The phylogeny of the Anderson's White-bellied Rat (*Niviventer andersoni*) based on complete mitochondrial genomes provides insight into its evolutionary history

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Abstract

Anderson's White-bellied Rat, *Niviventer andersoni* (Thomas, 1911) (Muridae, *Niviventer*) is an species endemic to China. In the present study, we have sequenced the first complete mitochondrial genome of *N. andersoni* using next-generation sequencing. The 16,291 bp mitochondrial genome consists of 22 transfer RNA genes, 13 protein-coding genes (PCGs), two ribosomal RNA genes, and one non-coding control region (D-Loop). Phylogenetic analyses of the nucleotide sequences of all 13 PCGs, PCGs minus ND6 and the entire mitogenome sequence except for the D-loop, produce nearly identical, well-resolved topologies. Our results support that *N. andersoni* clustered with *N. excelsior* and form a sister group with *N. confucianus*, and they statistically reject the hypothesis from one cytochrome b (cytb) gene tree that *N. confucianus* is sister to *N. fulvescens*. Our research may be helpful to further reconsideration of clearer taxonomy and improve our understanding of mitogenomic evolution in the genus *Niviventer*.

1. Introduction

Anderson's White-bellied Rat*, Niviventer andersoni* belongs to genus *Niviventer* , family Muridae, and order Rodentia. *N. andersoni* is a species endemic to China, and has the largest body-size when compared with congeneric species of *Niviventer* [1]. They live in various kinds of forest in both lowlands and mountains [2]. Fossil records showed that this species extended to the low altitude regions of Southeast China during the late Quaternary in Chongqing and Guizhou Provinces, suggesting its potential to expand southward the climate turned colder [3,4].

Niviventer contains 17 recognized species, with another 65 are recognized as synonyms, occurring from the Himalayas and China to the Great Sunda Islands [5]. All *Niviventer* species are distinguished from other murid rodents by the long, slender, flat craniums and the tail-tip on tails [6]. They inhabit a variety of habitats ranging from damp forests to dry valleys. They are also among the most common infectious agents in humans [7]. According to previous studies (Musser, 1981), the *Niviventer* was separated into two primary divisions: the *N. andersoni* -Division and the*N. niviventer* -Division [8]. Phylogenetic trees based on mitochondrial cytochrome b (cytb) gene showed that *N. andersoni*and *N. excelsior* were clustered together and comprised the*N. andersoni* -Division [6]. Meanwhile, *N. fulvescens*and *N. cremoriventer* were initially clustered together, with*N. confucianus* as the next closest relative and formed the sister group of, *N. niviventer* -Division. However, single gene sequences are sometimes limited in their provision of useful

data, since each gene evolves under different evolutionary pressures and time scales [9]. Compared to single mitochondrial gene sequences, complete mitochondrial genome sequences can provide improved resolution and sensitivity for investigations into the evolutionary relationships between closely related species [21,24].

Up to now, the complete mitogenomes of 4 species within the genus*Niviventer* were available in GenBank. Complete mitochondrial genomes have been used for taxonomic and phylogenetic analyses of diverse animal groups, due to its small size, maternal inheritance, low level of recombination and fast rate of evolution (particularly in rodents) [10-13]. The lack of genetic data has limited our understanding of the phylogeny of *N. andersoni* . In the present study, we sequenced the complete mitochondrial genome of *N. andersoni* . The study has provided the features of the *N. andersoni* mitochondrial genome and has allowed us to compare its phylogenetic relationships with several other rat species. Our findings provide useful genetic data for phylogenetic comparisons to other rodent species with complete mitogenome information.

2. Materials and methods

2.1 Sample collection and genomic DNA extraction

Individuals of *N. andersoni* were collected from Lufeng County, Yunnan province, China (24°57'45.774"; 102deg10'15.7296", H=1875.43m), in August 2018. These individuals were sacrificed and dissected for organ collection. The heart, liver, spleen, lung, kidney and muscle were kept in the cryopreservation tubes directly. All the samples were immediately put in liquid nitrogen for short storage, then transported to the laboratory in dry ice and stored at -80degC. DNA was extracted from the muscle using mitochondrial extraction kit (Solarbio) and stored at -80degC.

