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Tscherskia ningshaanensis: A neglected species based on phylogenetic and taxonomic analysis of *Tscherskia* and *Cansumys* (Cricetidae, Rodentia)

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Abstract

The greater long-tailed hamster is primarily distributed in North Korea, Siberia (Russia), and central and northern China, while the Gansu hamster is restricted to southern Gansu Province, China. The genera *Tscherskia* and *Cansumys* have each been considered monotypic. The taxonomic status of these two genera has long been debated, and the specific status of *T. ningshaanensis* has also been contentious. Researchers have variously treated *T. ningshaanensis* as a subspecies of either *T. triton* or *Can. canus*. In this study, we estimated the phylogeny, divergence times, species delimitation, and biogeographical history of *T. ningshaanensis* by using one mitochondrial (*CYT B*) and three nuclear loci (GHR, IRBP, and RAG1) and compared the external and skull morphology variations between *T. ningshaanensis* and *T. triton*. The results showed that: 1) The genus *Cansumys* is a distinct genus in Cricetinae; 2) The notion that the genus *Tscherskia* is a monotypic genus is unsupported, *T. ningshaanensis* and *T. triton* were identified within this genus; and 3) The formation of *T. ningshaanensis* may have been driven by uplift of the Qinling Mountains. We conclude that *T. ningshaanensis* is a valid species within the subfamily Cricetinae.

Key Words

Classification, morphology, Mt. Qinling, phylogeny, Rodentia, *Tscherskia*

Introduction

The greater long-tailed hamster (*Tscherskia triton* de Winton, 1899), family Cricetidae, Order Rodentia, is mainly distributed in North Korea, Siberia (Russia), and central and northern China, including the Provinces of Hebei, Shanxi, Shaanxi, Henan, Anhui, Jiangsu, Shandong, Heilongjiang, Jilin, Liaoning, as well as Inner Mongolia and Beijing (Smith and Xie 2008; Wilson et al. 2017; Wei et al. 2021). Currently, a single species with five subspecies (*T. t. triton*, *T. t. incanus*, *T. t. collinus*, *T. t. fuscipes*, and *T. t. nestor*) has been identified in the genus *Tscherskia* (Smith and Xie 2008; Wilson et al. 2017).

The species-level classification within *Tscherskia* has been controversial to date. In 1899, *Cricetus triton* was first described by de Winton (1899) from Shantung (= Shandong) Province, China. Based on morphological differences, geographical distribution, behavioral, and ecological characteristics, Thomas (1907, 1908) proposed that the classification of *C. triton* be revised from the genus *Cricetus* to *Cricetulus*, with subsequent studies recognizing one species and one subspecies within the genus *Cricetulus*. In 1907, *Cricetulus nestor* was described from Korea (Thomas 1907); and in 1908, *Cricetulus triton incanus* was described from Ko-lan-chow (= Kelan), Shan-si (= Shanxi), and Yen-an-fu (= Yan'an), Shen-si

(= Shaanxi) Provinces (Thomas 1908). Ognev (1914) described *Tscherskia albipes* from eastern Siberia, noting its larger body size and longer tail.

In 1925, two new subspecies were recognized: *Cricetulus triton collinus* from the base of Tai-pei-shan (= Taibaishan), Tsing-ling (= Qinling) Mountains, Shen-si (= Shaanxi) Province; and *Cricetulus triton fuscipes* from Peking, Chili (= Beijing, Hebei) Province (Allen 1925). Kishida (1929) described a new genus, *Asiocricetus*, from Korea, including *Asiocricetus bampensis* and *Asiocricetus yamashinai*. According to early taxonomic studies (e.g., Argyropulo 1933; Ellerman 1941), *Tscherskia* and *Asiocricetus* were regarded as subgenera of the genus *Cricetulus*. Furthermore, *T. albitpes*, *A. bampensis*, and *A. yamashinai* were treated as synonyms of *C. nestor* (Argyropulo 1933). In 1934, *Cricetulus triton meihsienensis* was described by Ho (1934) from Mei-hsi (= Meixian), Shen-si (= Shaanxi) Province, and was subsequently treated as a synonym of *C. t. collinus* (Ho 1934; Wilson et al. 2017). In 1985, *Cricetulus triton ningshaanensis* was described by Song (1985) based on its smaller body size, tail length, and color of tail from Ningshan, southern Shaanxi Province. Neumann et al. (2006) moved *C. triton* into *T. triton* and treated *Tscherskia* as a monotypic genus based on molecular phylogenetic studies, a view accepted by many researchers (Smith and Xie 2008; Wilson et al. 2017; Wei et al. 2021).

The classification status of Gansu hamster (*Cansumys canus* Allen, 1928) and *C. t. ningshaanensis* has been debated for a long time in China due to the significant morphological (particularly tail length and color) and distributional differences (Wang and Zheng 1973; Chen and Min 1982; Wang and Xu 1992; Gu et al. 2005). *Can. canus* was first described from Choni (= Zhuoni), southern Kansu (= Gansu) Province, China (Allen 1928). Later, Ellerman (1941) treated *Can. canus* as a subspecies of *Cricetulus triton*, a view that was accepted by some researchers (Ellerman and Morrison-Scott 1951; Wang and Zheng 1973). However, *Can. canus* was considered a species placed in *Cricetulus* based on its geographical distribution and tail haircoat (Chen and Min 1982). Ross (1988) supported the idea that *Cansumys* was a valid genus and *Can. canus* a separate species, which was subsequently widely accepted (Corbet and Hill 1992; Musser and Carleton 1993; Pavlinov et al. 1995a). In 1985, *C. t. ningshaanensis* was first described as a subspecies of *C. triton* (Song 1985), but some researchers placed *C. t. ningshaanensis* within *Can. can. ningshaanensis*, supporting *Cansumys* as a valid genus (Wang and Xu 1992; Lu 1997). Yang et al. (2003) claimed *Cansumys* was a separate genus based on karyotype analyses of *Can. can. ningshaanensis* from Ningshan, Shaanxi Province. Furthermore, Gu et al. (2005) analyzed the external morphology of *Can. canus* and *C. triton* from Zhuoni, southern Gansu Province, and the results supported that *Can. canus* and *C. triton* were two distinct species and that the status of *Can. can. ningshaanensis* warranted further investigation. However, Liao et al. (2007) treated *Can. canus* as a synonym of *C. triton* and *C. t. canus* as a subspecies of *C. triton* based on molecular analysis of

