

ORIGINAL ARTICLE

A new species of the genus *Crocidura* (Mammalia: Eulipotyphla: Soricidae) from Mount Huang, China

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Abstract In this study, a new species of white-toothed shrew, *Crocidura huangshanensis* Yang, BW Zhang & Li, **sp. nov.** was described based on five individuals collected from the Mt. Huang of China. Its morphological data and mtDNA sequences (Cyt *b*) were analyzed. Morphologically, *C. huangshanensis* Yang, BW Zhang & Li, **sp. nov.** is different from its congeners (*C. anhuiensis* and *C. attenuata*) by the body and skull smaller in size and the premolar and molar smoother, which are also different from other Southeast Asian *Crocidura*. The phylogenetic analysis shows that all individuals of *C. huangshanensis* Yang, BW Zhang & Li, **sp. nov.** merged into a single clade. In addition, the genetic distance between *C. huangshanensis* Yang, BW Zhang & Li, **sp. nov.** and other species of *Crocidura* is ranged from 9.8% to 15.3%, indicating a species-level divergence. The new species is currently distributed in the Wild Monkey Valley, located in Mt. Huang, China.

Key words White-toothed shrew, phylogenetics, morphology, Anhui Province.

1 Introduction

Crocidura is one of the largest genera of mammals with more than 199 species, which are widely distributed in the Palaearctic, Ethiopian and Oriental Regions (Hutterer, 2005; Mittermeier & Wilson, 2018; Zhang *et al.*, 2019). The genus was well worked recent years, especially in Southeast Asia. Dozens of new species from Asia were reported, such as *C. kegoensis* in Vietnam (Lunde *et al.*, 2004), *C. hikmiya* in Sri Lanka (Meegaskumbura *et al.*, 2007), *C. zaitsevi* and *C. sokolovi* in Vietnam (Jenkins *et al.*, 2007), *C. cranbrookii* in Myanmar (Jenkins *et al.*, 2009), *C. annamitensis* and *C. guy* in Vietnam (Jenkins *et al.*, 2009), *C. ninoyi* in Philippines (Esselstyn & Goodman, 2010), *C. phanluongi* in Vietnam and Cambodia (Jenkins *et al.*, 2010), *C. sapaensis* in Vietnam (Jenkins *et al.*, 2013), *C. gathornei* in India (Jenkins, 2013), *C. absconditus* in Indonesia (Esselstyn *et al.*, 2014), *C. umbra* in Indonesia (Demos *et al.*, 2017), and *C. anhuiensis* in China (Zhang *et al.*, 2019).

In China, the taxonomy of *Crocidura* was once confused (Allen, 1938; Ellerman & Morrison-Scott, 1951; Hutterer, 1993; Wolsan & Hutterer, 1998; Wang, 2003). Jiang (2017) reported that there were twelve species distributed throughout China, with *C. rapax* and *C. tanakae* as endemic species. Zhang *et al.* (2019) reported another new species from Anhui, China. Thus, 13 species were recorded in China now.

From May to July of 2017, five shrews were captured from a tea plantation of Fuxi Village, Huangshan City, Anhui

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Province, China by using the pitfall trap method (Goodman & Ingle, 1993). Referring to their genetic and morphological characters, these individuals were accordantly identified as a new species of the genus *Crocidura*.

2 Materials and Methods

2.1.1 Sampling

Five individuals of *Crocidura* were collected from Mt. Huang during May to July of 2017 (Fig. 1). Stuffed skin and skull specimens were prepared for further study. Muscle tissues were preserved in 100% ethanol for DNA extractions. The remaining carcasses were kept in 10% formalin and subsequently stored in 70% ethanol. All specimens and tissue samples were deposited in the Biological Museum of Anhui University, Anhui, China.

2.1.2 DNA extraction, Polymerase Chain Reactions (PCR) amplification, and sequencing

The DNA extraction used the standard proteinase K/phenol-chloroform protocol described by Sambrook (1989). The specific primers (L-14724-hk3/H-15915-hk3) for shrews were used to amplify the mtDNA Cytochrome *b* (Cyt *b*) gene (He *et al.*, 2010). PCR was performed for each reaction mixture (30 μ L volume), containing 1 μ L genomic DNA (concentration 50 ng/ μ L), 2.5 μ L of 10 \times EasyTaq Buffer, 1 μ L of MgSO₄, 2 μ L of dNTPs, 1 U EasyTaq polymerase, 0.3 mM of forward and reverse primers, with the remaining portion filled with double distilled water. The PCR program was as follows: initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30s, annealing at 55°C for 50s, and extension at 72°C for 90s, and finally, extension at 72°C for 10 min. The PCR products were detected by 1% TAE agarose gel electrophoresis and sent to the company for sequencing if there were clearly visible target bands.

2.2 Phylogenetic analysis

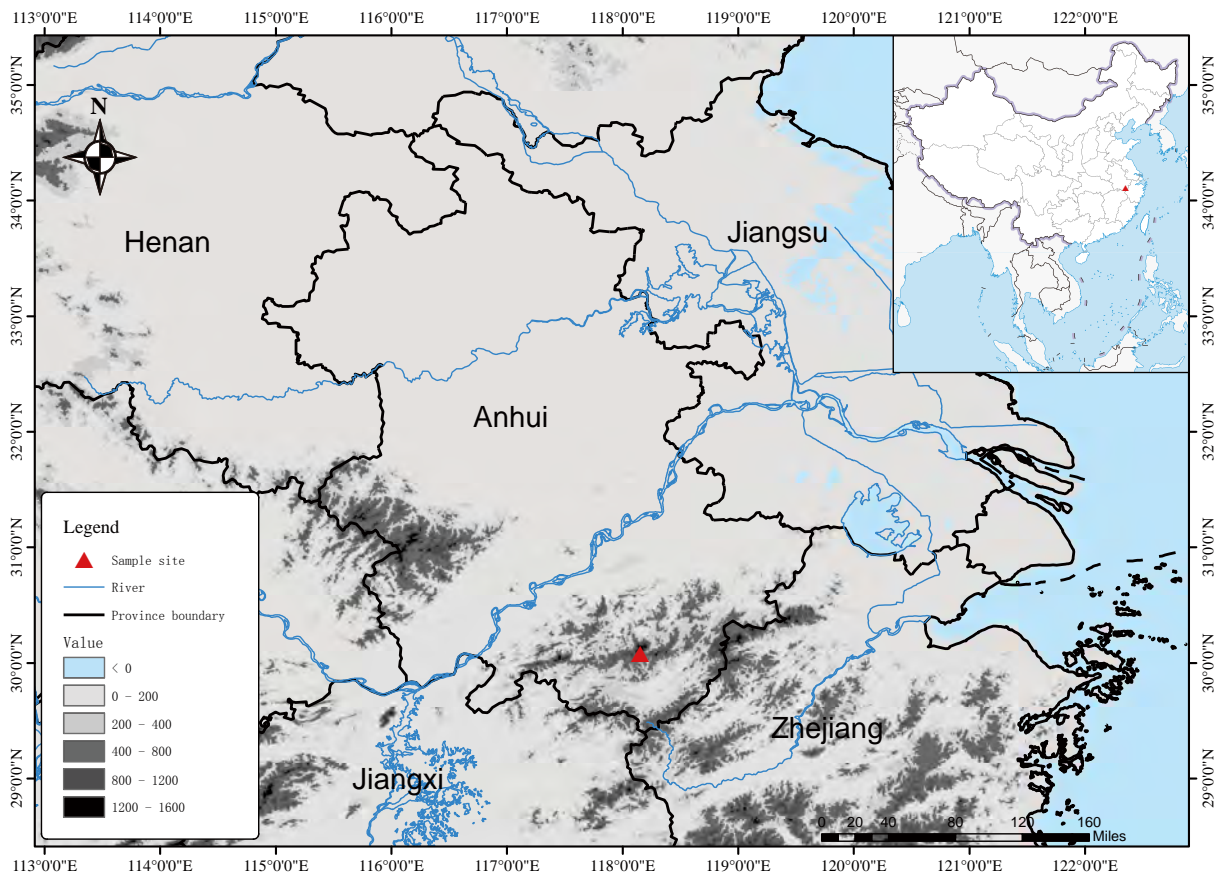


Figure 1. Sampling site (Red triangle) of *C. huangshanensis* Yang, BW Zhang & Li, **sp. nov.** in Mt. Huang.

