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First description of the female of *Achalinus sheni* (Serpentes, Xenodermidae), with expanded description of this species

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Abstract

Achalinus sheni Ma, Xu, Qi, Wang, Tang, Huang & Jiang, 2023 was originally described based on only male specimens from Lianyuan City and Nanyue District, Hunan, China. So far, no information on the females of this species is available. After molecular systematics and morphological characters of recently collected specimens from Xinshao County, Hunan, several specimens (3 males and 1 female) were identified as this species. Therefore, we provided supplementary descriptions of the female characteristics of this species in this study. Meanwhile, we extended the description of this species.

Key Words

Morphology, Phylogenetics, Xinshao County

Introduction

Achalinus Peters, 1869, commonly known as the odd-scaled snakes, is the most diverse genus of the family Xenodermidae (Uetz et al. 2022). It contains 28 recognized species, and is widely distributed in eastern and southeastern Asia, ranging from northern Vietnam to southwestern China, and partly into Japan, from which 21 species occur in China. In recent years, with extensive sampling and molecular phylogenetic methods, more than 20 members of the genus *Achalinus* have been discovered and described (Wang et al. 2019; Ziegler et al. 2019; Li et al. 2020; Luu et al. 2020; Miller et al. 2020; Hou et al. 2021; Huang et al. 2021; Li et al. 2021; Ha et al. 2022; Yang et al. 2022; Ma et al. 2023a, 2023b; Yang et al. 2023; Zhang et al. 2023; Li et al. 2024).

Achalinus sheni was described based on five specimens collected in Hunan Province, China: four male specimens (ANU20230012—ANU20230015) from Lianyuan City and one male specimen (CIB 119043) from Nanyue District (Ma et al. 2023b). Molecular phylogeny inferred from the mitochondrial *CO* gene fragment revealed that this new species is most closely related to *A. yunkaiensis*, but it can be distinguished from

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A. yunkaiensis by the following morphological characters: (1) relative length of supraocular and upper anterior temporal (supraocular equal to or longer than anterior temporal, SPOL/ATUL 0.99–1.20 vs. supraocular shorter than anterior temporal, SPOL/ATUL 0.55–0.83); (2) higher ventral + subcaudals counts in males (220–225 vs. 200–212); (3) higher ventral scale counts in males (161–170 vs. 150–162); (4) higher subcaudal scale counts in males (55–61 vs. 49–56). However, to date there have been no morphological comparisons between females of these two species.

During a herpetofaunal survey of Hunan, China in 2022, one female and three male *Achalinus* were collected from Xinshao County, Shaoyang City (Fig. 1). Morphological and molecular phylogenetic analyses revealed that the specimens are *A. sheni*. Here we provide morphological data for the newly collected female specimen of *A. sheni* , and extend the description of this species.