specimens from Gansu Province and Ningxia. Since then, there have been no arguments regarding the classification status of *C. triton* and *Can. canus* (Smith and Xie 2008; Wilson et al. 2017; Wei et al. 2021). However, we know little about why *T. t. ningshaanensis* was treated as a synonym of *T. t. incanus* (Wilson et al. 2017; Wei et al. 2021).

In this study, we evaluate specimens from *Tscherskia* and *Cansumys* collected from Gansu, Henan, Shandong, Shanxi, Shaanxi, Heilongjiang, Beijing, and Inner Mongolia Provinces. We compared the external and skull morphologies of these specimens and conducted a combined analysis of the DNA sequences of one mitochondrial and three nuclear genes. Our aims were to infer: 1) the phylogenetic relationship and status of the genera *Tscherskia* and *Cansumys*; 2) the molecular phylogeny among the subspecies of *Tscherskia*; and 3) the taxonomic status of *T. ningshaanensis*.

Materials and methods

## Specimen collection and DNA sequencing

We collected tissues from 27 specimens (*Tscherskia* and *Cansumys*) from Gansu, Henan, Shandong, Shanxi, Shaanxi, Heilongjiang Provinces, as well as Inner Mongolia Autonomous Region and Beijing, China (Fig. 1 and Suppl. material 1: table S1). Voucher specimens and liver or muscle tissue are deposited at the Institute of Biodiversity and Ecology (**IBE**), Zhengzhou University, Sichuan Academy of Forestry Sciences (**SAF**), Marine College, Shandong University (**SDU**), and College of Life Sciences, Sichuan Normal University (**SNU**), respectively. The genomic DNA of specimens was extracted from the liver and muscle tissues using a DNA extraction kit (Tiangen DNA Easy Blood and Tissue Kit, Beijing, China). One complete mitochondrial locus [Cytochrome b (*CYT B*)] and partial sequences of three nuclear genes [interphotoreceptor retinoid-binding protein (IRBP), growth hormone receptor (GHR), and recombination activating protein 1(RAG1)] were amplified. Primer pairs were obtained from the literature (Teeling et al. 2000; Galewski et al. 2006; He et al. 2010; Cheng et al. 2017) and are shown in Suppl. material 1: table S2. PCR products were sent to Sangon Biotech Co., Ltd. for sequencing.

Phylogenetic analyses and molecular dating

Phylogenetic analyses

Recovered DNA sequences were assembled and aligned individually using MEGA X (Kumar et al. 2018). Additionally, 49 sequences from 10 species were downloaded from GenBank (Suppl. material 1: table S3). *Lagurus lagurus* was used as the outgroup for all subsequent phylogenetic analyses.

We calculated Bayesian Inference (BI) and maximum likelihood (ML) using BEAST v1.7.4 (Drummond et al. 2012) and W-IQ-TREE (Trifinopoulos et al. 2016),
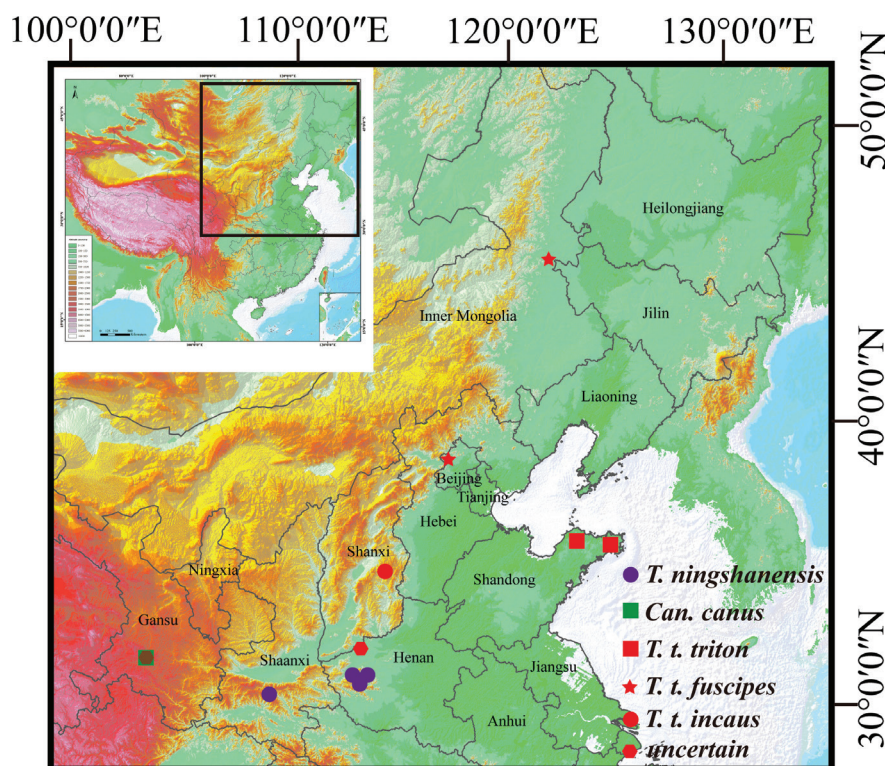
Figure 1. Collection sites of specimens of *Tscherskia* and Cansumys in China.