For the phylogenetic analyses, 40 sequences, belonging to 19 species of *Crocidura* from Southeast Asia and two species of *Suncus* (Soricidae) were downloaded from NCBI (Table 1). Five sequences obtained in this study were deposited in GenBank (GenBank Accession Numbers: MN364684–MN364688). SeqMan in Lasergene (Burland, 2000) was performed to splice the sequences, and Clustal W software (Larkin *et al.*, 2007) was used for sequence alignment. The Bayesian method was implemented to estimate evolutionary relationships among taxa, by using MrBayes 3.1.2 software and posterior probability (PP) strands for the trustworthiness of each branch. The starting tree was set to be a random tree, and the Markov Chain Monte Carlo (MCMC) program was set to four chains (three hot chains and one cold chain) running for 20,000,000 generations. Each 100 generations were sampled and 200,000 phylogenetic trees were eventually obtained, which were repeated to ensure the convergence of MCMC. When the average standard deviation of separation frequency was less than 0.01, the first 20,000 trees were abandoned as burnin samples. After abandoning the aging samples, the Bayesian posterior probability was calculated for the consensus tree which was constructed according to the remaining samples. In addition, the uncorrected *p*-distance of pairwise sequences/species was calculated using MEGA 6.0 (Tamura *et al.*, 2013).

2.3 Morphological analysis

Three species were measured for morphological analysis, including *C. anhuiensis* (*n*=6), *C. attenuata* (*n*=16) and *C. huangshanensis* Yang, BW Zhang & Li, **sp. nov.** (*n*=5). All the measurements were measured according to the method described by Meegaskumbura *et al.* (2007) and Jenkins *et al.* (2009). Nine external measurements of head and body, tail, hind foot and ear were performed by a ruler to the closest 0.01 mm, and 24 cranial measurements were measured using digital calipers, accurate to the nearest 0.01 mm. The weight (WT) of each specimen was weighed on an electronic scale, which was accurate to the nearest 0.01 g.

The external measurements were composed of length of head (HL), length of head and body (HBL), length of tail (TL), length of forefoot (FFL), length of hind foot (HFL), length of lower arm (LAL), length of tibia (TBL), and ear height (EH). The cranial measurements included: greatest length of skull (GL), basal length (BL), basilar length (BSL), condyloincisive length (CIL), palatal length (PL), palatilar length (PAL), post-palatal length (PPL), length of rostrum (LR), breadth of braincase (BB), least interorbital breadth (LIOB), breadth of palate between the buccal margins of second molars (PW1), breadth of palate between the lingual margins of last molars (PW2), breadth of rostrum at narrowest point (BR1), breadth of rostrum at broadest point (BR2), breadth of bony palate at the premolar (BPM), height of braincase (HB), Incisor-3rd unicuspid length (I-Un3), upper toothrow length (UTRL), mandible length (ML), length of dentary including incisors (LDI), length of dentary teeth excluding incisors (LDT1), length of dentary teeth including incisors (LDT2), depth of dentary (DD), and mandible height (MH).

SPSS (Version 21) was used for statistical analysis. Principal Component Analysis (PCA) was used to analyze the external and cranial data to determine the morphological differences between the new species and the two other species.

3 Results

3.1 Phylogenetic analysis

The Bayesian phylogenetic tree based on Cyt *b* gene shows that the five individuals from Mt. Huang are merged into a monophyletic clade (Fig. 2), which was temporarily named as *Crocidura* sp. For the genetic distance, the maximum genetic distance among the five individuals of *Crocidura* sp. is 0.009 (Table 2), while the genetic distances between *Crocidura* sp. and other species are from 0.098 (*C. lasiura*) to 0.153 (*C. sibirica*). The result indicates that the five individuals from Mt. Huang may be a different species rather than other known species of *Crocidura* from China.

3.2 Morphological analysis

The external (Table 3) and cranial (Table 4) measurements reveal that all external and skull data of *Crocidura* sp. are smaller/lighter than those in *C. anhuiensis* and *C. attenuata*. PCA of external measurements (Table 5) indicates that *Crocidura* sp. was separated from *C. anhuiensis* and *C. attenuata*. The first principal component (PC1) accounted for 47.5% of the variation, which was positively correlated with all variables. The second factor axis (PC2) accounted for 14.0% of the variation and was dominated by HL, HBL and FFL. As shown in the plot of PC1 and PC2, *Crocidura* sp. occurs in the most negative regions of the PC1 and PC2 axes (Fig. 3), indicating *Crocidura* sp. has the smallest external data among the three species.

Table 1. Samples used for molecular phylogenetic analysis in this study.

Species	Number of GenBank	Field number	Collection area	Reference
<i>C. attenuata</i>	AB175082	AMNH101492	Vietnam	Ohdachi <i>et al.</i> , 2004
	GU358515	AMCC101492	China	Esselstyn <i>et al.</i> , 2009
<i>C. fuliginosa</i>	FJ813925	IZEA3753	Malaysia	Esselstyn <i>et al.</i> , 2009
	GU981271	19701	China	He <i>et al.</i> , 2010
<i>C. indochinensis</i>	HM587024	ZISP:97670	Vietnam	Bannikova <i>et al.</i> , 2011
	HM587023	ZISP:97669	Vietnam	Bannikova <i>et al.</i> , 2011
<i>C. lasiura</i>	AB077071	HS1252	Vietnam	Ohdachi <i>et al.</i> , 2004
	HM586997	NED171/2004	Vietnam	Bannikova <i>et al.</i> , 2011
<i>C. leucodon</i>	EF417545	2003.217	Unknown	Dubey <i>et al.</i> , unpublished
	DQ994793	PMSSlovenia1	Unknown	Dubey <i>et al.</i> , unpublished
<i>C. rapax</i>	AB062686	SO-2000/12/23-1	China	Ohdachi <i>et al.</i> , unpublished
	AB175086	SO-03/3/15-3	China	Ohdachi <i>et al.</i> , unpublished
<i>C. shantungensis</i>	AB077081	SO2Kmisc62	Russia	Ohdachi <i>et al.</i> , 2004
	AB077082	SO2Kmisc61	Russia	Ohdachi <i>et al.</i> , 2004
<i>C. sibirica</i>	AB077086	SO2000/7/27-1	China	Ohdachi <i>et al.</i> , 2004
	AB077085	SO2Kmisc59	China	Ohdachi <i>et al.</i> , 2004
<i>C. tanakae</i>	AB175081	SO-03/3/15-5	China	Ohdachi <i>et al.</i> , unpublished
	HM587032	ZISP:99028	Vietnam	Bannikova <i>et al.</i> , 2011
<i>C. wuchihensis</i>	AB175085	AMNH101508	Unknown	Ohdachi <i>et al.</i> , unpublished
	GU358518	AMCC101508	Vietnam	Esselstyn & Oliveros, 2010
<i>C. anhuiensis</i>	MK546383	AhuHST1501	China	Zhang <i>et al.</i> , 2019
	MK546384	AhuHST1502	China	Zhang <i>et al.</i> , 2019
<i>C. ninoyi</i>	NC027244	FMNH145685	Philippine	Giarla <i>et al.</i> , 2015
	KR537878	FMNH145685	Philippine	Giarla <i>et al.</i> , 2015
<i>C. phanluongi</i>	HM587020	ZISP:97092	Vietnam	Bannikova <i>et al.</i> , 2011
	JX181939	ZIN:100306	Vietnam	Abramov <i>et al.</i> , 2012
<i>C. sokolovi</i>	HM586999	ZISP:96394	Vietnam	Bannikova <i>et al.</i> , 2011
	HM586998	ZISP:96393	Vietnam	Bannikova <i>et al.</i> , 2011
<i>C. zaitsevi</i>	HM587022	ZISP:97627	Vietnam	Bannikova <i>et al.</i> , 2011
	HM587021	ZISP:97628	Vietnam	Bannikova <i>et al.</i> , 2011
<i>C. absconditus</i>	KF801083	FMNH218756	Indonesia	Esselstyn <i>et al.</i> , 2014
	Kf801084	FMNH218747	Indonesia	Esselstyn <i>et al.</i> , 2014
<i>C. hikmiya</i>	EU122223	WHT6853	Sri Lanka	Meegaskumbura <i>et al.</i> , 2007
	EU122221	WHT6845	Sri Lanka	Meegaskumbura <i>et al.</i> , 2007
<i>C. kurodai</i>	GU358535	NTU985	China	Esselstyn & Oliveros, 2010
	GU358534	NTU981	China	Esselstyn & Oliveros, 2010
<i>C. horsfieldii</i>	EU122213	WHT6869	Sri Lanka	Meegaskumbura <i>et al.</i> , 2007
	AB175078	HA6214	Thailand	Ohdachi <i>et al.</i> , 2004
<i>C. huangshanensis</i> Yang, BW Zhang & Li, sp. nov.	MN364684	AhuHS04	China	This study
	MN364685	AhuHS05	China	This study
	MN364686	AhuHS06	China	This study
	MN364687	AhuHS07	China	This study
	MN364688	AhuHS10	China	This study
<i>Suncus murinus</i>	LC126596	SDO140819-3	Tanzania	Ohdachi <i>et al.</i> , 2016
<i>S. etruscus</i>	JN556043	Unknown	India	Meegaskumbura <i>et al.</i> , unpublished