2.2 Mitogenome sequencing, assembly and annotation

The mitochondrial DNA was subjected to random PCR (rPCR) as previously described [14]. The purified rPCR products were used to construct the sequencing library and sequenced on HiSeq-PE150 instrument (TIANGEN, Beijing, China). The raw reads were trimmed and filtered using Trimmomatic (Version 0.39) [15]. The cleaned reads were aligned to NCBI non-redundant protein sequence database using BLASTx by DIAMOND [16]. Mitochondrial reads were picked and de novo assembled into a complete mitochondrial genome using Geneious software package (Version 2019.1.1) [17]. Protein coding genes (PCGs) were annotated using the NCBI ORF Finder (*https://www.ncbi.nlm.nih.gov/orffinder/*) and BLASTx with the vertebrate mitochondrial genetic code. The tRNA genes were identified using the tRNAscan-SE Search Server under the default search mode, using the vertebrate mitochondrial genetic code source [18]. Composition skew analysis was calculated according to the formulas: AT skew = (A-T)/ (A + T) and GC skew = (G-C)/ (G + C) [19]. Relative synonymous codon usage (RSCU) values were calculated using CodonW 1.4.2 [20]. The circular mitochondrial genome map of*N. andersoni* was drawn using OGDRAW 1.3.1 [21].

2.3 Phylogenetic analysis

Phylogenetic analysis was performed comparing *N. andersoni* and 12 other rat mitogenomes downloaded from GenBank (Table 1). The nucleotide sequences were aligned using ClustalX with default settings before concatenation by DAMBE (Version 7.2) [22,23]. Models of evolution were evaluated using corrected Aikake Information Criteria (AICc) in jModelTest 2.1.10 to determine the best nucleotide substitution model [24]. Maximum likelihood (ML) analysis of the 13 PCGs in 13 species of rat was also performed using MEGA X [25]. The support values of the ML tree were evaluated via a bootstrap test with 1,000 iterations.

3. Results and discussion

3.1 Genome organization

After quality filtering the raw reads, a total of 1,578,672 high quality clean reads were obtained and used to assemble the *N. andersoni*mitochondrial genome. After obtaining the complete mitochondrial genome sequence of *N. andersoni* we deposited it in NCBI with GenBank accession number MW030174. The mitogenome of *N. andersoni* was a circular DNA molecule which was 16,291 bp in length. As shown in Fig. 1, the mitogenome organization of *N. andersoni* was similar to that of most all other rodents [26].

Thirty-seven typical mitochondrial genes were identified, including 13 PCGs, 22 tRNAs and 2 rRNAs (Table 2). Most of genes were encoded on the Heavy (H)-strand, while ND6 and 8 tRNAs were encoded on the Light (L)-strand.

The total base composition of *N. andersoni* mitochondrial genome was estimated to be 33.7% for A, 25.8% for C, 12.1% for G and 30.0% for T, which makes as AT and GC percentage of 61.6% and 38.4%, respectively, indicating that the mitochondrial genome is biased towards AT (Table 3). Such base composition biases have been reported to play a vital role in the replication and transcription of mitochondrial genome [27]. It also showed a negative GC skew value (-0.347), indicating that C is more common than G whereas the AT skewness was positive (0.092) suggesting that A occurs more frequently than T in the *N. andersoni* mitochondrial genome (Table 3).

3.2 Protein-coding genes (PCGs)

Total length of the 13 PCGs was 11,420 bp, which accounted for 70.1% of the mitogenome. Initiation codons of all PCGs in mitogenome of *N. andersoni* were typical ATN, except for ND1, which started with GTG. All PCGs of the mitogenome of *N. andersoni* terminated with complete (TAA) or truncated (T) stop codons, except for ND2 which terminated with CAT (Table 2). The relative synonymous codon usage (RSCU) values of PCGs are displayed in Table 4, which also shows that the protein-coding gene region has 3,805 codons. According to the RSCU analyses, CUA (L), AUU (I) and AUA (M) were the three most frequently used codons. Leucine, isoleucine and threonine were the most frequent PCG amino acids (Fig. 2). This may explain the negative GC-skew and positive AT-skew of PCGs.