respectively, based on *CYT B* and nuDNA (GHR + IRBP + RAG1). For the BI analyses, the best-fit evolutionary models for *CYT B* and the three nuclear loci were determined using the Akaike Information Criterion (AIC) implemented in JMODELTEST v2.1.10 (Suppl. material 1: table S4) (Darriba et al. 2012). We employed a relaxed, uncorrelated lognormal clock model, Yule process tree priors, and the default prior distribution of the program for the model parameters. Each analysis was run for 100 million generations, with samples taken every 5,000 generations (Drummond et al. 2012). TRACER v1.7.0 was used to assess the effective sample size (ESS) values (i.e., ESS > 200) (Rambaut et al. 2018). And the first 10% of the trees were treated as burn-in.

For the ML analyses, the prior value of the parameter used was the default value for W-IQ-TREE (Trifinopoulos et al. 2016). We employed ultrafast bootstrap analysis with 1,000 bootstrap replicates, the SH-aLRT branch test with 1,000 iterations, the maximum iterations set to 1,000 iterations, and a minimum correlation coefficient of 0.99.

Species delimitation

Firstly, we calculated the Kimura-2-parameter (K2P) distance between specimens of Cricetinae in this study based on the *CYT B* gene using MEGA X (Kumar et al. 2018). Species trees (*CYT B* + nuDNA combined) were calculated using the *BEAST model in BEAST v1.7.4 (Heled and Drummond 2010; Drummond et al. 2012). Eight lineages were treated as species in the *BEAST based on the results of the K2P distance and phylogenetic analyses. We used the Yule speciation model and the strict clock model for tree construction. Other parameters followed

BI settings. Each analysis was run for 100 million generations, with samples taken every 5,000 generations (Drummond et al. 2012).

Secondly, another species delimitation analysis was conducted using the program BPP v3.1 (Camargo et al. 2012; Yang and Rannala 2014). The BPP analyses were performed using dataset1 (*CYT B* + nuDNA combined) and dataset2 (nuDNA combined), respectively. And the best tree topology recovered by BEAST v1.7.4 was used as the guide tree. The validity of our assignment of Cricetinae species was tested in BPP v3.1. The species delimitation analysis only included individuals who possessed both mtDNA and nuDNA data. Two reversible jump Markov chain Monte Carlo (rjMCMC) algorithms for species delimitation (algorithms 0 and 1) were used, respectively. Each rjMCMC was run for 100,000 generations, with sampling every 100 generations following a pre-burn-in of 10,000 generations as determined by TRACER v1.7 (Rambaut et al. 2018).

Divergence-time analyses

Divergence times were estimated based on the three nuclear loci combined (*IRBP + GHR + RAG1*). The divergence time was estimated using BEAST v1.7.4. The prior for the age of the tree root was based on the results by Steppan et al. (2004) (mean = 19 ma, standard deviation = 1.5), as referenced in Lebedev et al. (2018). We used the Yule Process speciation model and the uncorrelated relaxed clock model for tree construction. The substitution rate model is set according to Bayesian trees. Each analysis was run for 100 million generations, with samples taken every 5,000 generations (Drummond et al. 2012).

Analyses of external morphological and skull features

The external morphological characteristics of specimens (*T. ningshaanensis* and *T. triton*) were compared based on specimens and data from a previous study. Following the original description by Song (1985), we examined *T. ningshaanensis* and compared it with all subspecies of *T. triton*.

A total of 15 specimens (*T. triton*: 7 and *T. ningshaanensis*: 8) were collected. For these specimens, we examined and measured several parameters, including external morphology and 11 craniodental measurements (Yang et al. 2005). External morphological data (including **W**: weight; **HBL**: head and body length; **TL**: tail length; **HL**: hindfoot length; **EL**: ear length) were measured by a digital scale (0.1 g) and measured (1 mm) from the original specimens; craniodental measurements (including **PL**: Profile length; **BL**: Basal length; **SUCL**: Short upper cranium length; **ZB**: Zygomatic breadth; **IOB**: Interorbital breadth; **CH**: Cranial height; **TBL**: tympanic bulla length; **UMRL**: Upper molar row length; **LMRL**: Lower molar row length; **ML**: Mandibular length; **CL**: Condyle length) were taken with digital calipers (0.01 mm). We compared specimens of *Tscherskia* based on measurements of external and skull morphology. Overall similarities between external morphology and skulls were assessed first through principal component analyses (PCA). The PCA was conducted at OriginLab (OriginLab Corporation, version 2024, USA).

Results

Sequence characteristics

We obtained ~3573 bp of sequence for most specimens, partitioned into 1140 bp of mitochondrial sequence (*CYT B* [1140 bp]) and 2433 bp of nuclear sequence (*IRBP* [895 bp], *GHR* [810 bp], and *RAG1* [728 bp]). All new sequences have been deposited in GenBank (accession numbers: *CYT B* PP975895–PP975921, *GHR* PP975932–PP975950, *RAG1* PP975951–PP975969, *IRBP* PP975970–PP975985).

