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The Complete Mitochondrial Genome of the *Niviventer lotipes* (Rodentia: Muridae) from China and its Phylogenetic Analysis

Yifei Zhang, Lu Zhang, Zhihui Zhang, Qiuying Guo and Zhu Liu*

College of Life Science and Technology, Mudanjiang Normal University, Mudanjiang, 157011, Heilongjiang, PR China

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Key words
***Niviventer lotipes*, Mitochondrial genome, Muridae, Phylogenetic relationships, *Niviventer*, mtDNA annotation**

ABSTRACT

Most of *Niviventer*'s phylogenetic analyses were performed using several mitochondrial (*CYTB, COI*) and nuclear genes (*RAG1, IRBP*) alone or in combination, but yielded inconsistent results. In order to further understand the phylogeny of *Niviventer lotipes*, we performed a phylogenetic analysis of *N. lotipes* with a partition model based on 13 mitochondrial protein-coding genes (PCGs) and 2 ribosomal RNA genes. The first complete mitochondrial genome of white-bellied rat (*Niviventer lotipes*) was determined using conventional PCR. The genome was 16322 bp in length and contained 13 protein-coding genes (PCGs), 2 ribosomal RNA genes (12S rRNA and 16S rRNA), 22 transfer RNA genes (tRNAs), one origin of light-strand replication and one control region. The basic composition of the whole genome includes, A (34.1%), T (28.3%), C (25.1%) G (12.5%) and AT-skew (0.092), GC-skew (-0.335). 13 PCGs encode a total of 3791 amino acids, of which Leu has the highest AARatio (15.59%). Phylogenetic analysis yielded three phylogenetic trees with similar topological, and the results support that genus *Niviventer* is divided into four groups, in which *N. lotipes* was clustered within the genus *Niviventer* and formed a division with *N. gladiusmaculus*, *N. pianmaensis*, *N. sacer*, *N. confucianus* and *N. niviventer*. The mitochondrial whole genome sequence of *N. lotipes* at the molecular level provides an important complement to the phylogenetic relationship of the genus *Niviventer*.

INTRODUCTION

The genus *Niviventer* (Rodentia: Muridae) dispersing from the Himalayas and China to the Greater Sunda Islands (Musser, 1981; Musser and Carleton, 1993). They have diverse and extensive habitats, from moist forests to dry valleys. They also carry a variety of human pathogens (Keesing *et al*., 2010). Current research shows, there are 19 recognized *Niviventer* species with another 64 recognized as synonyms or subspecies (Solari and Baker, 2007; Wei *et al*., 2021; Li *et al*., 2020).

Niviventer lotipes (Allen, 1926; Type Locality, Nada, Hainan, China) are endemic to China. At present distribution of *N. lotipes* is mainly found in southern China (Hainan, Guizhou, Hunan), southeastern China (Zhejiang, Fujian) and Xizang (Zhang *et al*., 2016). *N. lotipes* and *N. champa* (Robinson and Kloss, 1922) were once regarded as synonyms of *N. tenaster* (Thomas, 1916) (Li *et al*., 2008). A previous study conducted karyotyping and morphological analysis of three Rat species (Mammalia: Rodentia: Muridae) in Hainan Island, and identified *N. lotipes* as a valid species for the first time (Li *et al*., 2008).

As more *Niviventer* species were sequenced and analyzed, the results of phylogenetic analysis based on one or several mitochondrial and nuclear genes indicated that genus *Niviventer* should be divided into four groups, not two, among which *N. lotipes* included in *N. confucianus*-Division (Lu *et al*., 2015; Zhang *et al*., 2016; Ge *et al*., 2021). In addition, it also supporting that *N. lotipes* and *N. niviventer* were sister species (Lu *et al*., 2015). However, the phylogenetic analyses results of *N. lotipes* are always inconsistent in detail due to the subjective selection of gene sequences and the limited number of gene sequences used. Compared with individual mitochondrial gene sequences, complete mitochondrial genome sequences can provide higher resolution and sensitivity to better reveal evolutionary relationships between closely related species (Ladoukakis and Zouros, 2017; Wei *et al*., 2017; Liu *et al*., 2022). Since different evolutionary pressures and time scales result in different gene evolution (Choi and Kim,

* Corresponding author: swxlz0@126.com
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2017), so for phylogenetic analysis we use a partition model which allow each gene has its own substitution models and evolutionary rates (Chernomor *et al*., 2016).