Moreover, the PCA analysis of the skull shows that PC1 accounts for 74.3% of the variation, and is positively correlated with all variables, while PC2 accounts for 7.04% of the variation and is dominated by PW2, BR1 and BPM (Table 6). *Crocidura* sp. can also be separated from *C. anhuiensis* and *C. attenuata* in the PCA analysis by distributing in the negative region of PC1 but in the positive region of PC2 (Fig. 3), which indicate that the skull of the unnamed species is relatively small, and the PW2, BR1 and BPM are relatively large.

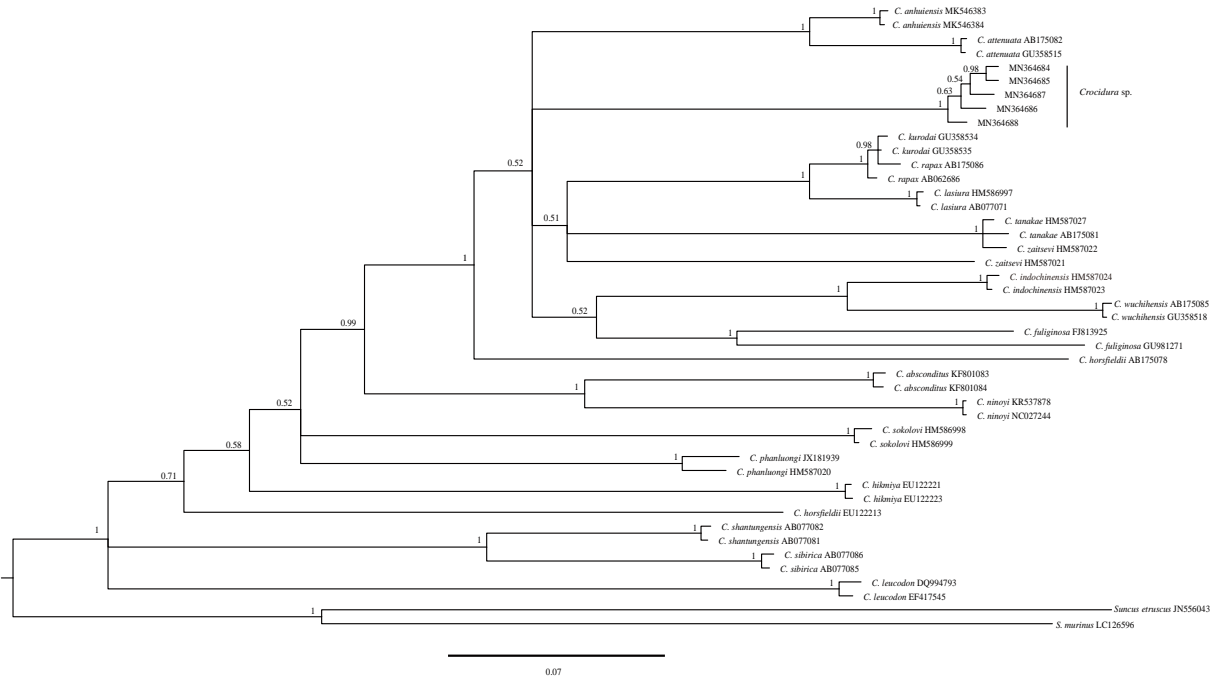


Figure 2. The Bayesian consensus tree of 20 Southeast Asia *Crocidura* species, resulted from analysis of mitochondrial DNA Cyt *b* gene. Numbers near the nodes are Bayesian posterior probabilities.

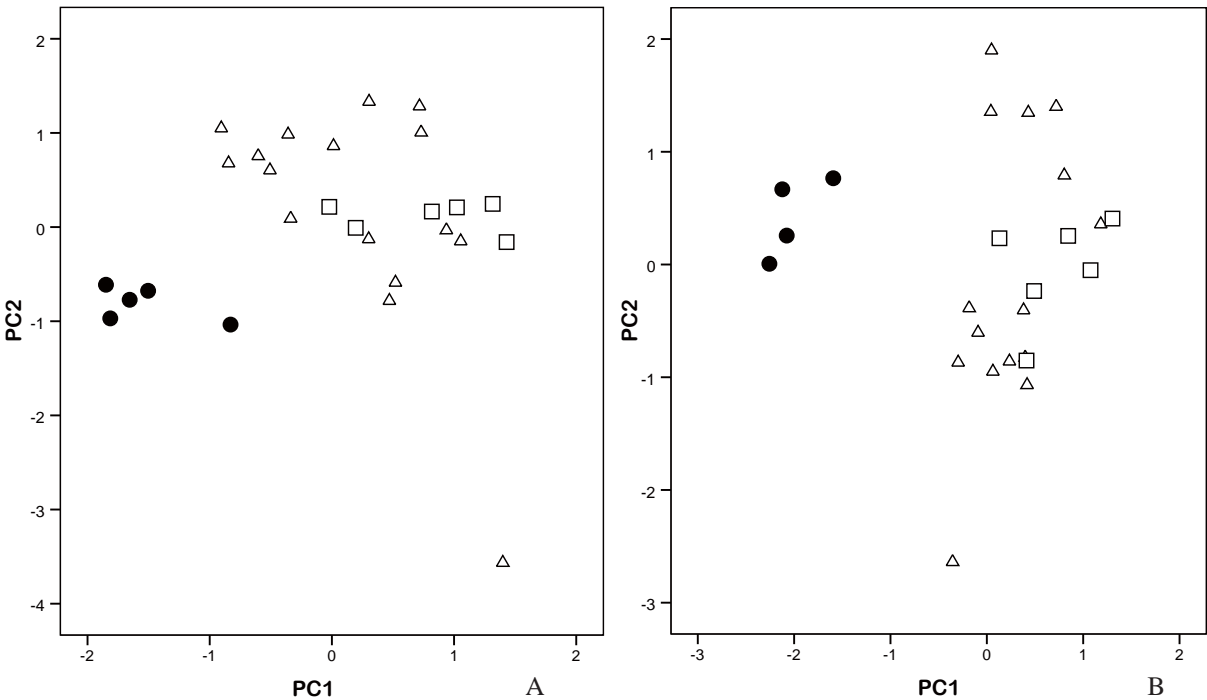


Figure 3. The PCA analyses among species of *C. anhuiensis* (hollow square), *C. attenuata* (hollow triangle) and *C. huangshanensis* Yang, BW Zhang & Li, **sp. nov.** (solid circle). A. Plot of the first 2 principal components from a PCA of 9 external body measurements. B. Plot of the first 2 principal components from a PCA of 24 cranial measurements.

Table 2. The genetic distances (uncorrected *p*-distance) among sequences and species used in this study.