Phylogenetic analyses

The concatenated BI and ML recovered the identical topology; therefore, only the BI tree is presented (Fig. 2). Most of the nodes were strongly supported [i.e., BEAST posterior probabilities (**PP**) ≥ 0.95, SH-aLRT values (**SH**) ≥ 80, ultrafast bootstrap values (**UBS**) (Huelsenbeck and Rannala 2004; Guindon et al. 2010; Minh et al. 2013)], with few exceptions based on combined *CYT B* and nuclear loci (Fig. 2a, b). The BI and ML results strongly supported sister relationships between *T. ningshaanensis* and *T. triton*, and both should be treated as single species, respectively [*T. ningshaanensis* (*CYT B*: **PP** = 1, **SH** = 99.9, **UBS** = 100; nuDNA: **PP** = 1, **SH** = 96.6, **UBS** = 97); *T. triton* (*CYT B*: **PP** = 1, **SH** = 100, **UBS** = 100; nuDNA: **PP** = 1, **SH** = 99.9, **UBS** = 100)] (Fig. 2a, b). The genus *Cansumys* was strongly supported as monophyletic based on *CYT B* (**PP** = 1, **SH** = 100, **UBS** = 100) (Fig. 2a). The BI and ML analyses based on *CYT B* indicate that *T. t. triton* is differentiated from the other subspecies, whereas *T. t. incanus* and *T. t. fuscipes* do not show distinct separation (Fig. 2a). The BI and ML analyses based on nuDNA results do not support the classification as a subspecies of *T. triton* (Fig. 2b). In addition, the species *Phodopus roborovskii* and *Urocricetus kamensis* were placed at the base of Cricetinae in both the analysis of *CYT B* and nuDNA results (*CYT B*: **PP** = 1, **SH** = 83, **UBS** = 85; nuDNA: **PP** = 0.99, **SH** = 97.3, **UBS** = 99) (Fig. 2a, b).

Species delimitation

Calculated K2P distances based on *CYT B* were as follows: between *T. ningshaanensis* and *Can. canus* (25.5%), between *T. ningshaanensis* and *T. triton* (15.1%), and between *Can. canus* and *T. triton* (23.8%) (Table 1). Additionally, BPP analysis results based on dataset1 and dataset2 supported *T. ningshaanensis* and *T. triton* as separate species (**PP** = 1.00), respectively. The BEAST tree analyses recovered the same topology as the BI and ML trees, with sister relationships between *T. ningshaanensis* and *T. triton* also strongly supported (**PP** = 0.99) (Fig. 3a).

Molecular divergence time

Our phylogenetic analyses based on nuDNA revealed highly concordant divergence time estimates (Fig. 3b). The species *Phodopus roborovskii* and *Urocricetus kamensis* were placed at the base of Cricetinae, with the divergence time result estimated to be in the latest Middle Miocene (12.73 Ma). Apart from the split between *T. triton* and *T. ningshaanensis* (3.88 Ma), intra-generic divergence events primarily occurred in the latest Pliocene.

Morphological and skull comparison

All external and skull measurements are provided in Table 2. The mean values of most measurements for *T. ningshaanensis* are smaller than those for *T. triton*, with significant differences in W, HBL, PL, BL, SUCL, ZB, CH, TBL, ML, and CL. However, the sizes of HFL (23.50–27.00, 24.79±1.15 vs 20.00–26.00, 23.24±2.17) and LMRL (5.28–5.41, 5.34±0.05 vs 5.13–5.77, 5.45±0.20) of *T. ningshaanensis* are bigger than those for *T. triton* (Table 2).
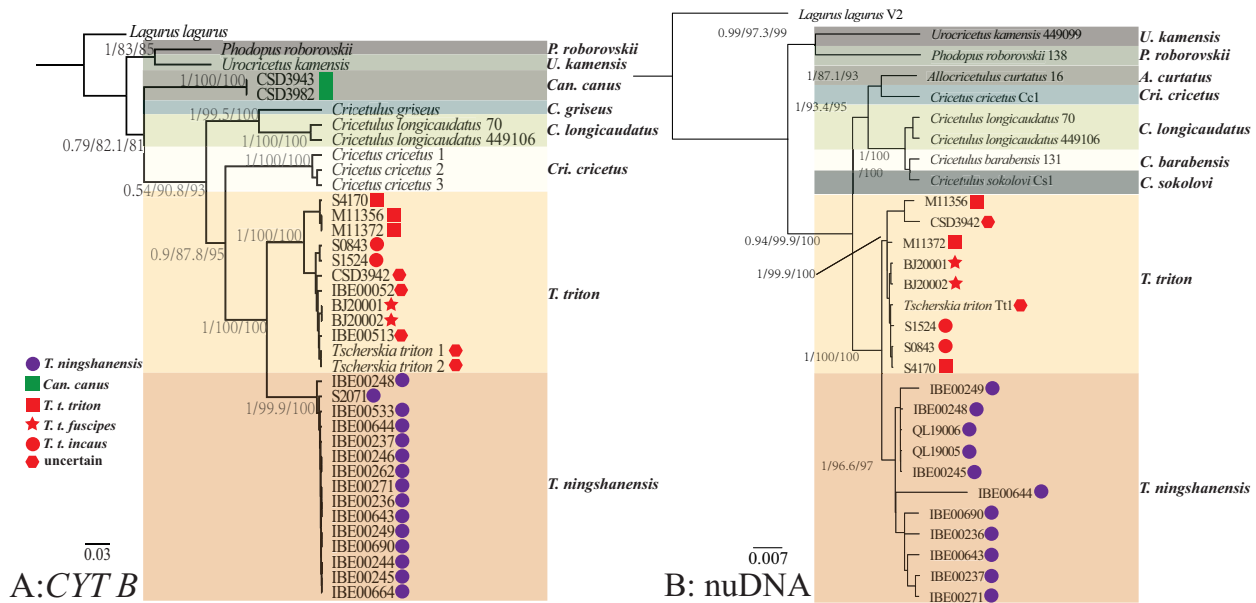
Figure 2. Maximum likelihood and Bayesian inference analysis results based on *CYT B* (**A**) and nuDNA (**B**). Left: BI posterior probabilities; middle: SH-aLRT values; right: ultrafast bootstrap values.