3.3 Ribosomal RNA and Transfer RNA genes

The mitogenome of *N. andersoni* contained the typical 22 tRNA genes throughout the genome and appeared to be highly A+T biased, ranging in length from 59 bp to 75 bp. Among these tRNA genes, eight tRNAs were encoded on the L-strand and the remaining 14 were encoded on the H-strand (Table 2). All the tRNA genes exhibited a typical cloverleaf structure, except trns1, which lacked a dihydroxyuridine arm that had been simplified to a ring shape. Loss of the DHU arm is common in the mitogenomes of many mammal animals [28].

The two rRNA genes (lrRNA, srRNA) encoding the small and large ribosomal subunits, were identified on the L- strand of *N. andersoni* , and were located between $tRNA^{Phe}$ and $tRNA^{Leu}$. The lrRNA and srRNA lengths are 1,567 and 957 bp, respectively. The A+T content of rRNA was 63.43%, and its AT-skew (0.204) and GC-skew (-0.099) showed that more As and Cs were present in the rRNA than As and Gs (Table 3).

3.4 Phylogenetic analysis

Based on 13 PCGs of 13 rat species, we established a phylogenetic tree by maximum likelihood method with 1,000 replications which set *Mus musculus* as outgroup (Fig. 3A). Some researchers have suggested that ND6 gene sequences should be excluded during phylogenetic analysis due to its high heterogeneity and consistently poor phylogenetic performance [29]. Thus, we constructed another phylogenetic tree based on PCGs excluding ND6 (Fig. 3B). The results of the two phylogenetic analyses were almost the same. When compared with other rat species, *N. andersoni* was phylogenetically closer to *N. excelsior* and clustered within genus *Niviventer* .

To further investigate the phylogenetic relationships of *N. andersoni* , the phylogenetic relationships were reconstructed based on the complete mitochondrial genome (Fig. 4). 13 species were used to preform phylogenetic analysis (Table 1). The D-loop region was excluded because of the rapid mutation rate in this region. The maximum likelihood tree was constructed based on the complete mitochondrial genome (except D-loop). The topologies of the maximum likelihood trees constructed based on the complete sequence and PCGs of the mitochondrial genome were identical.Our results were generally congruent with those from the previous study using only the cytb gene, except for the phylogenetic position of *N. confucianus* . Single cytb gene trees in previous studies showed that *N. confucianus* was closer to*N. fulvescens* and *N. cremoriventer* than to *N. andersoni* and *N. excelsior*[6,30,31]. Our results suggest that *N. andersoni* and

N. excelsior clustered together, then with *N. confucianus* , and formed a sister group of *N. fulvescens* and *N. cremoriventer* . Since each gene evolves under different evolutionary pressure and time scale, it has been known that one gene tree for a population may differ from other gene trees for the same population depending on the subjective selection of the genes [9]. The single mitochondrial gene tree and complete mitogenome tree were conflicting, suggesting that phylogenetic tree using complete mitochondrial genomes was warranted.

4. Conclusion

We have sequenced the complete mitochondrial genome of *N. andersoni* for the first time and compared it with closely related species of the family Muridae. The mitochondrial genome structural features were similar to other species in genus *Niviventer* . In the phylogenetic analysis of sequences of the 13 PCGs, the PCGs excluding ND6 and the complete mitogenome without D-loop, *N. andersoni* was consistently the most similar to *N. excelsior* , consistent with previous studies based on single cytb gene sequences and morphological characteristics. Phylogenetic analysis based on the complete mitogenome showed that *N. confucianus* had the closest relationship to *N. andersoni* and *N. excelsior* , rather than*N. fulvescens* and *N. cremoriventer* as previously suggested. The availability of complete mitochondrial genome of *N. andersoni* should be helpful to better understand evolution within the genus *Niviventer,* as well as its relationship to other murid rodents.

