the final version of the manuscript.

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