Table 1. K2P distances between species of *Cricetinae* based on the *CYT B* gene.

	Tsc nin	*Cri gri*	*Lag lag*	*Tsc tri*	*Cri cri*	*Pho rob*	*Cri long*	*Uro kam*
Tsc nin								
Cri gri	0.231							
Lag lag	0.269	0.224						
Tsc tri	0.151	0.229	0.258					
Cri cri	0.204	0.188	0.213	0.209				
Pho rob	0.257	0.225	0.222	0.263	0.231			
Cri long	0.236	0.143	0.236	0.223	0.208	0.240		
Uro kam	0.225	0.237	0.220	0.238	0.201	0.198	0.244	
Can can	0.255	0.222	0.221	0.238	0.208	0.212	0.212	0.217

Table 2. Some external and skull measurements (mm) used in PCA analyses of *T. ningshaanensis* and *T. triton*.

Measurement (min, max, mean ± SD)	*T. triton*	*T. ningshaanensis*
W	66.00 – 92.00, 80.67 ± 10.16	37.64 – 93.10, 52.44 ± 19.81
HBL	146.00 – 175.00, 128.00 ± 16.38	112.00 – 155.00, 129.29 ± 16.15
TL	65.00 – 90.00, 77.43 ± 8.28	76.00 – 114.00, 89.71 ± 12.62
HFL	20.00 – 26.00, 23.24 ± 2.17	23.50 – 27.00, 24.79 ± 1.15
EL	18.00 – 22.00, 20.26 ± 1.49	20.00 – 23.00, 21.71 ± 1.04
PL	33.43 – 37.68, 35.79 ± 1.56	29.33 – 37.07, 31.49 ± 2.65
BL	30.42 – 35.30, 32.13 ± 1.79	26.99 – 34.47, 29.03 ± 2.68
SUCL	33.43 – 38.94, 35.97 ± 1.86	30.24 – 37.93, 32.43 ± 2.74
ZB	16.87 – 20.22, 17.99 ± 1.06	15.54 – 19.02, 16.30 ± 1.34
IOB	4.88 – 5.54, 5.18 ± 0.22	4.66 – 5.47, 5.03 ± 0.26
CH	12.83 – 13.38, 13.11 ± 0.20	11.90 – 12.98, 12.31 ± 0.40
TBL	8.62 – 10.94, 10.06 ± 0.79	7.98 – 10.29, 8.63 ± 0.89
UMRL	5.03 – 5.57, 5.27 ± 0.17	4.94 – 5.34, 5.13 ± 0.16
LMRL	5.13 – 5.77, 5.45 ± 0.20	5.28 – 5.41, 5.34 ± 0.05
ML	22.46 – 24.70, 23.69 ± 0.74	19.23 – 25.06, 20.84 ± 2.02
CL	19.08 – 20.70, 19.71 ± 0.64	16.19 – 19.83, 17.18 ± 1.28

Note: W: weight; HBL: head and body length; TL: tail length; HL: hindfoot length; EL: ear length; PL: profile length; BL: basal length; SUCL: short upper cranium length; ZB: zygomatic breadth; IOB: interorbital breadth; CH: cranial height; TBL: tympanic bulla length; UMRL: upper molar row length; LMRL: lower molar row length; ML: mandibular length; CL: condyle length.
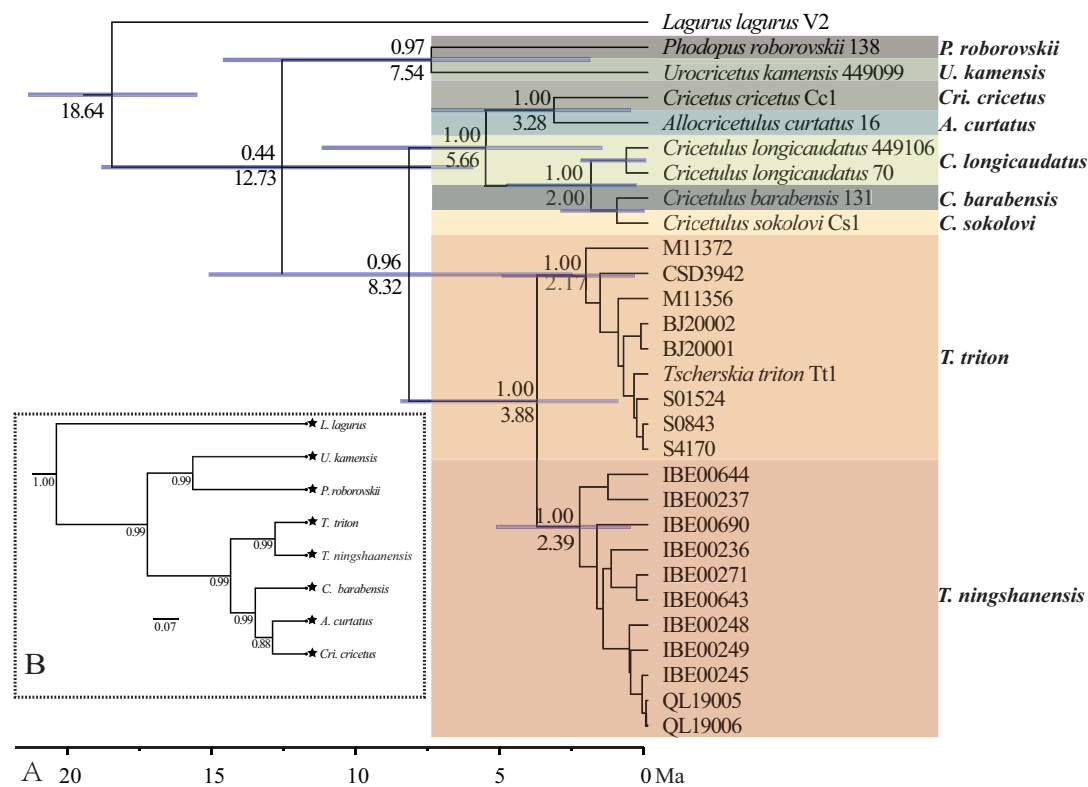
Figure 3. Divergence times estimated (**A**) and species delimitation (**B**) results in this study. a: black stars represents BPP species definition results; numbers of each node represent posterior probabilities (under); b: numbers of each node represent posterior probabilities (upper) and divergence times (under).