Until now, NCBI has included the mitochondrial genome sequences of most recognized *Niviventer* species except for *N. bukit*, *N. coninga, N. culturatus*. We sequenced and annotated the first mitochondrial whole genome sequence of *N. lotipes*. The mitochondrial whole genome sequence of *N. lotipes* at the molecular level provides an important complement to the phylogenetic relationship of the genus *Niviventer*. On the other hand, the topological differences between genome and gene trees also highlights the importance of the entire mitochondrial genome in rodent phylogenetic analysis.

MATERIALS AND METHODS

Sample collection and genomic DNA extraction

A muscle sample obtained from a female *Niviventer lotipes* captured from Tongren regions in Guizhou Province, China (27°69′14″N, 108°84′84″E) was stored at -75 °C in Animal and Plant Herbarium of Mudanjiang Normal University with voucher number HNSS2019004 (Zhu Liu, swxlz0@126.com) after preservation in 95% ethanol. Genomic DNA was extracted from muscle using the EasyPure genomic DNA kit (TransGen Biotech Co., Beijing, China). The 15 pairs of primers used in PCR were designed based on the existing *Nivienter* mitochondrial genome useing Editseq and Premier 6 (Liu *et al*., 2019, 2021).

PCR amplifcation and sequencing

PCR for fragmented amplification of mitochondrial whole genomes was performed in 20 µl reaction volumes. A 90 µl cocktail prepared by mixing 50.02 µL ultrapure water, 8.2 µL buffer, 6.56 µL dNTP, 4.92 µL $MgCl_2$, 1.64 µL primer, 0.82 µL Taq polymerase (Takara, China), was divided into five equal parts of 18 µL each, and then finally 2 µL DNA templates was added. The PCR cycle conditions were as follows: DNA denaturation temperature 94 °C for 4 min, followed by 39 cycles, DNA denaturation temperature 94 °C for 45 s, annealing temperature 52 °C for 1 min, extension temperature 72 °C for 1.5 min, and a final extension of 7 min at 72 °C. The PCR product was sequenced using the first-generation sequencing technology and the ABI 3730 sequencer (Ruiboxingke Biotechnology Co. Ltd., Beijing, China).

Sequence assembly, annotation and analysis

Referring to the existing *Nivienter* mitochondrial genome on NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi), the mitochondrial gene sequences of *N. lotipes* obtained by sequencing was assembled with DNASTAR software package to obtain a complete mitochondrial genome. Annotation of the whole mitochondrial genome with the MITOS web server (mitos2.bioinf.uni-leipzig.de/index.py), and the annotations were validated using BLAST (https://blast.ncbi.nlm.nih.gov) (Kamalakkannan *et al*., 2020). For tRNA genes analysis using tRNAscan-SE Search Server (http://trna.ucsc.edu/tRNAscan-SE/), the parameters are set to default (Lowe and Eddy, 1997). Composition skew analysis was calculated by the formula (Perna and Kocher, 1995): AT skew = (A–T)/(A + T) and GC skew = (G–C)/(G + C). Base composition and relative synonymous codon usage (RSCU) by using software PhyloSuite v 1.2.2 (Zhang *et al*., 2020). The circular mitochondrial genome map of *N. lotipes* was drawn using SnapGene v 6.0.2 (www.snapgene.com).

Phylogenetic analysis

The complete mitochondrial genome used for phylogenetic analysis was downloaded from the GenBank database (https://www.ncbi.nlm.nih.gov/genbank) (Table I). Software PhyloSuite v 1.2.2 was used to extract the desired genes from the downloaded mitochondrial genomes. Multiple sequences were aligned using MAFFT v 7.471 and MACSE v 2.03 with default settings (Katoh and Standley, 2013; Ranwez *et al*., 2018). Before using software PhyloSuite v 1.2.2 to concatenate the extracted gene sequences, Gblock (Talavera and Castresana, 2007) was used to cut all gaps generated after the alignment. The best-fit partition model (Table II) for ML analysis and BI analysis were evaluated using Bayesian information criterion in iqtree v 2.2 (Nguyen *et al*., 2015) and -MF+MERGE program. The MERGE strategy starts with the full partition model and subsequentially merges two genes until the model fit does not increase any further (Robert *et al*., 2012). Phylogenetic tree reconstruction using iqtree v 2.2 and Mrbayes v 3.2.7a for ML analysis and BI analysis, respectively. The standard Bootstrap test of ML tree were set to 1000 iterations. The MCMC settings for the BI tree were as follows: 10 million generations as 4 MCMC chains at the same time, sampling every 100 generations, Burnin Fraction is 10%, and number of runs is 2. We used tracer v 1.7.1 and ESS value > 200 (Rambaut *et al*., 2018) to evaluate the credibility of the results. Editing phylogenetic tree results using ITOL web server (https://itol.embl.de/).