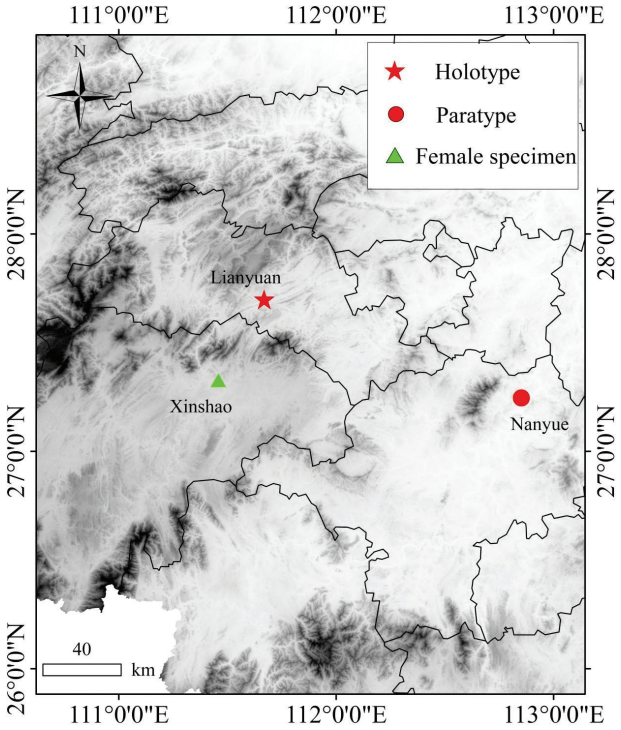

Figure 1. Sampling sites of *Achalinus sheni*: solid red star, the locality of the holotype; solid red circle, the locality of the paratype; solid green triangle, the locality of the newly collected female specimen.

Materials and methods

Sampling

Four odd-scaled snake specimens were collected from Xinshao County, Shaoyang City, Hunan Province, China (HNNU2022001—HNNU2022004). The four specimens were fixed in 75% ethanol and deposited in the Vertebrate Zoology Laboratory, College of Life Science, Hunan Normal University.

Morphological examination

Morphological descriptions followed Zhao (2006) and Ma et al. (2023b). Abbreviations in this study are as follows: snout-vent length (**SVL**): snout-vent length from tip of snout to anterior margin of the cloaca; tail length (**TaL**): tail length from posterior margin of cloaca to tip of tail; total length (**TL**): from snout tip to tail end; head length (**HL**): from the tip of snout to the posterior margin of mandible; head width (**HW**): from the widest part of the head in dorsal view; eye horizontal diameter (**ED**): from the most anterior edge of the eye to the most posterior edge; loreal height **(LorH**): measured from the highest point to the lowest point of the loreal in lateral view; loreal length (**LorL**): from the most anterior edge of the loreal to the most posterior edge of the loreal in lateral view; length of the suture separating the internasals (**LSBI**); length of the suture separating the prefrontals (**LSBP**); length of the supraocular (**SPOL**): horizontal distance between the anterior and posterior tips of the supraocular, and length of the upper anterior temporal (**ATUL**): horizontal distance between the anterior and posterior tips of the upper anterior temporal. We also directly compared the length of the sutures between the internasals and prefrontals (**LSBI vs. LSBP**).

The morphological features and their abbreviations are as follows: loreals (**Loreal**), supralabials (**SPL**), infralabials (**IFL**), the number of infralabials touching the first pair of chin shields (**IFL-1st Chin**), supraoculars (**SPO**), temporals (**TEM**), the number of anterior temporals touching the eye (**aTEM-Eye**), ventral scales (**VEN**), subcaudals (**SC**), cloacal plate entire or divided (**CP**), dorsal scale rows (**DSR**) (counted at one-head-length posterior of head, at midbody, at one-head-length anterior of cloacal plate). Bilateral scale counts were given as left/right.

Phylogenetic analyses

Genomic DNA was extracted from preserved liver tissue using the TIANamp Genomic DNA Kit. The fragment of the mitochondrial DNA gene encoding cytochrome c oxidase subunit □ (*CO*□) was amplified using the primer pairs Chfm4 and Chrm4 (Che et al. 2012). The PCR products were sequenced at Shanghai Map Biotech Co., Ltd. The homologous sequences of the *Achalinus* species and the outgroups species were downloaded from GenBank (Table 1).

The *CO*□ sequences (639 bp) were assembled using SeqMan in the DNASTAR software package (Burland, 2000), and compared and aligned using MEGA 7 software (Kumar et al. 2018). The uncorrected pairwise distances (*p*-distance) were calculated in MEGA 7. Maximum likelihood analysis (Nguyen et al. 2015) was executed using IQ-TREE 2 under the best-fit model TIM3 + F + I + G4 selected by Modelfinder according to AIC. Nodal support was estimated by 1,000 bootstrap replicates using the ultrafast bootstrap feature.

Table 1. Localities, voucher information and GenBank numbers for all samples used in this study.

