

Letter to the editor

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Molecular phylogeny and morphological comparisons of the genus *Hebius* Thompson, 1913 (Reptilia: Squamata: Colubridae) uncover a new taxon from Yunnan Province, China, and support revalidation of *Hebius septemlineatus* (Schmidt, 1925)

We describe a new species of the genus *Hebius* and provide evidence for the validity of *H. septemlineatus* **comb. nov.**. Morphological and molecular analyses of *Hebius* specimens collected in Yunnan Province, China, revealed three distinct lineages, namely the newly described *Hebius weixiensis* **sp. nov.**, as well as *H. octolineatus* (Boulenger, 1904), and *H. septemlineatus* **comb. nov.** (Schmidt 1925), which is removed from synonymy with *H. octolineatus*. Based on mitochondrial genealogy, *Hebius weixiensis* **sp. nov.** is sister to *H. septemlineatus* **comb. nov.**, while *H. octolineatus* is sister to *H. bitaeniatus*. The new species and *H. septemlineatus* **comb. nov.** showed considerable genetic divergence from their recognized congeners (uncorrected *P*-distance $\geq 3.9\%$). Furthermore, the new species and *H. septemlineatus* **comb. nov.** can be diagnosed from closely related congeners by a combination of pholidosis characters.

The genus *Amphiesma* Duméril, Bibron & Duméril, 1854 was long recognized as a monophyletic group with many species widely distributed across southern, eastern, and south-eastern Asia (Wallach et al., 2014). However, taxonomy within the clade has been in flux. Based on molecular analyses, Guo et al. (2014) split the genus *Amphiesma* (*sensu lato*) into three genera: i.e., *Amphiesma* (*sensu stricto*), *Herpetoreas* Günther, 1860, and *Hebius* Thompson, 1913. Recently, Kizirian et al. (2018) synonymized the monotypic genera *Parahelicops* Bourret, 1934, which contained *Parahelicops annamensis* Bourret, 1934, and *Pararhabdophis* Bourret, 1934, which contained *Pararhabdophis chapaensis* Bourret, 1934, with the genus *Hebius*. In addition, the generic

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assignments of some species have changed based on their systematic positions revealed in various studies; for example, multilocus phylogenetic analyses of mitochondrial and nuclear genes indicated that *Hebius pealii* (Sclater, 1891) and *Hebius xenura* (Wall, 1907) were actually nested within the genus *Herpetoreas*, and thus both species were moved to *Herpetoreas* (Das et al., 2020; Lalronunga et al., 2020).

Over the last several years, we collected a series of *Hebius* specimens during herpetological surveys in Yunnan, China, (Figure 1Ai). After euthanasia, liver tissue samples were taken and preserved in 85% ethanol, and specimens were fixed in 10% buffered formalin solution, then transferred to 70% ethanol after two days. All specimens and samples were deposited in the Kunming Institute of Zoology (KIZ), Chinese Academy of Sciences (CAS) (see Supplementary Text and Table S1).

Measurement and scale counting methods are presented in Supplementary Materials and Methods. To reconstruct the phylogeny of *Hebius*, we extracted total genomic DNA and amplified and sequenced a 978 bp fragment of the mitochondrial cytochrome *b* (*cyt b*) gene. Trees were

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Figure 1 Phylogenetic relationships of genus *Hebius*, and distribution and photo of *Hebius weixiensis* sp. nov., *H. septemlineatus* comb. nov. and *H. octolineatus*

Ai: Distribution of selected species of genus *Hebius* in Yunnan, China, including type locality of *Hebius weixiensis* sp. nov. in Weixi (blue circle), *H. septemlineatus* comb. nov. (blue triangle) between Tengchong and Longchuan, and *H. octolineatus* (white triangle) between Kunming and Chuxiong. Aii: Phylogenetic analyses using partitioned maximum-likelihood (ML) based on a 978 bp fragment of mitochondrial cytochrome *b* (cyt *b*) gene. Nodal support from partitioned Bayesian inference (BI) analyses are mapped on ML topology. Numbers before slashes indicate BI posterior probabilities (>95% retained) and numbers after slashes are ML bootstrap support values (>75 retained). Dashes indicate differential topology between BI and ML analyses. New sequences are indicated in bold. B: Holotype of *Hebius weixiensis* sp. nov. (KIZ 035739) in preservation, showing dorsal view (Bi), ventral view (Bii); dorsal head (Biii), ventral head (Biv), lateral right head view (Bv), lateral left head view (Bvi), and lateral mid-body close-up (Bvii). C: Specimens of *H. septemlineatus* comb. nov. (KIZ 037706) in life, showing dorsal view (Ci), ventral view (Cii); dorsal head (Ciii), ventral head (Civ), lateral right head view (Cv), lateral left head view (Cvi); (Cvii) lateral mid-body close-up, and dorsal mid-body close-up (Cviii). D: Adult female of *H. octolineatus* (KIZ 037733) in life, from Qipan Mountain, Kunming City, Yunnan, China, showing dorsal view (Di), lateral left head view (Dii), lateral right head view (Diii), and dorsal mid-body close-up (Div). Photos by S.B. Hou.