RESULTS AND DISCUSSION

Mitogenome organization

Accurate annotation of the closed-circle complete mitochondrial genome of 16322 bp in length has been

Table I. The complete mitochondrial genome used for this phylogenetic analysis. For sequences without annotation information in GenBank (*N. pianmaensis*, *N. niviventer*, *N. brahma*, *N.eha*, *N.huang*, *N. gladiusmaculus*, *N. fengi*, *N. mekongis)*, we use the MITOS web server for gene annotation.

Genus/ Species	Length (bp)	GeneBank No
Rattus		
R. andamanensis	16304	NC_046686
R. nitidus	16297	NC_040919
R. norvegicus	16313	NC_001665
R. rattus	16305	NC_012374
R. tanezumi	16306	NC_011638
Mus		
M. musculus	16303	LC644158
Niviventer		
N. andersoni	16291	MW030174
N. brahma	16289	MW193742
N. confucianus	16281	NC_023960
N. cremoriventer	16323	KY117573
N. eha	16301	MW193732
N. excelsior	16298	JQ927552
N. fengi	16300	MW193731
N. fulvescens	16301	KP455488
N. gladiusmaculus	16365	MW193728
N. huang	16372	MW193730
N. mekongis	16304	MW193741
N. niviventer	16302	MW193736
N. pianmaensis	16303	MW193740
N. sacer	16308	MZ935252
Leopoldamys		
L. edwardsi	16284	NC_025670
L. sabanus	15975	NC_035819

submitted to Genbank with accession number ON652843. The arrangement of the multiple genes is in line with other Muridae species and most mammals (Mouchaty *et al*., 2000; Nikaido *et al*., 2003; Liu *et al*., 2019, 2021). The complete mitochondrial genome of *N. lotipes* includes 13 protein coding genes, 2 *rRNA* genes, 22 *tRNA* genes, 1 origin of L strand replication and 1 control region (Fig. 1, Table III). The base composition of the whole genome is as follows: A (34.1%), T (28.3%), C (25.1%) G (12.5%), and A+T (62.4%), C+G (37.6%). In addition, AT-skew (0.092), GC-skew (-0.335) are calculated by formula AT skew = (A–T)/(A + T) and GC skew = (G–C)/ (G + C) which indicating As and Cs were more abundant than Ts and Gs (Table III). Most genes in the mitochondrial genome were encoded by heavy (H) strand except for ND6 and 8 tRNAs (trnaQ, trnaA, trnaN, trnaC, trnaY, trnaS2, trnaE, trnaP) which were encoded by the Light (L) strand. The intergenic spacer (IGS) varing from 1 to 32 bp, with the largest intergenic spacer between trnaN and trnaC. At the same time, overlap between genes varing from 1 to 43 bp, with the largest overlap between ATP8 and ATP6.

Table II. The best-fit partition model for phylogenetic analysis.

