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

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

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15

16 Running title: Expanded Description of *Achalinus sheni*

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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 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, out of which 21 are found 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; Zhang et al. 2023; Ma et al. 2023a; Yang et al. 2023; Ma et al. 2023b, Li et al, 2024).

Achalinus sheni was described based on five specimens were collected in the Hunan Province of China: four male specimens (ANU20230012—ANU20230015) were collected in Lianyuan City and one male specimen (CIB 119043) was collected in the Nanyue District (Ma et al. 2023b), molecular phylogeny inferred from the mitochondrial *COI* gene fragment revealed that this new species is most closely related to *Achalinus yunkaiensis*, but they can distinguished from *Achalinus 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) more ventral scales + subcaudals counts in males (220—225 vs. 200—212); (3) more ventral scales in males (161—170 vs. 150—162); (4) more subcaudals in males (55—61 vs. 49—56). But, there are no morphological comparisons between the female individuals of these two species.

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

Figure 1

Materials and methods

Sampling

Four odd-scaled snake specimens were collected from Xinshao County, Shaoyang City, Hunan Province of China (HNNU2022001 — HNNU2022004). The four specimens were collected in the field, 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 corner of the eye to the most posterior corner; loreal height (**LorH**): measured from the highest part to the lowest part of the loreal in lateral view; loreal length (**LorL**): from the most anterior loreal to the most posterior loreal in lateral view; length of the suture between internasals (**LSBI**); length of the suture between prefrontals (**LSBP**); length of supraocular (**SPOL**): horizontal distance between anterior and posterior tip of supraocular and length of upper anterior temporal (**ATUL**): horizontal distance between anterior and posterior tip of upper anterior temporal. We also directly compared the length of the sutures between internasals and prefrontals (**LSBI vs. LSPB**).

The scale 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**), subcaudal (**SC**), entire or divided of the cloacal plate (**Anal**), dorsal scale rows (**DSR**) (counted at one-head-length behind the head, at midbody, at one-head-length before

the 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 I (*COI*) 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 down loaded from GenBank (Table 1).

Table 1

The *COI* 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.

Results

Maximum likelihood trees showed consistent topology. The newly collected female specimen clustered with the specimens (including the holotype) of *Achalinus sheni* with 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 *Achalinus sheni* was only 0.7—0.8% (Table 2) .

Figure 2

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 and postoculars. Nostril at anterior part 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. 2 aTMP and 3 pTMP present. aTMPs elongated, upper one much smaller than the lower one; upper one in contact with eye, lower one also in contact with parietal scale. Supralabials 6, 4th—5th contact the eye, the last one much elongated. One mental scale present. Two chin shields, the anterior pairs longer than the posterior pairs. Infralabials 5, the first one contact with each other after the mental and before the 1st chin shields, 1st—3rd touch the 1st chin shields. Dorsal scales 23-23-23, strongly keeled, dorsum with no longitudinal vertebral stripe. Ventrals (VEN) 173. Subcaudals (SC) 45, uniserial, anal entire (Table 3) .

Table 3

Coloration of the female specimen in life. Scales possess a subtle iridescent quality. The dorsum's distinguishing characteristic is its 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 side, a grayish-white shade prevails, with the edges of the ventral scales gradually transitioning from gray-white to black. Ventral side of tail brownness.

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

Figure 3

Extended diagnosis. Upon examining adult male individuals of *Achalinus sheni* from Xinshao County, it was observed that they possess fewer ventral scales compared to specimens from the type locality. However, the number of subcaudal scales remains approximately the same between individuals from both locations. Upon inspection of adult female *Achalinus sheni* collected in Xinshao County, it was noted that they have extremely short tails, consequently exhibiting fewer subcaudal and more ventral compared to adult male individuals.

Comparison. Compared to male individuals of *Achalinus sheni* and *Achalinus yunkaiensis*, female individuals of both species exhibit similar differences in the number of ventral and sublabial. Specifically, female *Achalinus sheni* have more ventral scales and fewer sublabial scales. However, a distinction arises in the number of subcaudal, where female *Achalinus sheni* have fewer than female *Achalinus yunkaiensis*. Conversely, male *A. sheni* possess more subcaudal compared to male *Achalinus yunkaiensis* (Table 4) .

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 species and provides a detailed description and photograph of the female of the species. In terms of morphological characteristics, the male specimens collected in Xinshao County are close to the holotype specimen from Lianyuan City. Some differences were found in the observation of 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 difference is not obvious. *Achalinus sheni* live in soil burrows and are difficult to collect in the investigation process. Only one female specimen was collected in this study.

Whether some differences between the sexes of this species are stable, whether there are more differences, and the reasons for these differences still need to be continuously investigated and analyzed.