Species/individual	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
AhuHS04																								
AhuHS05	0.004																							
AhuHS06	0.004	0.006																						
AhuHS07	0.007	0.009	0.005																					
AhuHS10	0.004	0.006	0.000	0.005																				
<i>C. attenuata</i>	0.114	0.112	0.111	0.113	0.111																			
<i>C. fuliginosa</i>	0.116	0.116	0.115	0.116	0.115	0.109																		
<i>C. indochinensis</i>	0.118	0.118	0.118	0.118	0.118	0.113	0.106																	
<i>C. lasiura</i>	0.098	0.100	0.099	0.102	0.099	0.104	0.118	0.114																
<i>C. leucodon</i>	0.136	0.138	0.138	0.140	0.138	0.144	0.130	0.151	0.138															
<i>C. rapax</i>	0.102	0.106	0.103	0.106	0.103	0.110	0.114	0.112	0.037	0.132														
<i>C. shantungensis</i>	0.142	0.140	0.142	0.140	0.142	0.144	0.131	0.141	0.144	0.145	0.133													
<i>C. sibirica</i>	0.153	0.151	0.153	0.149	0.153	0.139	0.140	0.139	0.147	0.152	0.141	0.083												
<i>C. tanakae</i>	0.116	0.116	0.114	0.114	0.114	0.133	0.115	0.119	0.112	0.141	0.111	0.151	0.134											
<i>C. wuchihensis</i>	0.129	0.127	0.127	0.127	0.127	0.123	0.114	0.079	0.125	0.147	0.121	0.138	0.137	0.125										
<i>C. anhuiensis</i>	0.106	0.106	0.107	0.109	0.107	0.049	0.110	0.112	0.102	0.136	0.104	0.149	0.140	0.120	0.118									
<i>C. ninoyi</i>	0.134	0.134	0.134	0.138	0.134	0.140	0.128	0.132	0.134	0.143	0.143	0.141	0.143	0.130	0.136	0.137								
<i>C. phanluongi</i>	0.113	0.115	0.113	0.115	0.113	0.132	0.126	0.132	0.120	0.149	0.120	0.131	0.137	0.117	0.132	0.130	0.133							
<i>C. sokolovi</i>	0.121	0.121	0.123	0.122	0.123	0.140	0.133	0.144	0.134	0.141	0.126	0.139	0.143	0.134	0.147	0.138	0.130	0.127						
<i>C. zaitsevi</i>	0.119	0.119	0.117	0.117	0.117	0.134	0.116	0.116	0.109	0.142	0.108	0.149	0.132	0.011	0.125	0.121	0.131	0.117	0.134					
<i>C. hikmiya</i>	0.135	0.139	0.135	0.140	0.135	0.142	0.138	0.138	0.132	0.145	0.126	0.139	0.151	0.140	0.139	0.136	0.147	0.128	0.150	0.138				
<i>C. kurodai</i>	0.101	0.105	0.102	0.105	0.102	0.111	0.115	0.113	0.036	0.131	0.003	0.132	0.142	0.111	0.122	0.105	0.142	0.117	0.123	0.108	0.125			
<i>C. horsfieldii</i>	0.128	0.130	0.126	0.128	0.126	0.136	0.121	0.133	0.127	0.147	0.128	0.147	0.158	0.129	0.121	0.135	0.131	0.110	0.144	0.128	0.141	0.127		
<i>Suncus murinus</i>	0.165	0.165	0.163	0.163	0.163	0.186	0.164	0.162	0.187	0.179	0.183	0.166	0.184	0.184	0.159	0.182	0.179	0.153	0.173	0.184	0.168	0.180	0.172	
<i>S. etruscus</i>	0.162	0.166	0.166	0.162	0.166	0.176	0.169	0.164	0.158	0.164	0.163	0.175	0.174	0.167	0.172	0.167	0.162	0.157	0.147	0.170	0.174	0.160	0.161	0.154

Table 3. Weight (g) and external measurements (mm) of *C. anhuiensis*, *C. attenuata* and *C. huangshanensis* Yang, BW Zhang & Li, sp. nov.

	<i>C. anhuiensis</i> (n=6)		<i>C. attenuata</i> (n=16)		<i>C. huangshanensis</i> Yang, BW Zhang & Li, sp. nov. (n=5)	
	Range	Mean(SD)	Range	Mean(SD)	Range	Mean (SD)
WT	10.52–13.51	11.60(1.2)	5.78–14.04	8.80(1.9)	4.35–7.77	5.92(1.1)
HL	26.91–28.73	27.83(0.7)	17.10–30.16	27.91(3.0)	22.70–25.09	24.07(0.9)
HBL	60.88–78.10	69.51(6.5)	63.90–83.52	72.27(6.5)	50.45–61.97	56.55(3.9)
TL	48.34–59.24	53.09(4.0)	40.63–56.24	46.40(4.1)	39.64–45.03	41.77(2.0)
FFL	9.20–9.87	9.56(0.3)	6.93–9.95	9.01(0.8)	6.99–8.33	7.74(0.5)
HFL	13.52–14.83	14.31(0.4)	10.92–14.91	13.06(1.1)	11.84–13.82	12.59(0.7)
LAL	10.71–12.09	11.41(0.5)	9.97–12.33	10.85(0.9)	8.33–9.63	9.07(0.4)
TBL	17.14–18.41	17.70(0.4)	14.80–18.41	17.11(1.0)	14.55–15.76	15.30(0.4)
EH	7.74–9.24	8.77(0.5)	7.26–9.96	8.32(0.6)	7.86–8.70	8.12(0.3)

Table 4. Skull measurements (mm) of *C. anhuiensis*, *C. attenuata* and *C. huangshanensis* Yang, BW Zhang & Li, sp. nov.

	<i>C. anhuiensis</i> (n=6)		<i>C. attenuata</i> (n=16)		<i>C. huangshanensis</i> Yang, BW Zhang & Li, sp. nov. (n=5)	
	Range	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)
GL	19.70–21.02	20.27(0.5)	18.72–21.43	19.44(0.7)	16.40–17.07	16.65 (0.3)
BL	16.97–18.21	17.81(0.4)	16.31–18.27	17.29(0.6)	14.53–15.46	14.86 (0.4)
BSL	16.49–17.48	17.02(0.4)	14.97–17.43	16.57(0.7)	13.62–14.75	13.98 (0.5)
CIL	20.81–22.38	21.66(0.7)	19.22–22.61	20.63(0.7)	16.88–17.90	17.30 (0.4)
PL	8.27–9.29	8.68(0.3)	7.76–8.82	8.35(0.3)	6.66–7.25	6.96 (0.2)
PAL	7.42–8.41	7.89(0.4)	6.42–8.18	7.75(0.3)	5.87–6.58	6.18 (0.3)
PPL	8.66–9.86	9.23(0.4)	8.08–9.89	9.08(0.4)	7.66–8.22	7.91 (0.2)
LR	8.12–9.76	8.81(0.6)	7.27–8.78	7.93(0.4)	6.46–6.73	6.58 (0.1)
BB	9.12–9.85	9.61(0.2)	8.67–9.83	9.18(0.3)	8.15–8.47	8.27 (0.1)
LIQB	4.66–4.95	4.76(0.1)	4.20–5.06	4.65(0.2)	4.07–4.18	4.11 (0.0)
PW1	6.32–7.14	6.69(0.3)	5.66–6.89	6.41(0.3)	5.48–5.64	5.56 (0.1)
PW2	2.41–2.99	2.64(0.2)	1.99–2.94	2.57(0.3)	2.22–2.51	2.39 (0.1)
BR1	2.30–2.48	2.38(0.1)	1.66–2.52	2.08(0.2)	1.78–2.09	1.93 (0.1)
BR2	6.36–7.18	6.80(0.3)	6.32–6.94	6.64(0.2)	5.52–5.68	5.60 (0.1)
BPM	1.06–1.60	1.30(0.2)	1.22–1.86	1.54(0.2)	1.24–1.48	1.34 (0.1)
HB	4.97–5.51	5.24(0.2)	4.86–5.59	5.18(0.2)	4.47–4.62	4.54 (0.1)
I-UN3	4.45–4.81	4.61(0.1)	3.81–5.33	4.37(0.4)	3.05–3.67	3.42 (0.3)
UTRL	9.12–10.08	9.62(0.3)	8.79–9.93	9.34(0.2)	7.35–8.08	7.68 (0.3)
ML	10.21–11.58	10.81(0.5)	9.48–12.04	10.53(0.6)	8.26–8.70	8.48 (0.2)
LDI	13.09–14.80	14.34(0.6)	13.01–14.25	13.64(0.4)	10.48–11.42	10.93 (0.3)
LDT1	5.06–6.56	6.11(0.6)	5.20–7.07	6.08(0.5)	4.71–5.19	4.86 (0.2)
LDT2	7.79–8.90	8.41(0.4)	7.52–8.84	8.32(0.4)	6.35–6.79	6.63 (0.2)
DD	5.60–6.38	6.03(0.3)	5.44–6.52	6.02(0.3)	4.47–4.79	4.57 (0.1)
MH	4.76–5.50	5.15(0.3)	4.81–5.51	5.09(0.2)	4.03–4.29	4.11 (0.1)

4 Systematics

Crocidura huangshanensis Yang, BW Zhang & Li, sp. nov.