Species	Locality	Voucher NO.	Accession
A. sheni	Lianyuan, Hunan, China	ANU20230013	OR178146
A. sheni	Lianyuan, Hunan, China	ANU20230014	OR178147
A. sheni	Nanyue, Hunan, China	CIB 119043	OR189183
A. sheni	Xinshao, Hunan, China	HNNU2022001	PP854453
A. ater	Huaping Nature Reserve, Guangxi, China	SYSr00852	MN380334
A. dabieshanensis	Fuziling Provincial Reserve, Anhui, China	AHU2018EE0710	MW316598
A. damingensis	Shanglin, Nanning, Guangxi, China	ANU20220009	OP644487
A. dehuaensis	Dehua, Fujian, China	YBU13013	MZ442642
A. emilyae	HoanhBo, Quang Ninh, Vietnam	IEBR4465	MK330857
A. formosanus	Taiwan, China	RN2002	KU529452
A. huangjietangi	Huangshan, Anhui, China	HSR18030	MT380191
A. hunanensis	Huaihua, Hunan, China	CIB119039	OQ848425
A. juliani	HaLang, Cao Bang, Vietnam	IEBRA.2018.8	MK330854
A. meiguensis	Mianyang, Sichuan, China	GP835	MZ442641
A. nanshanensis	Nanshan National Park, Hunan, China	HNNU230903	OR523370
A. niger	Taiwan, China	RN0667	KU529433
A. ningshanensis	Ningshan, Shaanxi, China	ANU20220006	ON548422
A. panzhihuaensis	Yanbian, Sichuan, China	KIZ040189	MW664862
A. pingbianensis	Honghe, Yunnan, China	YBU18273	MT365521
A. quangi	northern Vietnam	sp4	OQ197471
A. rufescens	Hongkong, China	SYSr001866	MN380339
A. spinalis	Badagong Mountains, Hunan, China	SYSr001327	MN380340
A. timi	ThuanChau, Son La, Vietnam	IEBRA.2018.10	MK330856
A. tranganensis	NinhBinh, Vietnam	VNUFR.2018.21	MW023086
A. vanhoensis	VanHo, Son La, Vietnam	VNUFR.2019.13	ON677935
A. yangdatongi	Wenshan Nature Reserve, Yunnan, China	KIZ034327	MW664865
A. yunkaiensis	Dawuling Forestry Station, Guangdong, China	SYSr001443	MN380329
A. yunkaiensis	Dawuling Forestry Station, Guangdong, China	SYS r001502	MN380330
A. yunkaiensis	Dawuling Forestry Station, Guangdong, China	SYS r001503	MN380331
A. zugorum	Bac Me, Ha Giang, Vietnam	IEBR4698	MT502775
Fimbrios klossi	Quang Ngai, Vietnam	IEBR3275	KP410744
Parafmbrios lao	Louangphabang, Laos	MNHN2013.1002	KP410746
Xenodermus javanicus	Sumatera Barat, Indonesia	—	KP410747

Results

Maximum likelihood trees showed consistent topology. The newly collected female specimen clustered with the specimens (including the holotype) of *Achalinus sheni* and demonstrated strong support (ML = 97, Fig. 2). The genetic distance (uncorrected p-distance) between the newly collected female specimen and the specimens (including the holotype) of *A. sheni* was only 0.7–0.8% (Table 2).

Achalinus sheni Ma, Xu, Qi, Wang, Tang, Huang & Jiang, 2023

Specimen examined. HNNU2022001, adult female, collected by Hui Li, Lin Zhou on April 11, 2022, from Xinshao County (27°23'58"N, 111°33'44"E, 420 m a. s. l.), Shaoyang City, Hunan Province, China.