The PCA, based on 16 measurements [including external morphology (5) and skull (11) measurements], produced two axes (PC1: 10.20 and PC2: 2.69) with eigenvalues > 2.0, explaining 60.00% and 15.85% of the variance (75.85% total) (Table 3). PC1 was positively correlated with all variables (Table 3). PC2 was strongly correlated with TL and EL, loading > 0.5. The PCA results showed that most specimens of *T. ningshaanensis* and *T. triton* could be distinguished from each other based on 16 $\log_{10}$–transformed variables (Fig. 4).

Discussion

The classification status of *Can. canus* and *T. ningshaanensis* had been extensively discussed in previous studies (Yang et al. 2003; Gu et al. 2005; Liao et al. 2007). The results from this study provide molecular evidence into the classification status of *Can. canus* and *T. ningshaanensis*, encompassing almost all subspecies of *T. triton* found in China (except for *T. t. collinus*). Our phylogenetic and morphological results indicated that the genus *Cansumys* should be treated as a distinct genus, and *T. ningshaanensis* is a distinct species. The genetic distance values among three species based on *CYT B* indicated that *T. ningshaanensis*, *T. triton*, and *Can. canus* are all distinct species (> 11%) (Bradley and Baker 2001). The

Table 3. Character loadings, eigenvalues, and percent variance explained on the first two components of a PCA of *T. triton* and *T. ningshaanensis*.

Variables	PC1	PC2
W	0.31	0.02
HBL	0.26	0.00
TL	0.06	0.55
HFL	0.01	0.43
EL	0.02	0.52
PL	0.31	0.03
BL	0.30	0.11
SUCL	0.31	0.10
ZB	0.30	0.05
IOB	0.18	0.21
CH	0.29	–0.14
TBL	0.22	–0.11
UMRL	0.19	0.02
LMRL	0.14	–0.16
ML	0.31	0.03
CL	0.31	–0.05
Eigenvalues	10.20	2.69
Variance explained (%)	60.00	15.85

large genetic distance (20.8% – 25.5%) and phylogenetic analyses based on *CYT B* strongly supported the classification status of *Cansumys* as a distinct genus (**PP** = 1, **SH** = 100, **UBS** = 100, Table 1), which was consistent
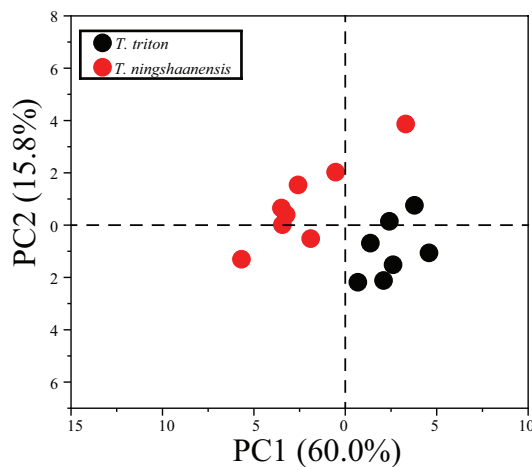
Figure 4. Results of principal component analysis (PCA) of *T. ningshaanensis* and *T. triton*.

with previous research (Smith and Xie 2008; Wilson et al. 2017; Wei et al. 2021). The calculated K2P distances based on *CYT B* of *T. triton* compared with other species of Cricetinae in this study ranged from 15.1% (*T. triton*) to 25.7% (*P. roborovskii*). The results from phylogenetic analyses based on *CYT B* and nuDNA loci strongly supported *T. ningshaanensis* as a separate species (*CYT B*: **PP** = 1, **SH** = 99.9, **UBS** = 100; nuDNA: **PP** = 1, **SH** = 96.6, **UBS** = 97); *T. triton* (*CYT B*: **PP** = 1, **SH** = 100, **UBS** = 100; nuDNA: **PP** =1, **SH** = 99.9, **UBS** = 100)] (Fig. 2a, b). The genus *Cansumys* was strongly supported as monophyletic based on *CYT B* (**PP** = 1, **SH** = 100, **UBS** = 100. Fig. 2a, b). However, the fine-scale subdivision of subspecies of *T. triton* indicates that additional studies are warranted to clarify the status of the described subspecies (Fig. 2a, b).

The growth of the Tibetan Plateau led to the uplift of the Qinling Mountains during the late Miocene to Pliocene (8–4 Ma) (Wang et al. 2011). This rapid uplift contributed to the biodiversity within the Qinling Mountains (Dong et al. 2022). The divergence time between *T. ningshaanensis* and *T. triton* was estimated to be approximately 3.88 million years ago. This divergence time suggested that the formation of *T. ningshaanensis* and *T. triton* was influenced by the uplift of the Qinling Mountains. In Europe, the earliest species of *Tscherskia* (*T. europaeus* and *T. janossyi*) was found at Csarnóta 2 (MN 15, ca. 5–3.5 Ma) in Hungary (Hír 1994; Venczel and Gardner 2005). The earliest known species of *Tscherskia* (*T.* sp.) was found in the Late Pliocene (2.58–3.60 Ma) from the Youhe Formation (ca. 3.40–2.59 Ma) (Yue and Xue 1996; Xie et al. 2021) in Linwei District, Weinan, Shaanxi Province, China. These findings suggested that the species of *Tscherskia* underwent rapid diversification during the late Pliocene (2.58–3.60 Ma). Fossils of *T. t. varians* were found from the late Middle Pleistocene to the Early Pleistocene in China (0.129 Ma–2.58 Ma) (Zheng 1984a, 1984b, 1993; Jin et al. 2009; Xie et al. 2023). This suggested that *T. triton*

underwent diversification during the early Pleistocene, which is consistent with the divergence time of *T. triton* estimated in this study.