Diagnosis. The new species has the size of the head and body easily distinguished from other known *Crocidura* species in the Southeast Asia region (Table 7). It is smaller than *C. hikmiya*, *C. sokolovi*, *C. cranbrookii*, *C. ninoyi*, *C. gathornei*, *C.*

absconditus, *C. anhuiensis*, *C. tanakae*, *C. attenuata* and *C. fuliginosa*, but larger than *C. kegoensis*, *C. zaitsevi*, *C. annamitensis* and *C. guy*. Additionally, the new species has the body size similar to *C. sapaensis* (HBL \pm SD 57.4 \pm 3.91, TL \pm SD 41.6 \pm 2.48 in mean); and skull size similar to *C. phanluongi* (UTRL \pm SD 7.7 \pm 0.3, BH \pm SD 4.4 \pm 0.19 in mean), *C. varax* (UTRL \pm SD 7.77 \pm 0.39, BB \pm SD 8.40 \pm 0.48, BH \pm SD 4.75 \pm 0.22 in mean) and *C. umbra* (UTRL \pm SD 7.63 \pm 0.12, BB \pm SD 8.27 \pm 0.19 in mean).

Table 5. Factor loadings of the 2 principal components (PC) axes based on 9 external variables from the *C. anhuiensis*, *C. attenuata* and *C. huangshanensis* Yang, BW Zhang & Li, sp. nov.

Variables	Component 1	Component 2
WT	0.045	0.002
HL	0.193	0.947
HBL	0.806	0.231
TL	0.814	0.092
FFL	0.598	0.127
HFL	0.61	-0.26
LAL	0.912	-0.007
TBL	0.869	0.01
EH	0.775	-0.469
Eigenvalues	4.272	1.262
Total variance explained (%)	47.466	14.027

Table 6. Factor loadings of the 2 principal components (PC) axes based on 24 skull variables from *C. anhuiensis*, *C. attenuata* and *C. huangshanensis* Yang, BW Zhang & Li, sp. nov.

Variables	Component 1	Component 2
GL	0.954	-0.014
BL	0.962	0.152
BSL	0.944	0.147
CIL	0.974	-0.074
PL	0.945	0.02
PAL	0.96	0.058
PPL	0.89	0.173
LR	0.874	0.158
BB	0.929	-0.118
LIQB	0.86	0.098
PW1	0.881	-0.161
PW2	0.494	0.75
BR1	0.51	0.524
BR2	0.94	-0.121
BPM	0.333	0.599
HB	0.866	-0.121
I-UN3	0.695	-0.106
UTRL	0.966	-0.114
ML	0.947	0.024
LDI	0.948	-0.138
LDT1	0.732	-0.304
LDT2	0.825	-0.377
DD	0.943	-0.04
MH	0.918	-0.169
Eigenvalues	17.834	1.691
Total variance explained (%)	74.310	7.047

Description. A middle-sized *Crociodura*. Weight approximately 4.35–7.77 g; length of head and body approximately 50.45–61.97 mm; length of tail ranging 39.64–45.03 mm ($n=5$).

Head small, with a long elephant-like nose, long mustache back to the base of the ear, small eyes, round ears, and an obvious undercoat on ear shells; pelage slightly metallic, gray-brown, slightly darker in dorsal; limbs slender, composed of five toes on each hind foot; small, fleshy protuberances and palmate rings present on foot, with moderate color; dorsal side of palm and sole pink, semi-naked, and ankles with hairs sparse and brown; tail with length about 76% of head and similar color as pelage, nearly naked, with sparse and long bristle hairs (Fig. 4).

Skull long and flat, without zygomatic arch, and with distinct ridges. First incisor of maxillary strong and curved; second single cusps with similar size as third, with a large and triangular premolar and a front cusp. Molar cusp sharp. First incisor teeth of mandibular extended forward, end of mandibular slightly bended upward; tip of premolar and molar teeth sharper than other tips. Suture line between occipital and parietal bones extended downward, at prominent position front of herringbone (Fig. 5).

Measurements of holotype (AhuHS10). WT 5.77; HL 23.49; HBL 61.97; TL 41.28; FFL 7.73; HFL 11.84; LAL 9.63; TBL 15.05; EH 8.03; GL 16.52; BL 14.82; BSL 13.9; CIL 17.24; PL 6.99; PAL 6.15; PPL 7.66; LR 6.55; BB 8.18; LIOB 4.08; PW1 5.48; PW2 2.37; BR1 1.91; BR2 5.52; BPM 1.25; HB 4.53; I-UN3 3.63; UTRL 7.83; ML 8.67; LDI 10.98; LDT1 4.71; LDT2 6.61; DD 4.47; MH 4.04.

Holotype. AhuHS10, female, (Fig. 2), coll. Liu Yang, 15 June 2017, Fuxi, Mt. Huang, Anhui Province, China (30.0828°N, 118.1506°E; elev. 616 m), dissected. The skin and skull tissues were preserved in dry, and the muscle tissues were kept in ethanol.

Paratypes. AhuHS04 (male, 50.45 mm HBL), AhuHS05 (female, 59.29 mm HBL), AhuHS06 (female, 55.75 mm HBL), AhuHS07 (female, 55.27 mm HBL), May to July, 2017, other data same as holotype.

Etymology. The specific name, *huangshanensis*, refers to its locality, Mt. Huang, Anhui Province, China.

Common names. We suggest “Huangshan white-toothed shrew” as the English common name of the species, and “黄山小麝鼯” as the Chinese common name.

Distribution. Only found in the type locality.