Description of the female specimen. Adult female with a total length of 345.1 mm (SVL 294.8 mm and TaL 50.3 mm), tail relatively short, TL/ToL 0.149, body slender and cylindrical. Head distinct from neck, rostral small, triangular, only the upper tip is visible from above. Head length 9.16 mm, head width 3.95 mm, HL/HW 2.32. Eyes small, eye width 1.10 mm. Length of the suture between the internasals (LSBI 1.40 mm) subequal to the length of the suture between the prefrontals (LSBP 1.31 mm). Frontal pentagonal pointed backwards, much shorter than the parietals; each parietal bordered with an elongated nuchal, with no preoculars or postoculars. Nostril in anterior portion of nasal scale, posterior margin of nostril with a distinct nostril cleft. A single loreal scale present, extending from the nasal to the eye, distinctly wider than high. Temporals 2+2+3. aTMPs elongated, upper aTMP much smaller than the lower aTMP; upper aTMP and lower aTMP in contact with eye, lower aTMP also in contact with parietal scale. Supralabials 6, 4th–5th contact the eye, the last much elongated. A single mental scale present. Two chin shields, the anterior pair longer than the posterior pair. Infralabials 5, the first contacting each other posterior to the mental and anterior to the 1st chin shields, 1st–3rd contact the 1st chin shields. Dorsal scales 23-23-23, strongly keeled, dorsum with an inconspicuous longitudinal vertebral stripe. Ventrals (VEN) 173. Subcaudals (SC) 45, not paired. Cloacal plate (CP) entire (Table 3).

Coloration of the female specimen in life. Scales possess a subtle iridescent quality which gives the dorsum a distinctive reflective brownish-black appearance. Dorsum dark brown and the five innermost dorsal scale rows a little darker, forming an inconspicuous longitudinal vertebral line. Chin shields are tan. On the ventral surface, an off-white shade prevails, with the edges of the ventral scales gradually transitioning from gray-white to black. Ventral side of tail brownish.

Coloration in preservative. (Fig. 3**)** The dorsal surface of the body uniformly brownish-black, slightly tinged with iridescence and the longitudinal vertebral line appears a little darker. Chin shields light brown. Ventrals generally pale brown, darker on both sides, free margins of ventral scales grayish-white. Ventral surface of tail light brown.

Extended diagnosis. Upon examining the additional adult males of *Achalinus sheni* from Xinshao County, it was observed that they possessed fewer ventral scales compared to specimens from the type locality. However, the number of subcaudal scales remained approximately the same for individuals from both locations. Upon inspection of adult female *A. sheni* collected in Xinshao County, it was noted that it exhibited an extremely short tail and consequently fewer subcaudals and more ventral compared to adult male individuals.

Comparison. Compared to male individuals of *A. sheni* and *A. yunkaiensis*, female individuals of both species exhibit similar differences in the number of ventral scales and subcaudals. Compared to males, both female *A. yunkaiensis*