The best-fit partition model for ML analysis (12 PCGs and 2 rRNA)	
GTR+F+I+G4	codon1 (atp6, cox1, cox2, cox3, cytb, nad1, nad3)
K3Pu+F+I	codon2 (atp6, cox1, cox2, cox3, cytb, nad1, nad4L)
TIM2+F+I+G4	codon3 (atp6, atp8, cox1, cox2)
TIM3+F+I+G4	codon1 (atp8, nad2, nad4); codon2 (atp8)
TIM2+F+I+I+R3	codon3 (cox3, cytb, nad1, nad2, nad3, nad4L, nad4, nad5)
TIM3+F+R2	codon2 (nad2, nad3, nad4, nad5)
TIM2+F+I+G4	codon1 (nad4L, nad5); 12s rRNA, 16s rRNA
The best-fit partition model for ML analysis (13 PCGs and 2 rRNA)	
GTR+F+I+G4	codon1 (atp6, cox1, cox2, cox3, cytb, nad1, nad3)
K3Pu+F+I	codon2 (atp6, cox1, cox2, cox3, cytb, nad1, nad4L, nad6)
TIM2+F+I+G4	codon3 (atp6, atp8, cox1, cox2)
TIM3+F+I+G4	codon1 (atp8, nad2, nad4); codon2 (atp8)
TIM2+F+I+I+R3	codon3 (cox3, cytb, nad1, nad2, nad3, nad4L, nad4, nad5)
TIM3+F+R2	codon2 (nad2, nad3, nad4, nad5)
TIM2+F+I+G4	codon1 (nad4L, nad5); 12s rRNA, 16s rRNA
TN+F+I+G4	codon1 (nad6)
TN+F+G4	codon3 (nad6)
The best-fit partition model for BI analysis (12 PCGs and 2 rRNA)	
GTR+F+I+G4	codon1 (atp6, cytb, nad1, nad3)
HKY+F+I	codon2 (atp6, cox1, cox2, cox3, cytb)
GTR+F+I+G4	codon3 (atp6, atp8, cox1, cox2)
GTR+F+I+G4	codon1 (atp8, nad2, nad4)
GTR+F+I+G4	codon2 (atp8, nad1, nad2, nad3, nad4L, nad4, nad5)
SYM+I+G4	codon2 (cox1, cox2, cox3)
GTR+F+I+G4	codon3 (cox3, cytb, nad1, nad2, nad3, nad4L, nad4, nad5)
GTR+F+I+G4	codon1 (nad4L, nad5); 12s rRNA, 16s rRNA

Table III. Features of the mitochondrial genome of *N. lotipes*.

Gene	Position		Codon		Anticodon	Size (bp)	Intergenic spacer (bp)	Strand
	From	To	Star	Stop				
trnF	1	69			GAA	69	1	H
rrnS	71	1026				956	0	H
trnV	1027	1096			TAC	70	0	H
rrnL	1097	2672				1576	-2	H
trnaL2	2671	2745			TAA	75	0	H
nad1	2746	3700	ATA	TAG		955	0	H
trnaI	3701	3769			GAT	69	-3	H
trnaQ	3767	3837			TTG	71	3	L
trnaM	3840	3908			CAT	69	0	H
nad2	3909	4944	ATC	TAG		1036	0	H
trnaW	4945	5010			TCA	66	1	H
trnaA	5012	5080			TGC	69	1	L
trnaN	5082	5152			GTT	71	32	L
trnaC	5184	5251			GCA	67	0	L
trnaY	5252	5318			GTA	67	1	L
cox1	5320	6867	ATG	TAA		1548	-6	H
trnaS2	6862	6927			TGA	69	3	L
trnaD	6931	6998			GTC	68	1	H
cox2	7000	7683	ATG	TAA		684	3	H
trnaK	7687	7750			TTT	64	1	H
atp8	7752	7955	ATG	TAA		204	-43	H
atp6	7913	8593	ATG	TAA		681	-1	H
cox3	8593	9376	ATG	TA		784	0	H
trnaG	9377	9444			TCC	68	0	H
nad3	9445	9792	ATC	TAA		348	1	H
trnaR	9794	9861			TCG	68	2	H
nad4L	9864	10160	ATG	TAA		297	-7	H
nad4	10154	11531	ATG	T		1378	0	H
trnaH	11532	11599			GTG	68	0	H
trnaS1	11600	11658			GCT	59	-1	H
trnaL1	11658	11728			TAG	71	6	H
nad5	11735	13558	ATA	TAA		1824	-23	H
nad6	13536	14054	ATG	TAA		519	0	L
trnaE	14055	14123			TTC	69	5	H
Cob	14129	15272	ATG	T		1144	0	H
trnaT	15273	15339			TGT	67	0	H
trnaP	15340	15407			TGG	68	0	L
D-loop	15408	16322				915		H