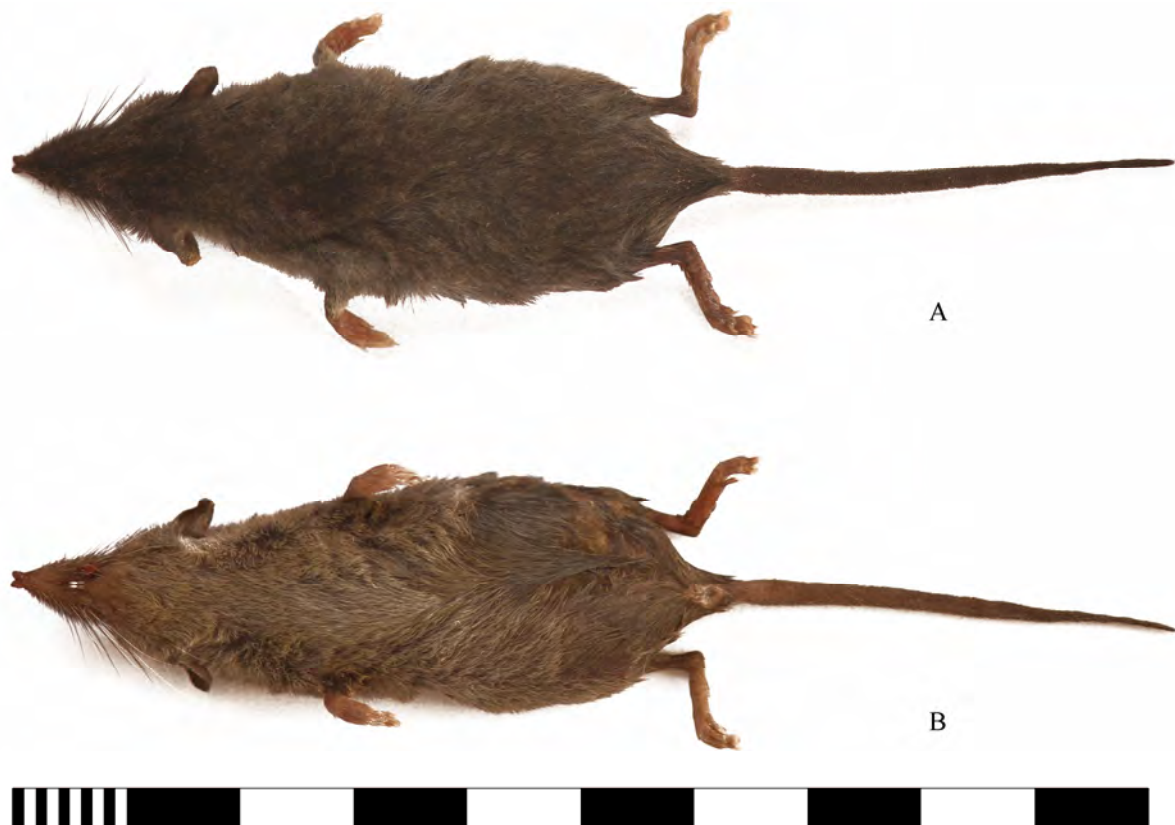


Figure 4. *Crociodura huangshanensis* Yang, BW Zhang & Li, **sp. nov.** (AhuHS10). A. Dorsal view. B. Ventral view.

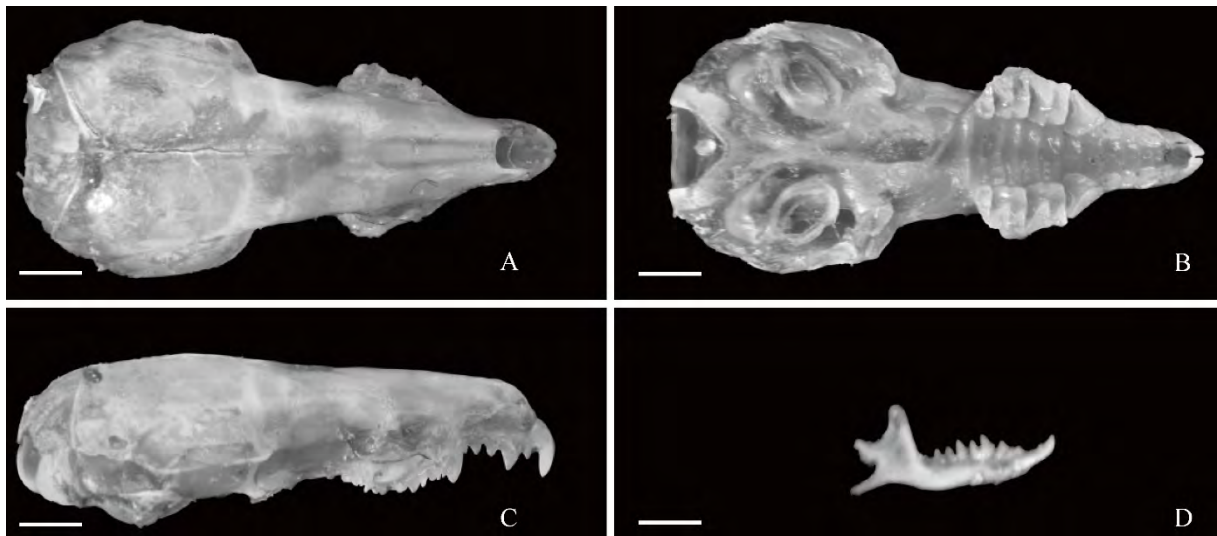


Figure 5. Skull of *Crocidura huangshanensis* Yang, BW Zhang & Li, **sp. nov.** (AhuHS10). A–C. Crania, dorsal, ventral and lateral view. D. Mandible. Scale bars = 2 mm.

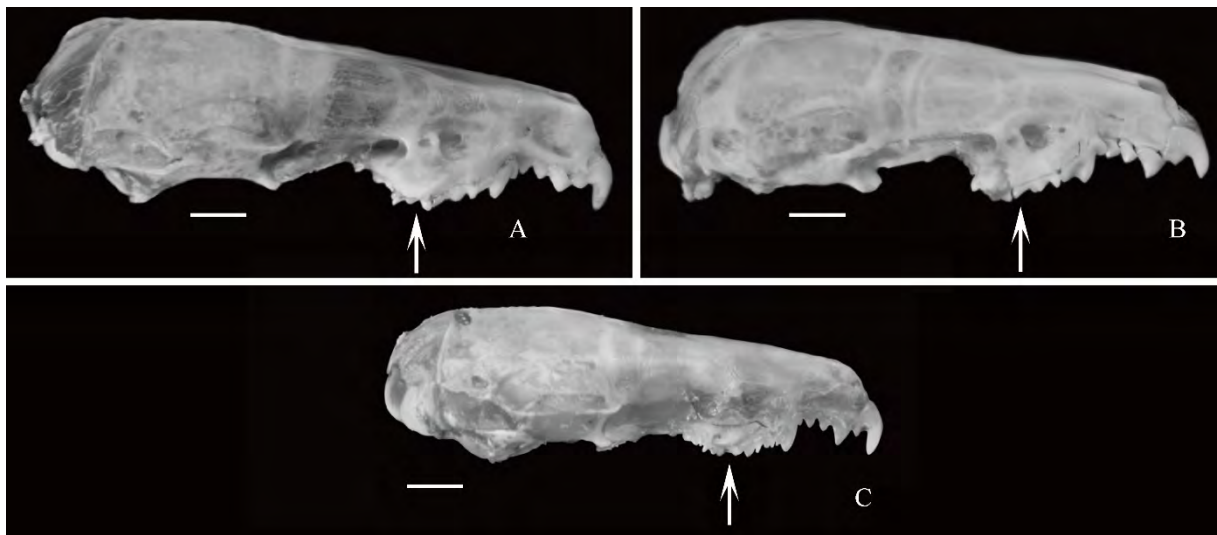


Figure 6. Crania of *Crocidura* spp., lateral view. A. *C. anhuiensis*, AhuHST1702. B. *C. attenuata*, AhuHS12. C. *C. huangshanensis* Yang, BW Zhang & Li, **sp. nov.**, AhuHS10. Scale bars = 2 mm.

5 Discussion

Before the work, 13 species of *Crocidura* has been reported in China (Jiang *et al.*, 2017; Zhang *et al.*, 2019), namely *C. attenuata*, *C. fuliginosa*, *C. gmelini*, *C. indochinensis*, *C. lasiura*, *C. leucodon*, *C. rapax*, *C. shantungensis*, *C. sibirica*, *C. tanakae*, *C. vorax*, *C. wuchihensis* and *C. anhuiensis*. In this study, the phylogenetic tree, which was constructed based on 43 sequences from 20 species of *Crocidura* (including the new species, but not including *C. vorax* of China), shows that *C. huangshanensis* Yang, BW Zhang & Li, **sp. nov.** can be distinctly distinguished from other species of *Crocidura* by its genetic distance. For *C. varax*, the new species can be distinguished from it by the smaller HBL and TL.

In Southeast Asia, dozens of species were newly reported recent years. Among them, 12 sequences from six species (*C. absconditus*, *C. ninoyi*, *C. phanluongi*, *C. zaitsevi*, *C. sokolovi* and *C. hikmiya*) were get from NCBI (Table 1). According to the results of the phylogenetic analysis, five of them were not closely related to *C. huangshanensis* Yang, BW Zhang & Li, **sp. nov.**, except *C. zaitsevi*. In the Bayesian tree, *C. zaitsevi* was close to *C. tanakae*, even it has smaller head and body size than the latter.

Table 7. Means (\pm SD) and ranges of external and cranial measurement (mm) of Southeast Asia *Crocidura*.