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

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

Table2. Uncorrected p-distances(%) among *Achalinus* species inferred from mitochndrial *COI* 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 <i>A. sheni</i>	0.7																										
3 <i>A. sheni</i>	0.7	0.0																									
4 <i>A. sheni</i>	0.8	0.2	0.2																								
5 <i>A. ater</i>	13.5	13.2	13.2	13.2																							
6 <i>A. dabieshanensis</i>	15.3	15.8	15.8	15.6	14.7																						
7 <i>A. damingensis</i>	14.4	14.0	14.0	14.0	8.0	15.8																					
8 <i>A. dehuaensis</i>	13.9	13.9	13.9	13.8	17.1	18.4	16.1																				
9 <i>A. emilyae</i>	14.2	13.7	13.7	13.5	11.9	17.7	13.4	15.7																			
10 <i>A. formosanus</i>	13.2	13.0	13.0	13.0	14.4	19.0	14.9	16.4	14.0																		
11 <i>A. huangjietangi</i>	13.6	13.6	13.6	13.3	15.0	8.9	16.3	16.5	14.5	15.6																	
12 <i>A. hunanensis</i>	12.5	12.1	12.1	12.3	7.3	16.9	6.1	14.9	13.2	13.7	16.8																
13 <i>A. juliani</i>	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 <i>A. meiguensis</i>	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 <i>A. nanshanensis</i>	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 <i>A. niger</i>	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 <i>A. ningshanensis</i>	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 <i>A. panzhihuaensis</i>	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 <i>A. pingbianensis</i>	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 <i>A. quangi</i>	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 <i>A. rufescens</i>	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 <i>A. spinalis</i>	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 <i>A. timi</i>	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 <i>A. tranganensis</i>	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 <i>A. vanhoensis</i>	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 <i>A. yangdatongi</i>	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 <i>A. yunkaiensis</i>	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 <i>A. zugorum</i>	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

Table 3. Main morphological characters of *Achalinus sheni*

VoucherNumber	HNUN2022001	HNUN2022002	HNUN2022003	HNUN2022004
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-1 st Chin	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
Anal	1	1	1	1

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

Species	<i>Achalinus sheni</i> .			<i>Achalinus yunkaiensis</i>
	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
Anal	1	1	1	1