reconstructed using partitioned maximum-likelihood (ML) and Bayesian inference (BI) analyses (see Supplementary Materials and Methods for details).

Both ML and BI analyses yielded nearly identical topologies, except for several nodes with low support (Figure 1Aii). The genus *Hebius* was recovered as a monophyletic group with strong support, and all newly collected specimens were nested within the genus (Bayesian posterior probability 1.00/ML bootstrap support 100, given in this order below). The newly collected specimens were recovered in three clades. The three specimens from Weixi County formed a monophyletic clade, which was sister to another monophyletic clade that included the three specimens from Longchuan County and Tengchong City (1.00/100). The three specimens from Kunming City and Chuxiong formed a monophyletic clade with *H. octolineatus* (1.00/99).

For the newly collected specimens, the three mtDNA clades were consistent with three distinct morphological groups. The uncorrected genetic distances among the three clades varied from 3.9% to 14.2% (Supplementary Table S2).

Taxonomic accounts

***Hebius weixiensis* sp. nov.** Hou, Yuan, Wei, Guo & Che (Figure 1Bi–Bvii)

Holotype: KIZ 035739, adult male, collected by S.B. Hou and P.F. Wei on 19 June 2019 from Shang Village (N27.130267°, E99.326753°; 2 426 m a.s.l.), Weixi County, Yunnan Province, China.

Paratypes: Three adult males (KIZ 035741, KIZ 035742, and KIZ 035776) and one adult female (KIZ 035740) collected by S.B. Hou and P.F. Wei on 19 June 2019 from same locality as holotype; three adult males (KIZ 028321, KIZ 028323, and KIZ 028324) and three adult females (KIZ 028318, KIZ 028325, and KIZ 028326) collected by K. Wang and J.L. Ren on 6 July 2016 from Gongnong Village, Pantiange Town, Weixi County, Yunnan Province, China; one adult male (KIZ 044335) collected by K. Wang from Shigu Township, Lijiang City; single adult male (CIB I10447) from Weixi, Yunnan (collector and collecting information unknown; CIB: Chengdu Institute of Biology).

Diagnosis: *Hebius weixiensis* sp. nov. can be distinguished from its recognized congeners by a combination of the following characters: (1) nasal divided, each half in contact with each other; (2) loreal pentagonal, entering orbit or not; (3)

supralabials eight; (4) preocular one or two; (5) postoculars three; (6) infralabials eight to ten; (7) dorsal scales 19–19–17 rows; (8) dorsal scale reduction from 19 to 17 rows at position of 96th to 101st ventrals; (9) ventrals 171–182; (10) subcaudals 74–88, paired; (11) anal divided; (12) no dorsal stripes or some dorsal stripes on posterior body; (13) no ventrolateral stripe; and (14) ventral body pale yellow.

Description of holotype: Body moderately stout, total length 567 mm (SVL 425 mm, TaL 142 mm); tail slender, 25.0% total length; head length moderate (HL 16.7 mm, 3.9% of SVL), oval in shape, distinct from neck; eyes large, pupil suborbicular; rostral semicircular, invisible from above; nasal divided, lateral nostril piercing medially; internasals paired, trapezoidal in shape, narrower end facing; prefrontals paired, larger than internasals; frontal hexagonal, spear like, longer than width, tip facing posteriorly and inlaid by both parietals; parietal paired, large, contacting five small nuchal scales; loreal pentagonal, slender, entering orbit; preocular single; supraocular single; postoculars three; temporals 2+1+3; supralabials eight, fourth and fifth entering orbit, seventh largest; mental triangle, inlaid completely by first pair of infralabials; infralabials nine; two pairs of chin shields; dorsal lanceolate, imbricated, 19–19–17 rows, distinctively keeled except outer most two rows; dorsal scale rows reduced from 19 to 17 between 99th and 101st ventrals; ventrals 175; subcaudals paired, 87 excluding tip; cloacal plate divided.