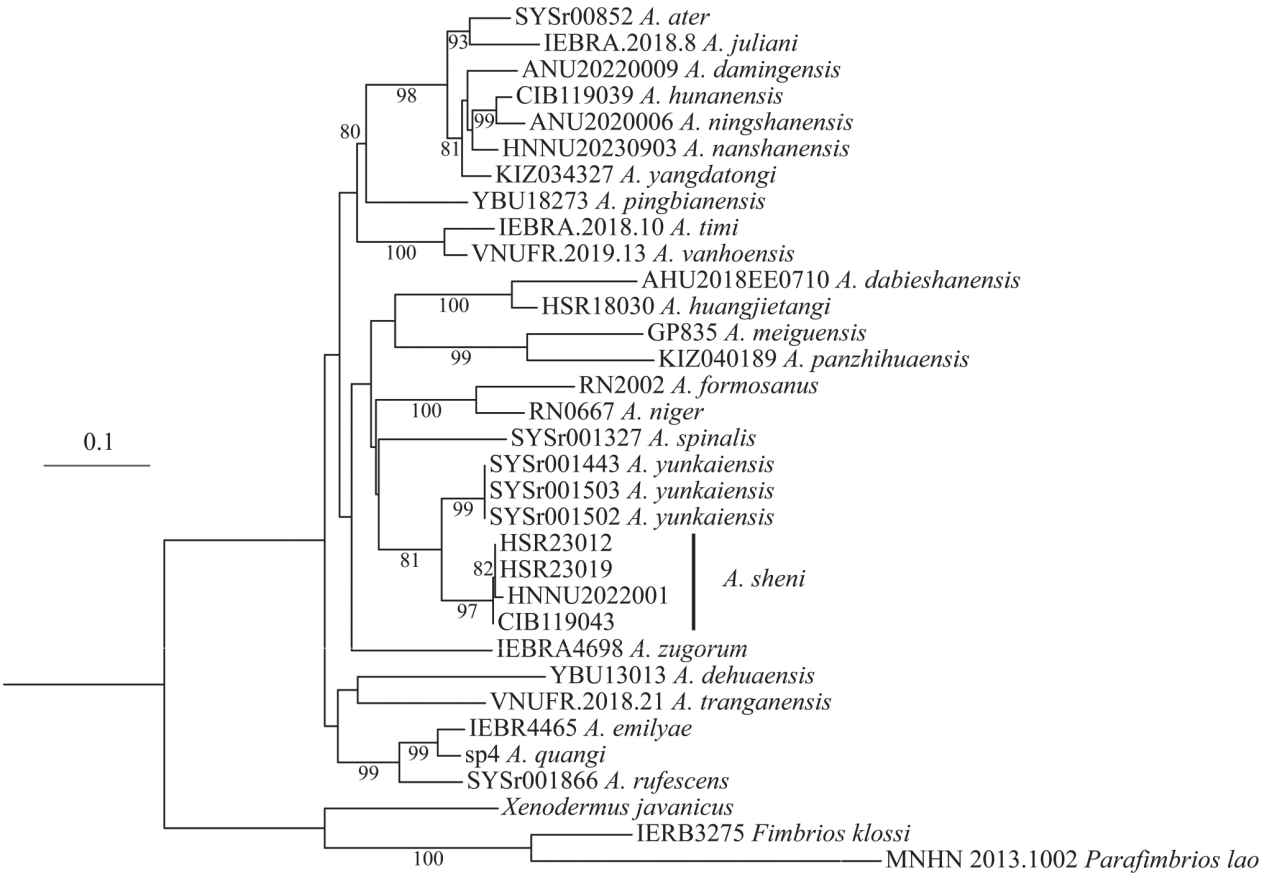

Figure 2. Phylogenetic tree of the genus *Achalinus* inferred from *CO* gene fragments (629 bp) using Maximum Likelihood. The numbers above the branches represent the supporting values.