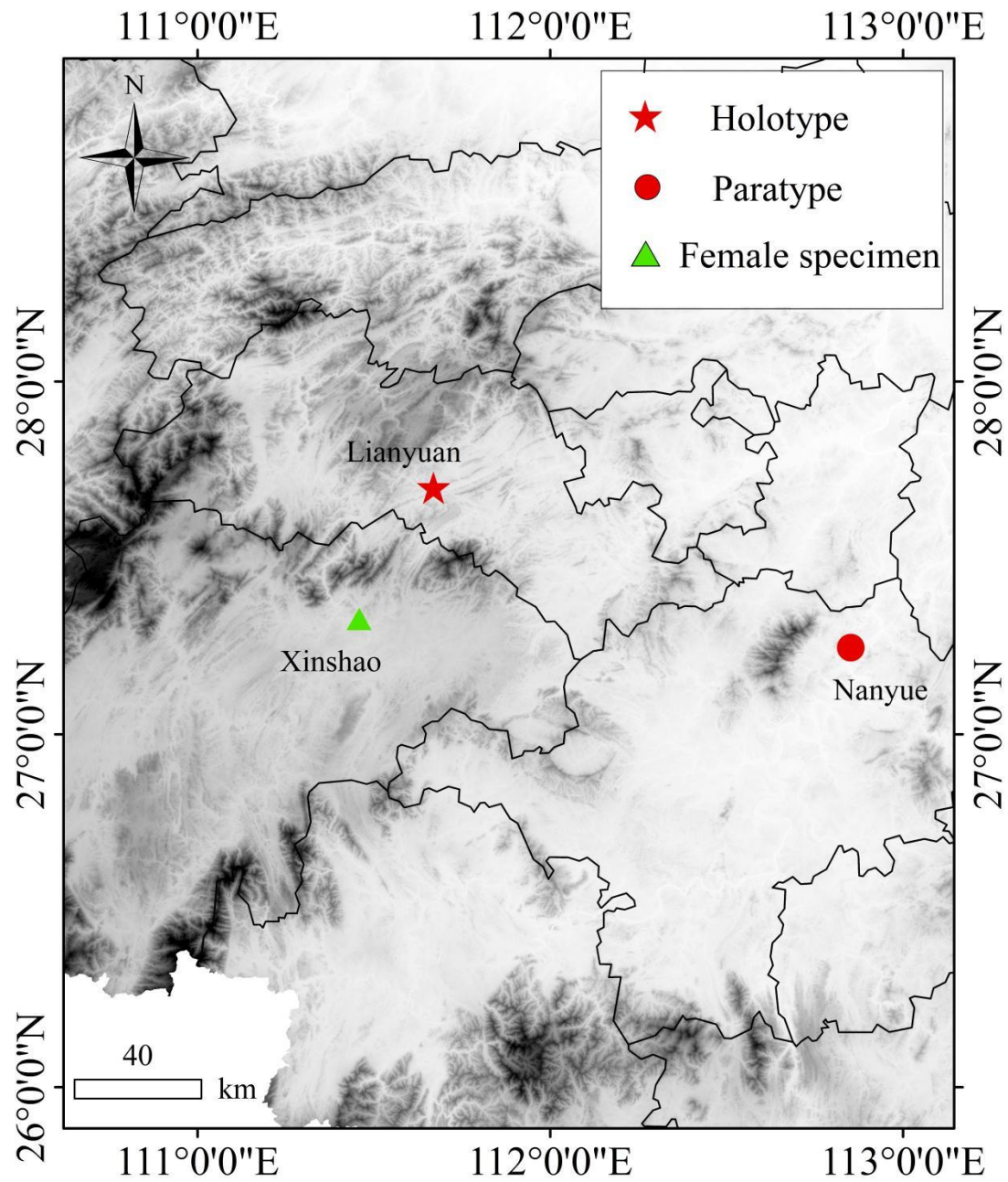


Figure 1. Sampling sites of *Achalinus sheni*: solid red star, the locality of the holotype; solid red circle, the locality of the paratype; solid green triangle, the locality of the newly collected female specimen

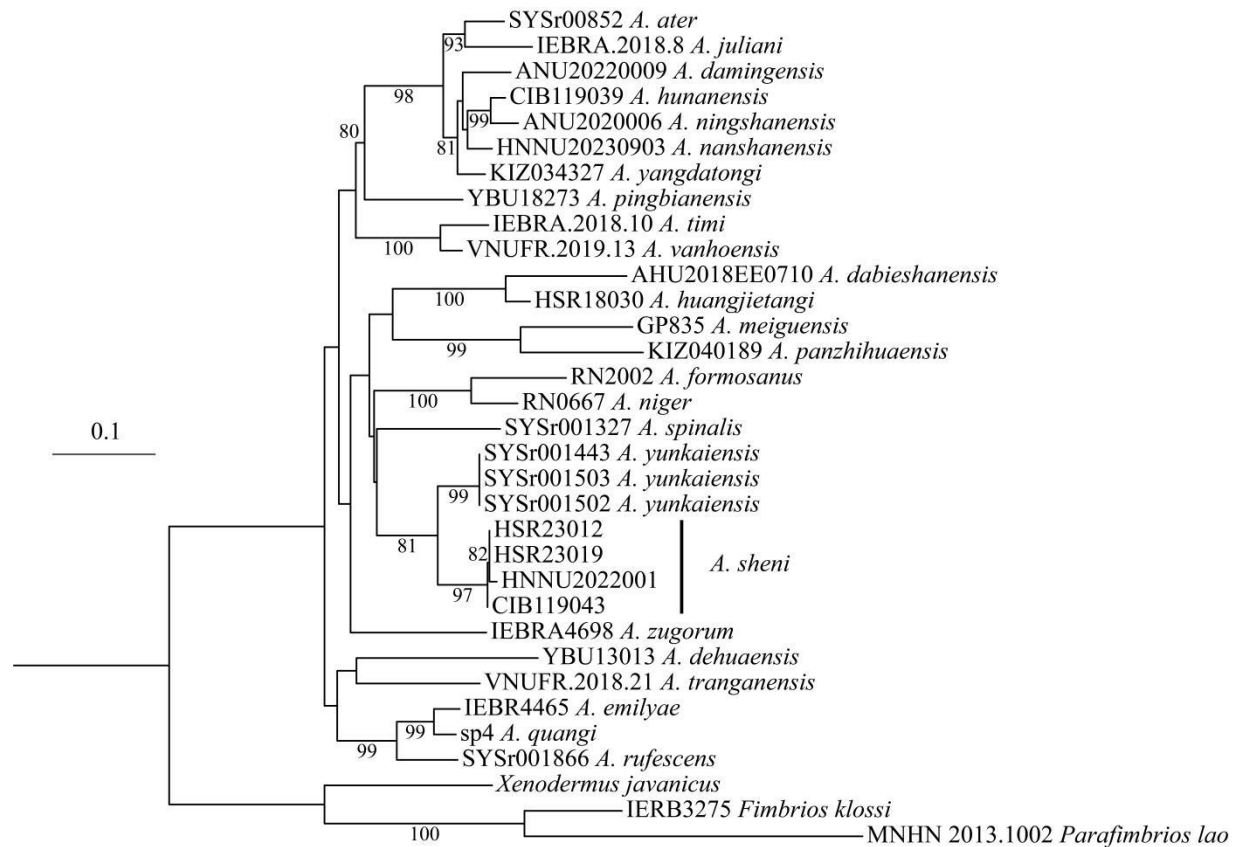


Figure 2. Phylogenetic tree of the genus *Achalinus* inferred from CO1 gene fragments (629 bp) using Maximum Likelihood. The numbers above the branches represent the supporting values



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.