Acknowledgements and declaration of interest

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Ethics approval and consent to participate

Sample collection and all the experiments in this study were under the ethics approval by the Yunnan Institute of Endemic Disease Control and Prevention with the animal ethics approval number: DLDXLL2017007.

Data accessibility

The following information was supplied regarding the availability of DNA sequences: The complete mitogenome of *Niviventer andersoni* is deposited in GenBank of NCBI under accession number MW030174.

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Figure Legends

Fig. 1. Mitochondrial genome map of *Niviventer andersoni* .**Fig. 2.** The relative synonymous codon usage (RSCU) of in the mitogenome of *Niviventer andersoni* . The box below the bar chart represents all codons encoding each amino acid, and the height of the column above represents the sum of all RSCU values.**Fig. 3.** The maximum likelihood analyses of phylogenetic relationship based on (A) 13 PCGs and (B) 12 PCGs of 13 rat species.

Fig. 4. The maximum likelihood analyses of phylogenetic relationships based on complete mitochondrial genome minus the D-loop.

Table 1 Complete mitochondrial genomes used for phylogenetic analysis in this study

Table 2 Characteristics of the mitochondrial genome of

Niviventer andersoni

Table 3 Nucleotide composition and AT-GC skewness of the*Niviventer andersoni* mitogenome

Table 4 Relative synonymous codon usage and codon numbers in*Niviventer andersoni* mitochondrial protein-coding genes

Table 1 Complete mitochondrial genomes used for phylogenetic analysis in this study

Genus	Species	Common name	GenBank
Leopoldamys	*Leopoldamys edwardsi*	Edwards's long-tailed giant rat	NC_025670.1
	Leopoldamys sabanus	long-tailed giant rat	MN964122.1
Mus	*Mus musculus*	house mouse	NC_005089.1
Niviventer	*Niviventer confucianus*	Chinese white-bellied rat	NC_023960.1
	Niviventer cremoriventer	dark-tailed tree rat	NC_035822.1
	Niviventer excelsior	large white-bellied rat	NC_019617.1

Genus	Species	Common name	GenBank
Rattus	*Niviventer fulvescens*	Chestnut white-bellied rat	NC_028715.1
	Rattus andamanensis	Indochinese forest rat	NC_046686.1
	Rattus baluensis	summit rat	NC_035621.1
	Rattus norvegicus	Norway rat	NC_001665.2
	Rattus tanezumi	Oriental house rat	NC_011638.1
	Rattus tiomanicus	Malayan field rat	MN126562.1

Gene	Start	Stop	Length(bp)	Start Codon	Stop Codon	Strand	A+T
tRNA-Phe	1	68	68			H	67.6%
s-rRNA	69	1025	957			H	62.6%
tRNA-Val	1026	1093	68			H	58.8%
l-rRNA	1094	2660	1567			H	63.9%
tRNA-Leu2	2661	2735	75			H	54.7%
ND1	2736	3690	955	GTG	T–	H	57.6%
tRNA-lle	3691	3759	69			H	72.5%
tRNA-Gln	3757	3827	71			L	62.0%
tRNA-Met	3831	3899	69			H	52.2%
ND2	3900	4935	1036	ATC	CAT	H	63.7%
tRNA-Trp	4936	5001	66			H	63.6%
tRNA-Ala	5003	5071	69			L	69.6%
tRNA-Asn	5073	5143	71			L	66.2%
tRNA-Cys	5178	5245	68			L	50.0%
tRNA-Tyr	5246	5311	66			L	54.5%
COX1	5313	6857	1545	ATG	TAA	H	59.7%
tRNA-Ser2	6855	6923	69			L	59.4%
tRNA-Asp	6927	6994	68			H	82.4%
COX2	6996	7679	684	ATG	TAA	H	59.5%
tRNA-Lys	7683	7747	65			H	66.2%
ATP8	7748	7951	204	ATG	TAA	H	64.2%
ATP6	7909	8589	681	ATG	TAA	H	62.7%
COX3	8589	9372	784	ATG	T–	H	57.4%
tRNA-Gly	9373	9440	68			H	64.7%
ND3	9441	9788	348	ATC	TAA	H	62.9%
tRNA-Arg	9790	9857	68			H	80.9%
ND4L	9860	10156	297	ATG	TAA	H	63.3%
ND4	10150	11527	1378	ATG	T–	H	62.0%
tRNA-His	11528	11595	68			H	73.5%
tRNA-Ser1	11596	11654	59			H	62.7%
tRNA-Leu1	11654	11724	71			H	66.2%
ND5	11725	13554	1830	ATA	TAA	H	61.3%
ND6	13532	14050	519	ATG	TAA	L	61.3%
tRNA-Glu	14051	14119	69			L	69.6%
CYTB	14125	15283	1159	ATG	TAA	H	58.3%
tRNA-Thr	15269	15335	67			H	68.7%
tRNA-Pro	15336	15402	67			L	62.7%
D-loop	15403	16291	889			H	64.2%