	References	HBL	TL	HFL	UTRL	BB	BH	PPL
<i>C. huangshanensis</i>	This study	56.55 \pm 3.9 (<i>n</i> =5)	41.77 \pm 2.0 (<i>n</i> =5)	12.59 \pm 0.7 (<i>n</i> =5)	7.68 \pm 0.3 (<i>n</i> =4)	8.27 \pm 0.1 (<i>n</i> =4)	4.54 \pm 0.1 (<i>n</i> =4)	7.91 \pm 0.2 (<i>n</i> =4)
Yang, BW Zhang & Li, sp. nov.								
<i>C. kegoensis</i>	Lunde <i>et al.</i> , 2004	48 (<i>n</i> =1)	27 (<i>n</i> =1)	5 (<i>n</i> =1)	6.5 (<i>n</i> =1)	7.4 (<i>n</i> =1)	3.8 (<i>n</i> =1)	/
<i>C. hikmiya</i>	Meegaskumbura <i>et al.</i> , 2007	70.1 \pm 3.9 (<i>n</i> =7)	74.9 \pm 2.2 (<i>n</i> =7)	17.4 \pm 1.6 (<i>n</i> =7)	/	9.0 \pm 0.1 (<i>n</i> =7)	4.9 \pm 0.1 (<i>n</i> =7)	9.5 \pm 0.2 (<i>n</i> =7)
<i>C. zaitsevi</i>	Jenkins <i>et al.</i> , 2007	53 \pm 3 (<i>n</i> =12)	37 \pm 2 (<i>n</i> =12)	10 \pm 1 (<i>n</i> =12)	6.51 \pm 0.16 (<i>n</i> =12)	7.51 \pm 0.18 (<i>n</i> =6)	3.75 \pm 0.12 (<i>n</i> =11)	/
<i>C. sokolovi</i>	Jenkins <i>et al.</i> , 2007	72.67 \pm 3.77 (<i>n</i> =3)	66 \pm 1.41 (<i>n</i> =3)	14 \pm 0 (<i>n</i> =3)	8.75 \pm 0.37 (<i>n</i> =3)	9.52 \pm 0.14 (<i>n</i> =3)	5.2 \pm 0.09 (<i>n</i> =3)	/
<i>C. cranbrookii</i>	Jenkins <i>et al.</i> , 2009	76.13 \pm 7.77 (<i>n</i> =8)	74.13 \pm 7.16 (<i>n</i> =8)	14.94 \pm 0.64 (<i>n</i> =9)	9.29 \pm 0.29 (<i>n</i> =17)	9.31 \pm 0.2 (<i>n</i> =14)	5.14 \pm 0.13 (<i>n</i> =14)	/
<i>C. annamitensis</i>	Jenkins <i>et al.</i> , 2009	54 (<i>n</i> =3)	32 (<i>n</i> =3)	9.33 (<i>n</i> =3)	6.48 (<i>n</i> =3)	7.2 (<i>n</i> =1)	3.7 (<i>n</i> =1)	/
<i>C. guy</i>	Jenkins <i>et al.</i> , 2009	49.5 \pm 2.27 (<i>n</i> =4)	35.85 \pm 1.40 (<i>n</i> =4)	9.5 \pm 0.41 (<i>n</i> =4)	6.49 \pm 0.14 (<i>n</i> =4)	7.23 \pm 0.17 (<i>n</i> =4)	3.58 \pm 0.1 (<i>n</i> =4)	/
<i>C. ninoyi</i>	Esselstyn & Goodman, 2010	90.6 \pm 4.8 (<i>n</i> =5)	72.3 \pm 3.7 (<i>n</i> =6)	15.3 \pm 1 (<i>n</i> =65)	10.7 \pm 0.34 (<i>n</i> =3)	9.9 \pm 0.1 (<i>n</i> =3)	/	9.88 \pm 0.2 (<i>n</i> =3)
<i>C. phanluongi</i>	Jenkins <i>et al.</i> , 2010	59.6 \pm 4.33 (<i>n</i> =13)	44.4 \pm 2.32 (<i>n</i> =13)	11.7 \pm 0.69 (<i>n</i> =13)	7.7 \pm 0.3 (<i>n</i> =12)	7.9 \pm 0.24 (<i>n</i> =12)	4.4 \pm 0.19 (<i>n</i> =12)	/
<i>C. sapaensis</i>	Jenkins <i>et al.</i> , 2013	57.4 \pm 3.91 (<i>n</i> =20)	41.6 \pm 2.48 (<i>n</i> =20)	/	7.0 \pm 0.17 (<i>n</i> =21)	7.7 \pm 0.19 (<i>n</i> =19)	4.1 \pm 0.17 (<i>n</i> =19)	/
<i>C. gathornei</i>	Jenkins, 2013	82.8 \pm 4.5 (<i>n</i> =5)	67 \pm 5.1 (<i>n</i> =5)	/	10.8 \pm 0.1 (<i>n</i> =4)	9.7 \pm 0.2 (<i>n</i> =3)	/	/
<i>C. absconditus</i>	Esselstyn <i>et al.</i> , 2014	/	88.3 \pm 3.5 (<i>n</i> =4)	16 (<i>n</i> =1)	8.67 \pm 0.15 (<i>n</i> =4)	8.78 \pm 0.23 (<i>n</i> =4)	/	9.13 \pm 0.29 (<i>n</i> =4)
<i>C. umbra</i>	Demos <i>et al.</i> , 2017	67.9 \pm 2.02 (<i>n</i> =15)	52.6 \pm 3.72 (<i>n</i> =15)	12.1 \pm 0.66 (<i>n</i> =15)	7.63 \pm 0.12 (<i>n</i> =15)	8.27 \pm 0.19 (<i>n</i> =15)	/	8.17 \pm 0.16 (<i>n</i> =15)
<i>C. anhuiensis</i>	Zhang, Zhang & Li, 2019	69.50 \pm 7.08 (<i>n</i> =6)	53.10 \pm 4.33 (<i>n</i> =6)	14.31 \pm 0.46 (<i>n</i> =6)	9.62 \pm 0.3 (<i>n</i> =6)	9.61 \pm 0.26 (<i>n</i> =6)	5.24 \pm 0.20 (<i>n</i> =6)	9.23 \pm 0.4 (<i>n</i> =6)
<i>C. attenuata</i>	Motokawa <i>et al.</i> , 2001	66.97 \pm 8.56 (<i>n</i> =14)	45.62 \pm 7.16 (<i>n</i> =14)	12.25 \pm 0.98 (<i>n</i> =14)	9.34 \pm 0.2 (<i>n</i> =14)	8.96 \pm 0.48 (<i>n</i> =14)	5.06 \pm 0.32 (<i>n</i> =14)	9.08 \pm 0.4 (<i>n</i> =14)
<i>C. shantungensis</i>	Jiang & Hoffman, 2001	58.7 \pm 3.57 (<i>n</i> =16)	36.0 \pm 4.36 (<i>n</i> =16)	11.2 \pm 0.79 (<i>n</i> =16)	6.91 \pm 0.14 (<i>n</i> =16)	7.49 \pm 0.17 (<i>n</i> =16)	3.94 \pm 0.20 (<i>n</i> =16)	7.57 \pm 0.33 (<i>n</i> =16)
<i>C. horsfieldii</i>	Jiang & Hoffman, 2001	61.1 \pm 6.18 (<i>n</i> =13)	40.8 \pm 5.97 (<i>n</i> =13)	11.0 \pm 1.04 (<i>n</i> =13)	7.07 \pm 0.33 (<i>n</i> =13)	8.17 \pm 0.25 (<i>n</i> =13)	4.39 \pm 0.21 (<i>n</i> =13)	8.04 \pm 0.18 (<i>n</i> =13)
<i>C. fuliginosa</i>	Jiang & Hoffman, 2001	86.3 \pm 6.7 (<i>n</i> =73)	74.1 \pm 5.4 (<i>n</i> =73)	16.5 \pm 1.1 (<i>n</i> =73)	10.06 \pm 0.35 (<i>n</i> =73)	10.33 \pm 0.35 (<i>n</i> =73)	5.47 \pm 0.22 (<i>n</i> =73)	10.44 \pm 0.36 (<i>n</i> =73)
<i>C. varax</i> J	iang & Hoffman, 2001	67.3 \pm 9.77 (<i>n</i> =12)	46.7 \pm 3.34 (<i>n</i> =12)	12.3 \pm 0.78 (<i>n</i> =12)	7.77 \pm 0.39 (<i>n</i> =12)	8.40 \pm 0.48 (<i>n</i> =12)	4.75 \pm 0.22 (<i>n</i> =12)	8.88 \pm 0.39 (<i>n</i> =12)
<i>C. rapax</i>	Jiang & Hoffman, 2001	64.1 \pm 4.51 (<i>n</i> =10)	42.2 \pm 2.80 (<i>n</i> =10)	11.9 \pm 0.60 (<i>n</i> =10)	7.57 \pm 0.30 (<i>n</i> =10)	8.32 \pm 0.35 (<i>n</i> =10)	4.51 \pm 0.19 (<i>n</i> =10)	8.55 \pm 0.34 (<i>n</i> =10)
<i>C. indochinensis</i>	Lunde <i>et al.</i> , 2004	66 \pm 0 (<i>n</i> =2)	48.5 \pm 2.1 (<i>n</i> =2)	13 \pm 0 (<i>n</i> =2)	7.5 \pm 0.1 (<i>n</i> =2)	8.1 \pm 0.1 (<i>n</i> =2)	4.4 \pm 0.1 (<i>n</i> =2)	/
<i>C. wuchihensis</i>	Jenkins <i>et al.</i> , 2007	58.9 \pm 3.3 (<i>n</i> =9)	38.6 \pm 2.3 (<i>n</i> =9)	11.3 \pm 0.8 (<i>n</i> =9)	6.9 \pm 0.3 (<i>n</i> =10)	7.6 \pm 0.1 (<i>n</i> =10)	4.0 \pm 0.1 (<i>n</i> =10)	/
<i>C. tanakae</i>	Motokawa <i>et al.</i> , 2001	73.36 \pm 6.67 (<i>n</i> =11)	52.50 \pm 4.22 (<i>n</i> =11)	13.05 \pm 0.54 (<i>n</i> =11)	9.33 \pm 0.19 (<i>n</i> =11)	/	5.01 \pm 0.15 (<i>n</i> =11)	/