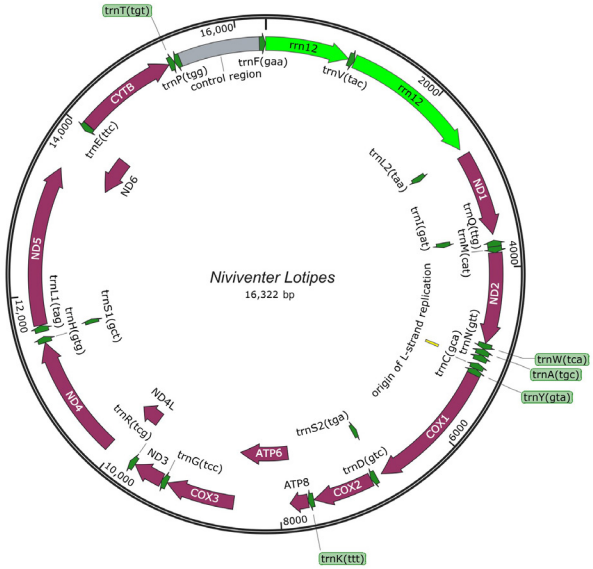

Fig. 1. The gene map of *N. lotipes* mitochondrial genome.

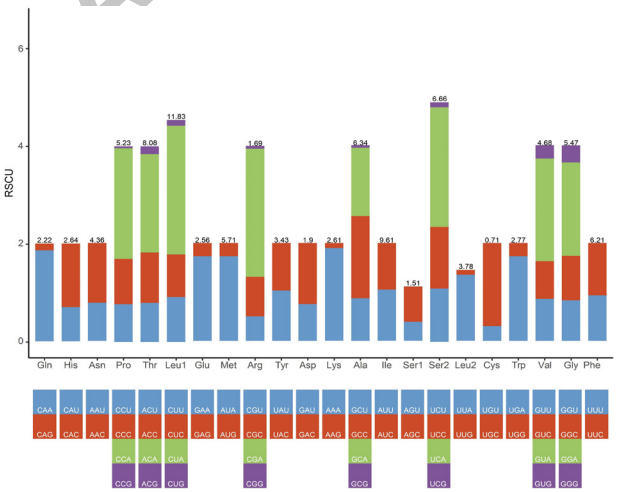

Fig. 2. The relative synonymous codon usage (RSCU) in the mitogenome of *N. lotipes*.

PCGs and codon usage

The complete mitochondrial protein-coding gene is 11402 bp in length and accounts for 69.9% of the entire mitochondrial genome. The AT and GC skews of PCGs were positive (0.031) and negative (-0.364), respectively as observed in the case of whole mitochondrial genome (Table IV). Thirteen PCGs were found in the mitochondrial genome of *N. lotipes*. Except for NAD1, NAD2 and NAD5, which use ATA, ATC, and ATA as the start codons, respectively, the rest all use ATG as the start codon. CYTB, COX3 and NAD4 use incomplete stop codons (T--), the rest use TAA or TAG as stop codons (Table III).

Table IV. Composition and skewness of *N. lotipes* mitogenome.

	A	T	C	G	A+T%	AT-skew	GC-skew	Size(bp)
Mitogenome	34.1%	28.3%	25.1%	12.5%	62.4%	0.092	-0.335	16322
PCGs	31.7%	29.8%	26.3%	12.3%	61.5%	0.031	-0.364	11402
nad1	30.9%	28.2%	29.8%	11.1%	59.1%	0.046	-0.458	955
nad2	35.8%	27.8%	28.7%	7.7%	63.6%	0.126	-0.576	1036
cox1	28.9%	31.3%	23.3%	16.4%	60.2%	-0.04	-0.174	1548
cox2	33.2%	28.5%	25.0%	13.3%	61.7%	0.076	-0.305	684
cox3	30.0%	28.8%	26.7%	14.5%	58.8%	0.020	-0.294	784
atp6	32.3%	28.5%	28.3%	10.9%	60.8%	0.063	-0.446	681
atp8	37.7%	29.9%	24.5%	7.8%	67.6%	0.116	-0.515	204
nad3	31.3%	31.0%	26.1%	11.5%	62.3%	0.005	-0.389	348
nad4	33.6%	28.4%	27.6%	10.3%	62.0%	0.083	-0.457	1378
nad4l	31.3%	33.3%	25.3%	10.1%	64.6%	-0.031	-0.429	297
nad5	33.4%	28.7%	28.0%	9.9%	62.1%	0.076	-0.478	1824
nad6	22.9%	44.3%	6.9%	25.8%	67.2%	-0.318	0.576	519
Cob	30.2%	28.5%	29.4%	12.0%	58.7%	0.028	-0.421	1144
tRNSs	34.8%	31.0%	15.8%	18.4%	65.8%	0.057	0.076	1499
rRNAs	38.6%	25.3%	19.7%	16.4%	63.9%	0.208	0.092	2532
CR	35.6%	29.0%	24.1%	11.3%	64.6%	0.103	-0.364	915