In addition, we compared the distribution range, external morphology, and skull morphology of *T. ningshaanensis*, *T. triton*, and *Can. canus*. The fact that both *T. triton* and *Can. canus* were found at Muer of Zhuoni, Gansu Province, assisted the conclusion that *T. triton* and *Can. canus* are distinct species. The results of external morphology analyses showed *T. ningshaanensis* and *T. triton* could be distinguished from each other with many distinguishable features. *T. t. triton* (de Winton 1899) has a dorsal coloration that is uniformly drab, with whitish underparts (Suppl. material 2). *T. t. collinus* (Allen 1925) is similar to *T. t. triton* but is much darker with a slightly longer tail. Its dorsal coloration is between drab and mouse gray, with warm buff sides to the head and body. The chin, feet, wrists, and a small median spot on the throat have clear white hairs. The tail is blackish-brown and thinly covered with short, appressed hairs, with many whitish hairs on the lower side. *T. t. fuscipes* (Allen 1925) is similar in general appearance to *T. t. triton* but has ankles and a basal part of the metatarsals that are dusky. Its entire dorsal area is nearly uniformly buffy, with hairs that are entirely black or have a fine black tip. The tail is thinly covered with hairs, dusky above and whitish below. *T. t. incanus* (Thomas 1908) is similar in general appearance to *T. t. triton*, with a white dorsum pedis and dorsal and belly hairs that are pale. *Can. canus* (Allen 1928) has a dorsal surface of the body and tail that is generally gray. The middle of its back has slaty-gray hairs with short whitish tips, interspersed with numerous all-black hairs, and there is a faint wash of buffy color on the sides of the body, while its tail is thickly covered with fine hairs.