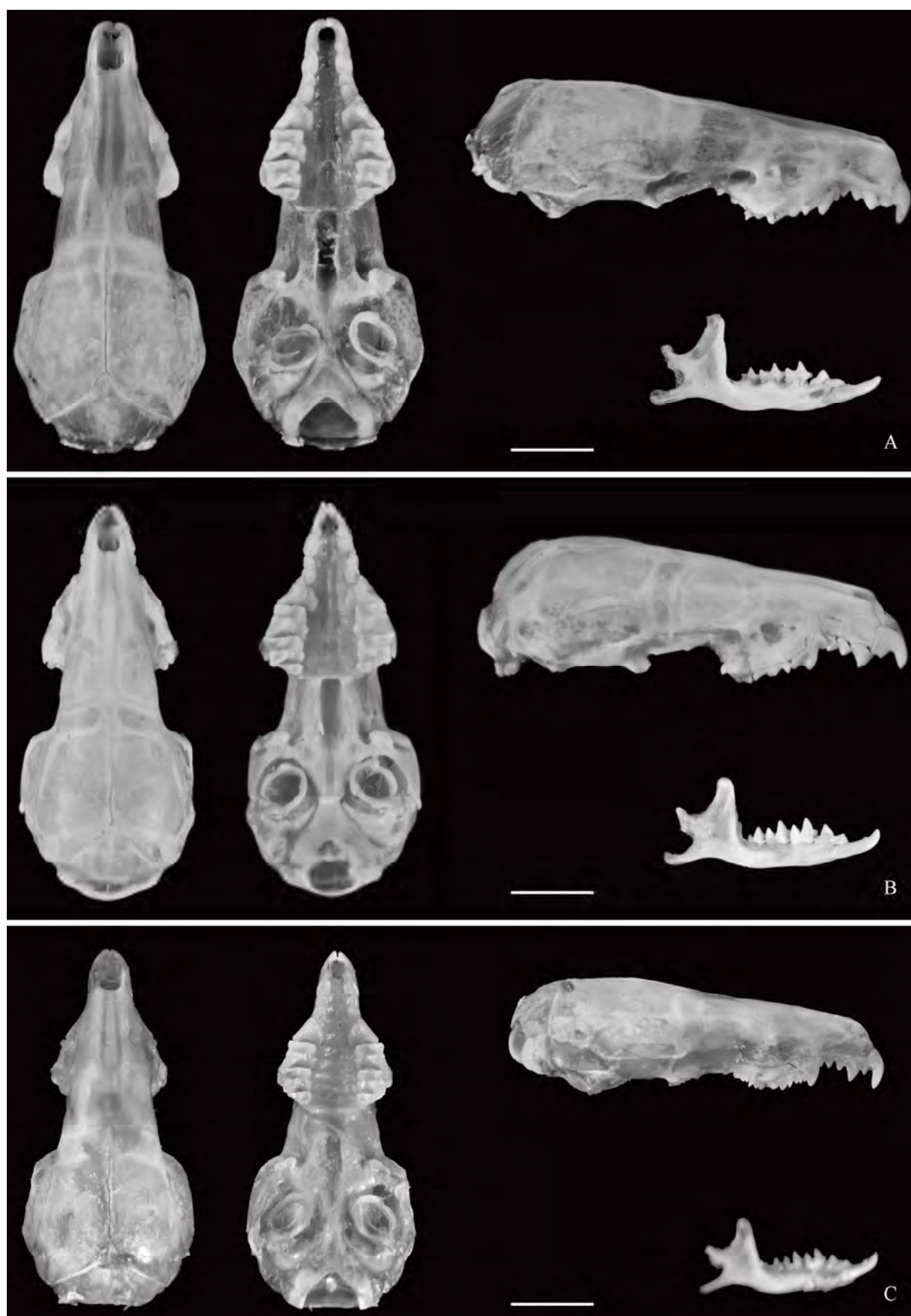


Figure 7. Comparison of crania (dorsal, ventral and lateral view) and mandibles of *Crocidura* spp. A. *C. anhuiensis*, AhuHST1702. B. *C. attenuata*, AhuHS12. C. *C. huangshanensis* Yang, BW Zhang & Li, **sp. nov.**, AhuHS10. Scale bars=4 mm.

However, as the five sequences of the new species are merged into a single clade, and has enough genetic distance with other species, so it is a quite different species rather than known species in the phylogenetic tree.

According to the phylogenetic analysis, a comparative morphological analysis was done to distinguish the new species and its two sympatries, *C. anhuiensis* and *C. attenuata*. The new species has the shape and color similar to *C. anhuiensis* and *C. attenuata*, but different from the latter two by its small body and skull size. An analysis of the two sets of PCA (Fig. 3) was done to separate these three species, the new species is completely away from other two. In the skull, the premolar and molar of *C. huangshanensis* Yang, BW Zhang & Li, **sp. nov.** are smoother than those of *C. anhuiensis* and *C. attenuata* (Fig. 6). At the same time, their mandibles also have visible differences in tooth roots (Fig. 7).

In addition, the genetic distances between *C. huangshanensis* Yang, BW Zhang & Li, **sp. nov.** and *C. anhuiensis* are reached to 10.6–10.9%, and reached to 11.1–11.4% with *C. attenuata* (Table 2). These are the interspecific level according to Bradley & Baker (2001).

In Anhui Province, China, four species of *Crocidura* were recorded, *C. shantungensis*, *C. attenuata*, *C. lasiura* and *C. anhuiensis* (Wang, 1990; Ding, 2012; Zhang *et al.*, 2019). Among them, *C. shantungensis* was only found in the plains, *C. attenuata* was found in the mountain forest area, while *C. lasiura* and *C. anhuiensis* were only reported in Dangtu County and Mt. Huang, respectively (Wang, 1990; Wang, 2003; Ding, 2012; Zhang *et al.*, 2019). The new species has the closest genetic distance with *C. lasiura* (9.8%), but is significantly smaller than the latter (HBL 1010 mm, TL 600 mm, HFL 160 mm in mean; Ding, 2012).

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