Coloration: In life, dorsal surface of head olive brown, with randomly scattered dark gray to black speckles. Dorsum curry colored, with some glossy yellow stripes, especially on anterior of body. Venter pale yellow, with grayish black markings at outer margins of ventral scales on posterior of body, grayish black blotches continuing to tip of tail. In preservative, dorsal surface of head curry colored, with gray dots; ventral surface of head white; dorsal surface of anterior body brown-black, posterior body black; venter pale-yellow.

Variation: Measurements, body proportions, and scale counts are listed in Table 1.

Comparisons: *Hebius weixiensis* sp. nov. can be easily distinguished from all recognized congeners by a series of contrasting morphological characters, including body pattern, morphometry, and scalation. Among the six morphological characteristics selected (DSR, VS, SC, TaL/ToL, dorsal stripes, ventrolateral stripe), there are at least two morphological differences between the new species and its

Table 1 Morphometric and pholidosis characters of holotypes of *Hebius weixiensis* sp. nov., *H. octolineatus*, and *H. septemlineatus* comb. nov.

Species	Voucher No.	Sex	ToL	SVL	TaL	TaL/ToL (%)	HL	HW	HH	SPL	IFL	Loreal-orbit	PIO	TMP	DSR	VS	SC
<i>Hebius weixiensis</i> sp. nov.	KIZ 028321	M	532	404	128	24.1	15.4	8.2	5.6	3-2-3	8(4/5)	Yes	1	3	1+1+3	19-19-17	174 83
	KIZ 028323	M	638	475	163	25.5	18.9	9.7	6.0	3-2-3	10(5)	No	1/2	3	2+1+3	19-19-17	176 84
	KIZ 028324	M	598	445	153	25.6	18.2	8.8	7.0	3-2-3	10(5)	No	2	3	1+1+3	19-19-17	179 85
	KIZ 035739	M	567	425	142	25.0	16.7	9.4	6.0	3-2-3	9(5)	Yes	1	3	2+1+3	19-19-17	175 87
	KIZ 035776	M	350	290	60.0 (broken)	Broken tail	12.3	5.3	4.4	3-2-3	9(5)	Yes	1	3	2+1+3	19-19-17	179 43+
	KIZ 035740	M	552	442	110.0 (broken)	Broken tail	17.9	8.9	6.3	3-2-3/3-3-3	9(5)	No	2	3	2+1+3	19-19-17	172 53+
	KIZ 035741	M	391	295	96	24.6	13.4	6.3	4.6	2-2-3/3-2-3	9(5)	Yes	1	3	2+2+3/2+1+3	19-19-17	173 77
	KIZ 035742	M	415	335	80.0 (broken)	Broken tail	14.8	7.9	5.5	3-2-3	9(5)	Yes	1	3	2+1+3/1+1+2	19-19-17	178 56+
	KIZ 028318	F	680	520	160	23.5	17.2	10.6	7.4	3-2-3	10(5)	No/Yes	2/1	3	2+1+3	19-19-17	173 74
	KIZ 028325	F	501	380	121	24.2	16.8	8.6	5.7	3-2-3	10(5)	No	2/1	2/3	2+2+2	19-19-17	171 77
<i>Hebius octolineatus</i>	KIZ 028326	F	375	287	88	23.5	13.6	6.4	6.5	3-2-3	10(5)	No	1/2	3	2+2+2	19-19-17	174 77
	KIZ 044335	M	630	470	160	25.4	19.1	8.2	6.3	2-3-3	10(5)	Yes	1	3	1+1+3	19-19-17	182 88
	CIB 110447 (Zhao et al., 1998)	M	630	476	154	24.4	-	-	-	2-2-3	9(5)	Yes	-	-	1+1/1+2	19-19-17	181 83
	KIZ 037734	M	487	363	127	25.9	16.5	8.7	7.3	3-2-3/4-2-3	10(5)	No	1	3	2+2	19-19-17	154 77
	KIZ 026445	M	266	204	62	23.3	12.1	6.1	5.5	3-2-3	10(5)	No	1	3	2+2+3	19-19-17	153 80
	KIZ 037733	F	586	445	141	24.1	20.5	9.4	9.4	3-2-3	10(5)	No	1	3	1+1+3/2+1+3	19-19-17	154 70
	KIZ 037735	F	701	556	145 (broken)	Broken tail	12.5	8.6	11.1	3-3-3/3-2-3	9(5)	No	2	3	1+1+3/1+2	19-19-17	150 54+
	KIZ 03204	F	652	504	148	22.7	23.7	12.3	9.7	3-2-3	10(5)	No	1	3	1+1+3/2+3	19-19-17	153 71
	KIZ 037706	M	624	453	171	27.4	18.8	10.6	7.5	3-2-3/2-2-3	10(5)	No	1	3	2+2+3	19-19-17	172 96
	KIZ 037697	M	697	520	177	25.4	18.9	11.2	9.2	3-2-3	10(5)	No	1	3	2+2+3	19-19-17	175 82
<i>Hebius septemlineatus</i> comb. nov.	KIZ 048610	M	264	198	66	25.0	11.2	5.9	4.4	3-2-3	10(5)	No	1	3	2+1+2	19-19-17	164 84
	KIZ 048611	M	271	204	67	24.7	12.5	5.8	4.5	3-2-3	10(5)	No	1	3	1+3	19-19-17	171 90
	KIZ 037712	F	550	408	142	25.8	14.6	7.7	6.4	3-2-3/2-2-3	10(5)	No	2	3	1+3	19-19-17	166 82
	KIZ 037711	F	428	316	112	26.2	15.7	7.4	5.3	3-2-3/2-2-3	10(5)	No	2	3	1+3	19-19-17	172 80
	KIZ 048609	F	415	321	94 (broken)	Broken tail	15.8	8.3	5.6	3-2-3	10(5)	No	1	3	2+2	19-19-17	165 68+