In the original description, Song (1985) presented several morphological characters to distinguish it from other subspecies of *T. triton*: 1) smaller body size compared to other subspecies of *T. triton*; 2) entire dorsal surface dark grayish–brown, covered with long black hair, while the ventral grayish–white with the medial part of the body hair being gray and the distal part being whit; 3) center of the chest and feet white, ankles taupe covered with thick haired; 4) long tail, with the tail length nearly 66% of the body length; 5) the tail appears bicolored, with the basal part being grayish-brown and the distal part white, covered with dense hair; 6) white tail percentage, with the white length nearly 40%–60% of the tail; 7) intumescent tail base; and 8) less developed supraorbital ridge (Figs 5, 6). *T. ningshaanensis* and *T. triton* (including four subspecies) can be clearly distinguished from each other based on morphological characteristics (body grayish-brown, long tail with two color rears, 40–60% being white). We believed that the aforementioned evidence supports the conclusion that *T. ningshaanensis* should not be treated as a synonym of *T. t. incanus*.
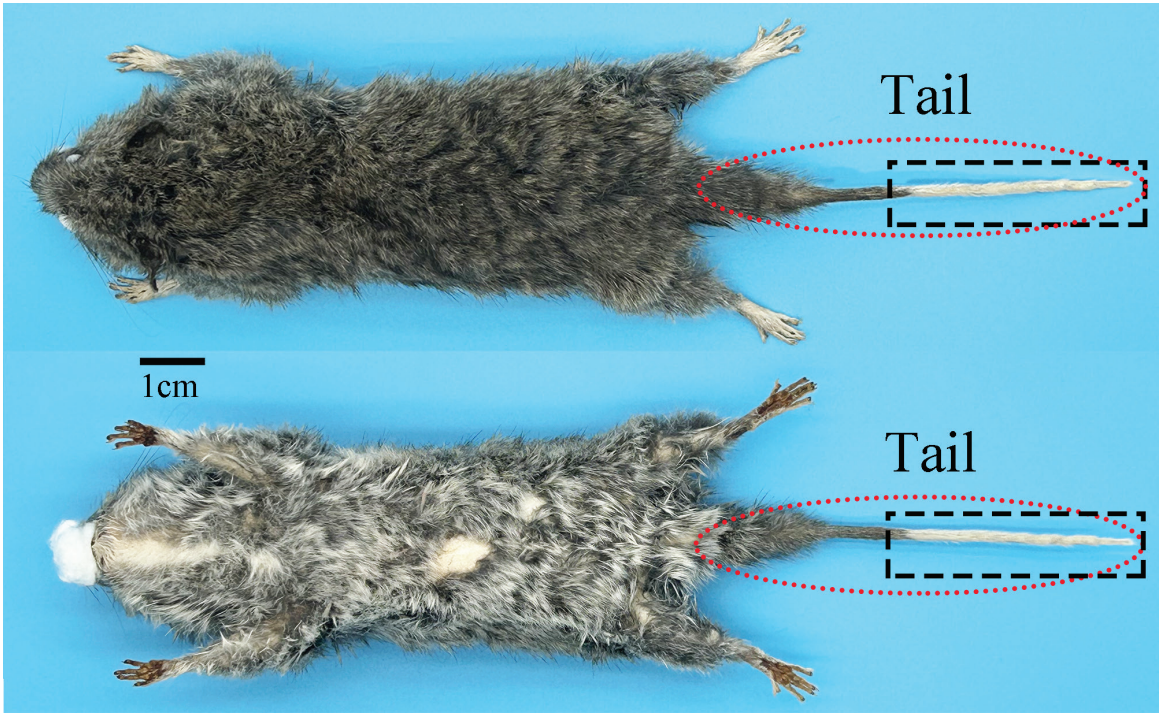
Figure 5. Dorsal and ventral views of *T. ningshaanensis*.
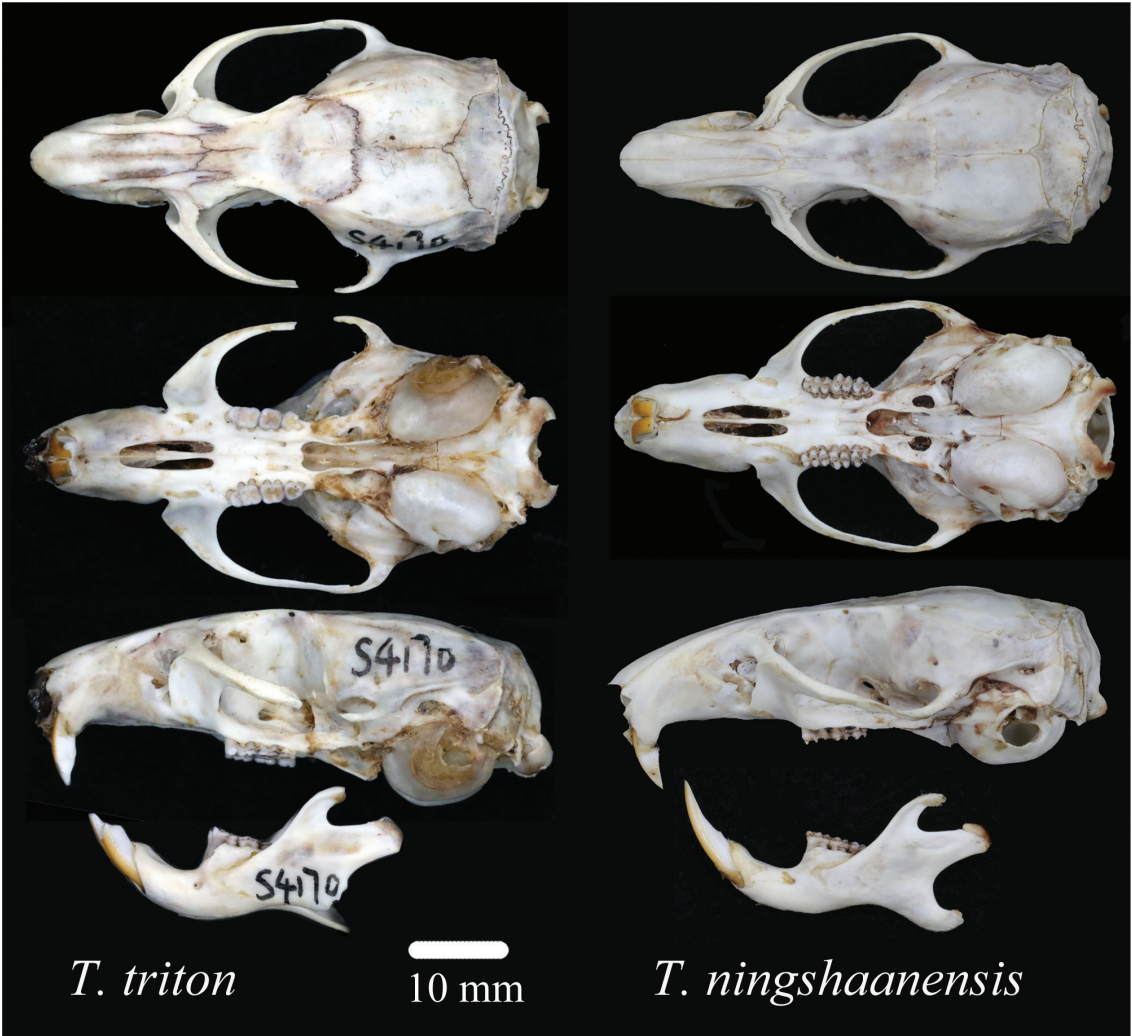

Figure 6. Dorsal, ventral, and lateral views of the skull and lateral views of the mandible, *T. ningshaanensis* and *T. t. triton*.

Conclusions

In this study, we used morphology and molecular phylogeny to investigate the taxonomy, phylogenetic relationships, and evolutionary history of the genera *Tscherskia* and *Cansumys*. The results supported the following conclusions: 1) the genus *Cansumys* is valid and distinct, possibly monotypic as only *Can. canus* is currently described; 2) the genus *Tscherskia* consists of *T. ningshaanensis* and *T. triton*, and *T. ningshaanensis* is not a synonym of *T. t. incanus*; and 3) the uplift of the Qinling Mountains likely facilitated the geographical isolation of ancestral species, further promoting the speciation of *T. ningshaanensis*.

Acknowledgments

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Supplementary material 1

Supplamentary information

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Data type: docx

Explanation note: **table S1.** Sampling localities of genera *Tscherskia* and *Cansumys* in this study. **table S2.** Primers used for PCR and sequencing in this study.**table S3.** Sampling information including localities and GenBank accession numbers for species used in this study. **table S4.** The best molecular evolution model according to the Akaike Information Criterion (AIC) used in phylogenetic reconstructions based on jModeltest v2.10

Link: https://doi.org/10.3897/zse.100.128567.suppl1

Supplementary material 2

Dorsal and ventral views of *T. triton*

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Data type: psd

Link: https://doi.org/10.3897/zse.100.128567.suppl2

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