Relative synonymous codon usage (RSCU) shows that serine and leucine are encoded by six synonymous codons, and the remaining amino acids are encoded by two or four synonymous codons, which is consistent with the mitochondrial genome study of *N. andersoni* (Liu *et al*., 2022). Moreover, the most commonly used codons are Leu-CUA (259), Ile-AUU (191), Ile-AUC (173), Met-AUA (187), and the synonymous codon usage of each amino acid, more favor codons whose third codon position is related to adenine (Fig. 2, Table II).

Ribosomal RNAs, transfer RNAs and CR

The lengths of 12S rRNA and 16S rRNA are 956 bp and 1576 bp, respectively, they are sandwiched by trnaF and trnaL2 and separated by trnaV. The AT-skew value was positive (0.205), and GC-skew value was negative (−0.091) which revealed more adenine and cytosine nucleotide prevalence among rRNAs (Table III, IV). The tRNAs are between 59 and 75 bp in length. The mitochondrial genome of *N. lotipes* encodes a total of 22 tRNAs, of which14 tRNAs were encoded by heavy (H) strand, 8 tRNAs were encoded by the Light (L) strand. Among the 22 tRNAs encode 20 amino acids, of which Leu and Ser are encoded by two anticodons, TAA, TAG and TGA, GCT, respectively (Table III). Except for trnaS1, which lacks the dihydrouridine arm, the secondary structures of all tRNAs are cloverleaf structures. Loss of the dihydrouridine arm is common in the mitogenomes of many mammal animals (Wolstenholme, 1992). The length of L-strand replication origin (OL) is 31bp, located between trnN and trnaC, and had the potential to fold into a stable stem-loop secondary structure. The control region (CR) is 914 bp in length and is sandwiched by trnP and trnaF. Three domains were defined in *N. lotipes* mitochondrial genome control region: the extended termination associated sequence (ETAS) domain, the central conserved domain (CD) and the conserved sequence block (CSB) domain (Zhang *et al*., 2009).

Phylogenetic analysis

Based on 13 PCGs and 2 rRNAs of 23 species, we performed a phylogenetic analysis using a partition model under the ML method, and obtained a phylogenetic tree (Fig. 3A). Considering that the evolution rates of different codon sites are different (Rokas and Charlesworth, 2001), we partitioned not only different genes but also different sites of codons for each PCG in the partition model. A previous study showed that nad6 has high heterogeneity and consistently poor phylogenetic performance (Miya and Nishida, 2000). Thus, we constructed a phylogenetic tree based on PCGs excluding nad6 using a partition model under the ML and BI method, respectively (Fig. 3B, C).