Table 2 Characteristics of the mitochondrial genome of *Niviventer andersoni*

Table 3 Nucleotide composition and AT-GC skewness of the *Niviventer andersoni* mitogenome

Niviventer andersoni	Size (bp)	A	G	T	C	A+T	AT skewness	GC skewness
mitogenome	16291	33.65	12.53	27.97	25.85	61.62	0.092	-0.347
PCGs	12309	28.96	11.51	27.29	25.01	56.25	0.030	-0.370
tRNAs	1499	34.62	18.55	30.29	16.54	64.91	0.067	0.057
rRNAs	2524	38.19	16.48	25.24	20.09	63.43	0.204	-0.099
Control region	889	34.31	11.36	29.92	24.41	64.23	0.068	-0.365

Table 4 Relative synonymous codon usage and codon numbers in*Niviventer andersoni* mitochondrial protein-coding genes

Codon	Count	RSCU	Codon	Count	RSCU	Codon	Count	RSCU	Codon	Count	RSCU
UCA(S)	115	1.83	AUG(M)	32	0.28	GGU(G)	35	0.67	UAG(*)	0	0.00
UCC(S)	72	1.14	AUA(M)	198	1.72	GGG(G)	22	0.42	UAA(*)	8	4.00
UCG(S)	10	0.16	AAC(N)	102	1.31	GGC(G)	59	1.12	AGA(*)	0	0.00
UCU(S)	55	0.87	AAU(N)	54	0.69	GGA(G)	94	1.79	AGG(*)	0	0.00
ACA(T)	159	2.05	CCU(P)	35	0.70	CAC(H)	71	1.46	GCU(A)	67	1.11
ACU(T)	59	0.76	CCG(P)	4	0.08	CAU(H)	26	0.54	GCG(A)	8	0.13
ACC(T)	86	1.11	CCC(P)	52	1.04	AUU(I)	200	1.08	GCC(A)	91	1.50
ACG(T)	6	0.08	CCA(P)	109	2.18	AUC(I)	170	0.92	GCA(A)	76	1.26
GUU(V)	35	0.86	CAA(Q)	81	1.88	AAA(K)	94	1.86	UGU(C)	5	0.33
GUG(V)	15	0.37	CAG(Q)	5	0.12	AAG(K)	7	0.14	UGC(C)	25	1.67
GUC(V)	41	1.01	CGA(R)	43	2.69	CUA(L)	251	2.19	GAU(D)	25	0.66
GUA(V)	71	1.75	CGC(R)	14	0.88	CUC(L)	97	0.85	GAC(D)	51	1.34
UGA(W)	98	1.85	CGG(R)	1	0.06	CUG(L)	19	0.17	GAG(E)	13	0.28
UGG(W)	8	0.15	CGU(R)	6	0.38	CUU(L)	91	0.80	GAA(E)	81	1.72
UAC(Y)	69	1.08	AGC(S)	38	1.33	UUA(L)	116	1.76	UUU(F)	104	0.88
UAU(Y)	59	0.92	AGU(S)	19	0.67	UUG(L)	16	0.24	UUC(F)	132	1.12

Highlights

The first complete mitochondrial genome sequence and annotation of*Niviventer andersoni* .

Phylogenetic relationships among major lineages of Muridae were reconstructed using mitochondrial genomes.