Table 2. Uncorrected p-distances (%) among *Achalinus* species inferred from mitochndrial *CO* gene.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1 HNNU2022001																											
2 *A. sheni*	0.7																										
3 *A. sheni*	0.7	0.0																									
4 *A. sheni*	0.8	0.2	0.2																								
5 *A. ater*	13.5	13.2	13.2	13.2																							
6 *A. dabieshanensis*	15.3	15.8	15.8	15.6	14.7																						
7 *A. damingensis*	14.4	14.0	14.0	14.0	8.0	15.8																					
8 *A. dehuaensis*	13.9	13.9	13.9	13.8	17.1	18.4	16.1																				
9 *A. emilyae*	14.2	13.7	13.7	13.5	11.9	17.7	13.4	15.7																			
10 *A. formosanus*	13.2	13.0	13.0	13.0	14.4	19.0	14.9	16.4	14.0																		
11 *A. huangjietangi*	13.6	13.6	13.6	13.3	15.0	8.9	16.3	16.5	14.5	15.6																	
12 *A. hunanensis*	12.5	12.1	12.1	12.3	7.3	16.9	6.1	14.9	13.2	13.7	16.8																
13 *A. juliani*	14.4	14.0	14.0	14.2	7.0	15.8	8.7	15.1	12.9	12.5	14.6	8.8															
14 *A. meiguensis*	14.6	14.1	14.1	13.9	15.4	17.7	16.8	18.1	15.4	15.6	15.2	16.4	16.8														
15 *A. nanshanensis*	13.5	13.9	13.9	14.0	6.7	16.0	5.4	14.7	13.4	15.1	16.6	4.7	8.2	17.7													
16 *A. niger*	12.7	12.5	12.5	12.5	13.5	15.8	14.4	16.2	12.4	9.2	13.9	13.2	12.5	13.9	13.5												
17 *A. ningshanensis*	14.5	14.1	14.1	14.2	7.6	17.2	7.8	16.5	14.1	14.8	17.2	3.4	9.6	17.0	5.1	14.6											
18 *A. panzhihuaensis*	14.6	14.6	14.6	14.6	16.2	16.6	15.5	15.3	16.6	16.0	15.2	16.2	15.5	11.6	15.5	14.4	17.4										
19 *A. pingbianensis*	11.5	11.5	11.5	11.6	11.6	15.3	10.9	15.0	12.8	14.5	13.0	11.1	1.0	16.8	11.5	11.8	11.7	14.9									
20 *A. quangi*	15.1	14.5	14.5	14.3	12.2	18.1	13.2	15.9	3.3	14.2	15.0	13.2	12.9	15.2	13.0	11.9	13.4	16.9	13.8								
21 *A. rufescens*	13.7	13.5	13.5	13.3	12.9	16.9	13.9	14.4	8.0	14.0	14.3	12.1	12.5	17.3	12.2	12.7	12.3	16.0	12.8	7.9							
22 *A. spinalis*	12.0	11.5	11.5	11.3	15.4	16.6	15.4	14.4	14.4	14.0	13.4	13.9	14.4	16.0	14.4	13.7	15.6	15.8	13.2	14.0	13.0						
23 *A. timi*	14.2	13.9	13.9	13.8	13.2	16.4	13.5	15.9	13.4	13.5	14.8	12.0	14.4	15.8	13.9	11.9	13.6	15.5	11.8	13.5	14.2	14.4					
24 *A. tranganensis*	14.0	13.7	13.7	13.7	12.9	15.3	14.4	14.0	12.0	17.1	13.4	14.0	13.9	16.4	13.7	14.5	15.2	16.4	13.2	12.4	11.7	15.1	14.0				
25 *A. vanhoensis*	14.1	13.8	13.8	13.6	13.1	15.5	12.6	16.0	12.2	14.0	14.6	11.5	13.6	15.6	12.4	12.8	12.1	15.5	10.8	12.4	13.8	12.9	5.2	13.3			
26 *A. yangdatongi*	14.0	13.7	13.7	13.8	6.2	16.6	5.6	14.0	12.8	14.4	14.6	5.1	7.3	17.1	4.4	13.7	5.9	15.5	11.3	12.6	11.5	14.2	13.1	12.8	11.3		
27 *A. yunkaiensis*	6.5	6.5	6.5	6.2	13.2	14.9	12.7	15.1	13.5	12.4	12.5	12.0	12.9	15.8	12.7	12.4	13.7	15.7	11.6	14.0	13.7	12.2	14.2	13.9	13.6	12.0	
28 *A. zugorum*	10.9	10.9	10.9	10.8	13.7	15.3	12.9	14.7	12.5	13.4	4.3	11.8	13.4	15.0	13.0	13.2	12.8	15.3	10.8	13.2	13.7	13.4	13.5	12.0	11.9	12.2	11.2