For abbreviations, see Supplementary Materials and Methods. M: Male; F: Female. -: Not available.

congeners. *Hebius weixiensis* sp. nov. differs from 12 species in DSRM (19 vs. 17 or 15), 35 species in VS (171–182 vs. less than 170 or more than 183), 26 species in SC (74–88 vs. less than 66 or more than 89), 19 species in TaL/ToL (23.5%–25.6% vs. more than 26.2%), 22 species in dorsal stripe (none vs. at least one), and 27 species in ventrolateral stripe (absent vs. present). A detailed comparison is provided in Supplementary Table S3.

Etymology: The specific name “*weixiensis*” is derived from the type locality of the new species in Weixi County, Yunnan Province, China. We propose “Weixi Keelback Snake” as its English common name and “维西腹链蛇” (Wei Xi Fu Lian She) as its Chinese common name.

Distribution: The new species is known only from Weixi County and Shigu Township in Lijiang, Yunnan Province, China.

***Hebius septemlineatus* comb. nov. (Schmidt, 1925) (Figure 1Ci–Cviii)**

Natrix septemlineata Schmidt, 1925.

Type locality: Tengyueh (now Tengchong City), Yunnan Province, China.

Holotype: AMNH 21051, collected by R. C. Andrews and E. Heller in May 1917.

Chinese Name: We suggest “Tengchong Keelback Snake” as its English name and “腾冲腹链蛇” (Teng Chong Fu Lian She) as its Chinese name.

Materials: *Hebius septemlineatus* comb. nov., $n=7$ (Supplementary Text).

Revised diagnosis: *Hebius septemlineatus* comb. nov. can be distinguished from its recognized congeners by a combination of the following characters: (1) TaL/ToL 23.5%–25.6%; (2) internasals narrowed anteriorly; (3) nasal divided, each half in contact with each other; (4) nostrils lateral; (5) loreal almost quadrilateral, higher than width, separated from orbit; (6) supralabials usually eight; (7) preocular one or two; (8) postoculars usually three; (9) mental triangles; (10) infralabials nine or ten, first pair in contact with each other, first–fifth infralabials in contact with anterior chin shields; (11) dorsal scales 19-19-17 rows; (12) dorsal scale rows reduced from 19 to 17 at position of third and fourth dorsal scale rows and from 97th to 101st ventrals; (13) ventrals 164–175; (14) subcaudals 80–96, paired; (15) anal divided; (16) four dorsal stripes on each side, not including ventrolateral stripe; and (17) ventral pale yellow.

Variation: Measurements, body proportions, and scale counts are listed in Table 1.