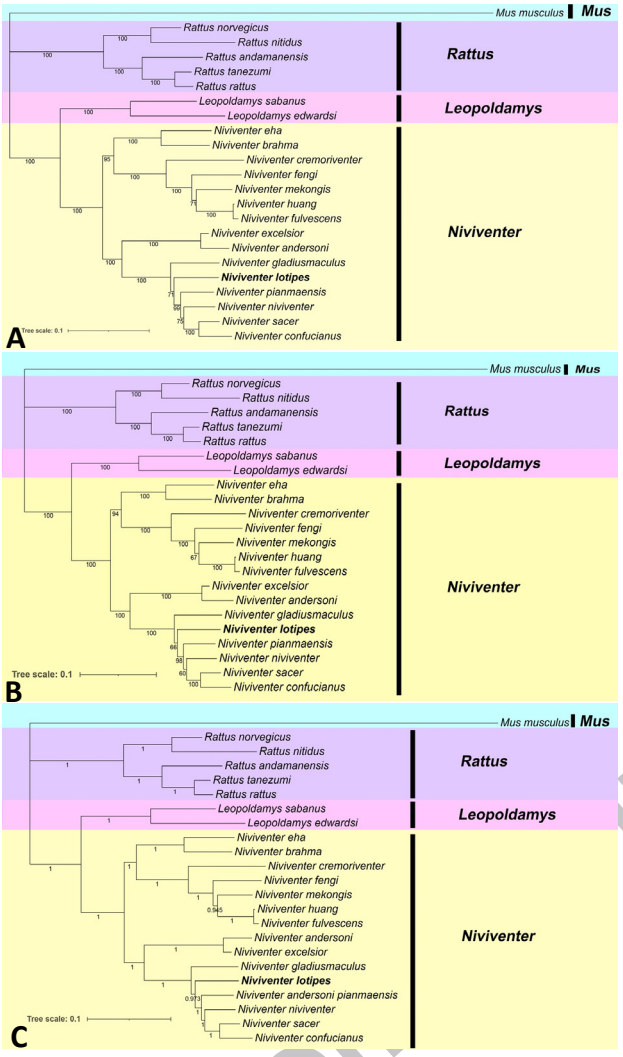

Fig. 3. Maximum likelihood analysis was performed based on (A) 12, (B) 13 protein-coding genes and (C) 12 PCGs and 2 rRNAs from 23 species.

The topology of the three phylogenetic trees is the same. The bootstrap value of the phylogenetic tree containing nad6 in the ML analysis (bootstrap value=60 - 100) was lower than the bootstrap value of the phylogenetic tree that did not contain nad6 (bootstrap value=71-100). Both the ML analysis and the BI analysis indicated that the genus *Niviventer* was divided into four groups, which was consistent with the previous phylogenetic analysis based on one or several mitochondrial and nuclear genes (Lu *et al*., 2015; Zhang *et al*., 2016; Ge *et al*., 2021). Among them *N. brahma* and *N. eha* form a division, and *N. andersoni* and *N. excelsior* form the other, which is also consistent with previous findings (Zhang *et al*., 2016; Liu *et al*., 2022). In addition, *N. huang* is phylogenetically closest to *N. fulvescens*, and form a division with *N. mekongis*, *N. fengi*, and *N. cremoriventer*. The last division is formed by *N. gladiusmaculus*, *N. lotipes*, *N. pianmaensis*, *N. sacer*, *N. confucianus*, *N. niviventer,* where *N. sacer* is phylogenetically closest to *N. confucianus*.

In this study, we obtained the first complete mitochondrial genome of *N. lotipes* and performed phylogenetic analysis based on 13 PCGs and 2 rRNA genes of *N. lotipes*. Compared with previous phylogenetic analysis results based on single or several mitochondrial genes, the results of this study provide a different perspective on the phylogenetic positioning of *N. lotipes* and further explore phylogenetic relationships within the genus *Niviventer*.

However, it is impractical to fully elucidate the phylogeny of the genus *Niviventer* based solely on mitochondrial genomic data. In the phylogenetic analysis using nuclear genes and mitochondrial genes respectively, the results are often inconsistent (Lu *et al*., 2015; Zhang *et al*., 2016; Ge *et al*., 2021), which suggests that there may be imperfect taxonomy and inter-specific gene flow within the genus *Niviventer* (Zhang *et al*., 2016; Barbosa *et al*., 2018; Ge *et al*., 2021). Therefore, more genetic data of the genus *Niviventer*, especially complete nuclear genome data, are still needed. Of course, morphological data is essential in species classification, and only when molecular data and morphological data are combined can the phylogenetic analysis of the genus *Niviventer* be better completed.

CONCLUSION

We sequenced and annotated the first mitochondrial whole genome sequence of *N. lotipes*. The mitochondrial genome structure of *N. lotipes* is consistent with other murine species and most mammals. The results of phylogenetic analysis showed that the genus *Niviventer* was divided into four groups, in which *N. lotipes* was clustered within the genus *Niviventer* and formed a division with *N. gladiusmaculus*, *N. pianmaensis*, *N. sacer*, *N. confucianus* and *N. niviventer*. The mitochondrial whole genome sequence of *N. lotipes* at the molecular level provides an important complement to the phylogenetic relationship of the genus *Niviventer*.

DECLARATIONS

Acknowledgement

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IRB approval

The experiment was approved by the Institutional Review Board (IBR number, IACUC-DWZX-2021-401).

Ethics statement

The specimen collection protocol was approved by Laboratory Animal Welfare Ethics Committee of Mudanjiang Normal University and licensed by the local government. The specimens collected were neither non-protected animals nor endangered.

Data availability

Data will be made available on request.

Generative AI and AI-assisted technology statement

The authors have declared that no generative AI or AI-assisted technologies were used to create this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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