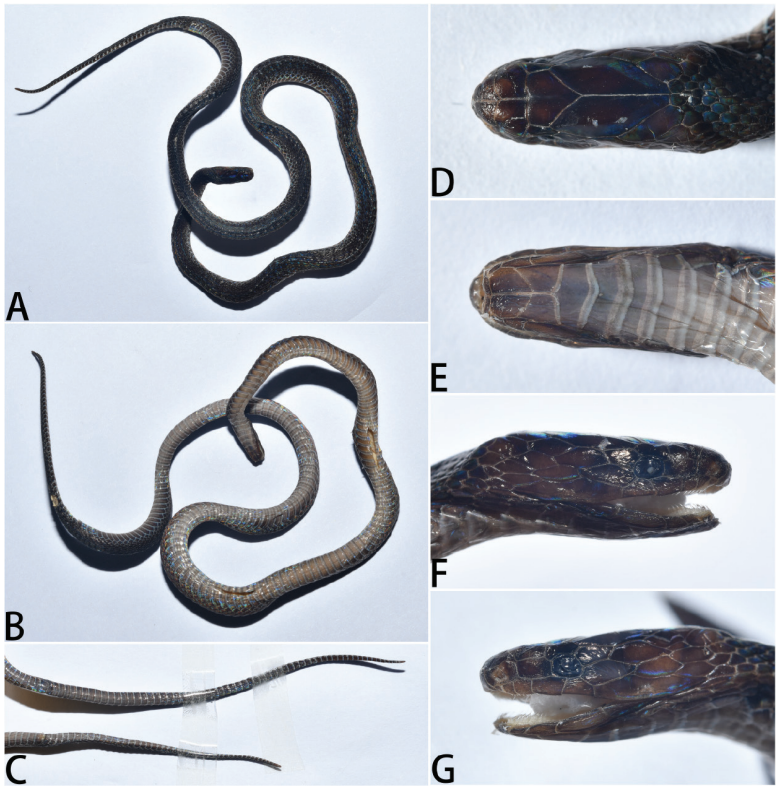

Figure 3. Adult female (HNNU2022001) of *Achalinus sheni*. **A.** Dorsolateral view; **B.** Ventral view; **C.** The tail of the male and female; **D.** Dorsal head view; **E.** Ventral head view; **F.** Light side of head view; **G.** Ventral head view. Photos by Le-Qiang Zhu.

Table 3. Main morphological characters of *Achalinus sheni*.

Voucher number	HNNU2022001	HNNU2022002	HNNU2022003	HNNU2022004
Sex	**Adult female**	**Adult male**	**Adult male**	**Adult male**
SVL	294.8	270.8	239.9	211.5
TaL	50.34	74.3	67.7	59.2
TL	345.1	345.1	307.6	270.7
TaL/TL	0.149	0.215	0.220	0.219
HL	9.16	8.76	7.86	7.65
HW	3.95	4.01	3.94	3.26
ED	1.10	0.97	1.02	0.76
SPL	6	6	6	6
SPL-Eye	4^{th}–5^{th}	4^{th}–5^{th}	4^{th}–5^{th}	4^{th}–5^{th}
IFL	5	5	5/6	5
Chin	2	2	2	2
IFL-1stChin	1^{st}–3^{rd}	1^{st}–3^{rd}	1^{st}–3^{rd}	1^{st}–3^{rd}
Loreal	1	1	1	1
LorH	0.88	0.87	0.93	0.73
LorL	1.38	1.14	1.31	1.00
LorH / LorL	0.63	0.76	0.71	0.73
LSBI vs LSBP	=1	=1	=1	=1
SPO	1	1	1	1
SPOL	1.49	1.15	1.15	1.33
TEM	2+2+3	2+2+3	2+2+3	2+2+3
aTEM-Eye	2	2	2	2
ATUL	1.63	1.41	1.71	1.46
SPOL / ATUL	0.91	0.82	0.67	0.91
DSR	23-23-23	23-23-23	23-23-23	23-23-23
VEN	173	156	156	158
SC	45	61	61	62
VEN+SC	218	217	217	220
CP	Entire	Entire	Entire	Entire

and female *A. sheni* have fewer sublabial scales, which is consistent with the common characteristics of this genus. However, the number of sublabial scales of *A. sheni* is even less than that of female *A. yunkaiensis* (Table 4).