Distribution: This species is known only from western Yunnan, China, including Tengchong and Longchuan.

Systematics: This species has only been mentioned by Schmidt (1925). It was subsequently synonymized with *Natrix octolineatum* (now *Hebius octolineatus*) by Pope (1935:112). Here, it is validated to a full species.

***Hebius octolineatus* (Boulenger, 1904) (Figure 1Di–Div)**

Materials: *Hebius octolineatus*, $n=5$ (details in Supplementary Text).

Diagnosis: *Hebius octolineatus* can be distinguished from its recognized congeners by a combination of the following

characters: (1) TaL/ToL 20.7%–25.9%; (2) internasals narrowed anteriorly; (3) nasal divided, each half in contact with each other; (4) nostrils lateral; (5) loreal almost quadrilateral, higher than wide, not entering orbit; (6) supralabials usually eight; (7) preocular one or two; (8) postoculars three; (9) infralabials nine or ten, first pair in contact with each other, first–fifth infralabials in contact with anterior chin shields; (10) mental triangles; (11) dorsal scales rows 19-19-17; (12) dorsal scale rows reduced from 19 to 17 at position of third and fourth dorsal scale rows and from 81st to 86th ventrals; (13) ventrals 150–154; (14) subcaudals 70–80, paired; (15) anal divided; (16) four dorsal stripes on each side, not including ventrolateral stripe, two yellow and two grayish-black; (17) black zigzag lateral line, formed by outer edges of ventral shields; and (18) venter pale yellow.

Description and variation: Detail measurements, body proportions, and scale counts are listed in Table 1.

Distribution: The species is found in Yunnan, China, including Kunming and Chuxiong.

Systematics: Boulenger (1904) described three new species of *Tropidonotus* from Yunnan Fu (now Kunming City), Yunnan Province, China, each based on a single specimen: *T. octolineatus*, *T. quadrilineatus*, and *T. pleurotaenia*. Subsequently, *T. quadrilineatus* and *T. pleurotaenia* were synonymized with *Natrix octolineatum* (now *Hebius octolineatus*) by Pope (1935). The clarifications of *H. septemlineatus* comb. nov. and *H. octolineatus* are consistent with the original descriptions (Supplementary Table S4).

Hebius octolineatus has been extensively confused with *H. septemlineatus* comb. nov. in the literature. Previous descriptions of *H. octolineatus* were based on specimens collected in western Yunnan and Sichuan, rather than the type locality (Yang and Rao, 2008; Zhao et al., 1998; Zhao, 2006). Despite the confusion, *H. octolineatus* can be rather easily separated from *H. septemlineatus* comb. nov. by (1) number of ventrals (164–175 vs. 150–154), (2) number of subcaudals (80–96 vs. 70–80), (3) more posterior occurrence of reduction in dorsal scale rows from 19 to 17 (97th to 101st ventrals vs. 81st to 86th ventrals), (4) fourth and fifth supralabials yellow (vs. fourth and fifth supralabials yellow with back edge black); (5) vertebral behind head yellow and immediately after dark, continued to base of tail (vs. dorsum pale grayish brown).

The genus *Hebius* is a species diverse group. The description of *Hebius weixiensis* sp. nov. and revalidation of *Hebius septemlineatus* comb. nov. bring the total species number of the genus to 48, including 23 species in China (with 18 in Yunnan alone). The species diversity of *Hebius* may be underestimated, and further study based on extensive samples is highly desirable.

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 prefix <http://zoobank.org/>.

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SCIENTIFIC FIELD SURVEY PERMISSION INFORMATION

The collection of all animals used in this study obeyed the Wildlife Protection Act of China. Collection permits were issued by the Kunming Institute of Zoology, Chinese Academy of Sciences (BBCJ-2014-001). All relevant protocols of the IACUC (IACUC R13-11) and Animal Care and Ethics Committee at the Kunming Institute of Zoology were followed for the proper treatment of animals in the field.

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

J.C. and P.G. conceived and designed this study. Z.Y.Y., P.F.W., J.M.C., and G.G.Z. conducted the field surveys. G.H.L. and W.J.S. sorted out samples. S.B.H. and Z.Y.Y. performed the experiments, measured the specimens, and analyzed the data. S.B.H. wrote the manuscript. S.B.H., P.G., Y.H.W., and J.C. discussed and revised the manuscript. All authors read and approved the final version of the manuscript.

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