Discussion

Only five male specimens of the *Achalinus sheni* have been described in previous studies. This study reports the first discovery of a female of the same species and provides a detailed description and photograph of the female. In terms of morphological characteristics, the male specimens collected in Xinshao County are close to the holotype specimen from Lianyuan City. Some differences were noted in the female specimen: Tail relatively short, TL/ToL 0.149, which was different from that of the male specimens (0.183–0.224); the tail of the female specimen is suddenly tapered, distinct from the elongated tail of the male specimens.

Xinshao County and Lianyuan City are geographically adjacent, and the climate is not obviously different. *Achalinus sheni* is fossorial and difficult to find. Only one female specimen was collected in this study. Whether the apparent differences between the sexes of this species are consistent, whether there are further differences, and the reasons for these differences have not yet been fully investigated and analyzed.

Table 4 Comparing the differences between *Achalinus sheni* and *Achalinus yunkaiensis* in different regions from a taxonomical perspective.

Species	*Achalinus sheni*			*Achalinus yunkaiensis*
	Lianyuan City & Nanyue District	Xinshao County		*Guangdong*
Sex	**Males(n=5)**	**Males (n=3)**	**Female**	**Female**
SVL	121.8–292.2	211.5–270.8	294.8	204–386.3
TaL	27.2–80.3	59.2–74.3	50.3	52–72.8
TL	149.0–371.3	270.7–345.1	345.1	256–448.1
TaL/TL	**0.183 ~ 0.224**	**0.215–0.220**	**0.149**	**0.156–0.204**
HL	10.07–10.95	7.65–8.76	9.16	-
HW	5.96–7.25	3.26–4.01	3.95	-
ED	1.09–1.11	0.76–1.02	1.10	-
SPL	6	6	6	6
SPL-Eye	4^{th}–5^{th}	4^{th}–5^{th}	4^{th}–5^{th}	4^{th}–5^{th}
IFL	**5 (rarely 6)**	**5 (rarely 6)**	**5**	**6**
Chin	2	2	2	2
IFL-1stChin	1^{st}–3^{rd}	1^{st}–3^{rd}	1^{st}–3^{rd}	1^{st}–3^{rd}/4^{th}
Loreal	1	1	1	1
LorH	0.69–0.93	0.73–0.93	0.88	0.74–1.2
LorL	1.29–1.71	1.00–1.31	1.38	1.51–2.2
LorH / LorL	0.53–0.57	0.71–0.76	0.63	0.49–0.55
LSBI vs LSBP	=1	=1	=1	=1
SPO	1	1	1	1
SPOL	1.21–1.59	1.15–1.33	1.49	1.26–1.60
TEM	2+2+3	2+2+3	2+2+3	2+2+3/4
aTEM-Eye	2	2	2	2
ATUL	1.20–1.48	1.41–1.71	1.63	1.93–2.90
SPOL / ATUL	0.99–1.16	0.67–0.91	0.91	0.55–0.65
DSR	23-23-23	23-23-23	23-23-23	23-23-23
VEN	**161–170**	**156–158**	**173**	**144–156**
SC	**55–61**	**61–62**	**45**	**51–55**
VEN+SC	220–225	217–220	218	195–205
CP	Entire	Entire	Entire	Entire

Author contributions

Yi-han Ma: Conceptualization, Writing - original draft. Jia-yu Liu: Data curation, Investigation. Hui Li: Data curation, Formal analysis. Lin Zhou: Investigation. Yu-hao Xu: Methodology. De-yong Peng: Investigation. Zhi-qiang Zhang: Funding acquisition, Methodology. Xiao-yang Mo: Writing - review and editing, Methodology.

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