RESEARCH ARTICLE Indian Journal of Animal Research, Volume 58 Issue 12: 2023-2031 (December 2024)

Phylogenetic Insights from the Mitochondrial Genome of *Eothenomys olitor* (Thomas, 1911): A Cricetidae Perspective

Juan Zhang[1#], Chengyao Yang[1#], Chaoyang Luo[2], Wanlong Zhu[1], Yuan Mu[2]

10.18805/IJAR.BF-1833

ABSTRACT

Background: The mitochondrial genome serves as a pivotal molecular marker for delving into systematic relationships and adaptive mechanisms. The genus *Eothenomys* (Cricetidae: Arvicolinae) has consistently attracted attention due to its similar biological characteristics and the analysis of its mitochondrial genome offers a fresh perspective for comprehending the phylogeny and adaptation within Cricetidae.

Methods: We sequenced and analyzed the entire mitochondrial genome of *E. olitor* and utilized the 13 PCGs derived from this genome to delineate the phylogenetic relationships within the Cricetidae and further evaluated the evolutionary rate of each PCG.

Result: The findings revealed that the mitochondrial genome of *E. olitor* was 16,351 bp in length, including 13 PCGs with ATN initiation codons, 22 tRNAs, 2 rRNAs and 1 D-loop. Apart from the *nad6* and 8 tRNAs positioned on the J-strand, the majority of the genetic elements were situated on the N-strand. The phylogenetic examination yielded a robust phylogenetic tree for Cricetidae, but the placement of some genera remained contentious, which needs more integrative and effective data. Among of them, *E. olitor* was a sister of *E. chinensis* and *Eothenomys* was monophyletic. Moreover, a selective analysis showed that evolutionary rates are significant differences among the 13 PCGs. Among these genes, *atp8* a high evolutionary rate, while *cox1* has a low. This research uncovered the mitochondrial genome analysis of *E. olitor*, offering valuable perspectives the phylogeny and adaptation within Cricetidae.

Key words: Adaptive evolution, Cricetidae, *Eothenomys olitor*, Mitochondrial genome, Phylogeny.

INTRODUCTION

Cricetidae, one of the largest families in mammals, was also an extremely diverse group of rodents, which result in the diverse and complex classification and phylogeny (Wilson and Reeder, 2005). This lineage was abundant in a variety of habitats, including grasslands, shrubberies, hills or plains and others. Previous research suggested that Cricetidae species distributed in China can be divided into four main subfamilies: Cricetinae, Gerbillinae, Myospalacinae and Microtinae/Arvicolinae (Luo *et al*., 2000). However, due to different taxonomic views, limited specimens and the large increasing number of species, the classification of extant Cricetidae has been always changing. According to the latest view, Gerbillinae cluster with Muridae and Myospalacinae with Spalacidae, because their morphological traits and habits were significantly different from those of other Cricetidae species (Wilson and Reeder, 2005; Ding, 2017). The taxonomic position of *Arvicolia* has been always discussed, some were support (Abramson *et al*., 2021; Wang *et al*., 2022) and some were against (Steppan and Schenk, 2017; Liu *et al*., 2017). Additionally, Pavlinov and Lissovsky (2012), Wilson *et al*. (2017) and Liu *et al*. (2017, 2020) confirmed that the genus status for *Alexandromys*, *Crazeomys* rather than subgenus. The genus *Eothenomys*, found in the Hengduan mountain regions and adjacent areas, has exhibited rapid adaptive evolution during adaptive radiation. In recent years, more and more new species was identified (Zeng *et al*., 2013; Tang *et al*., 2021; Wang *et al*., 2022). However, the absence of effective molecular markers has resulted in the phylogenetic trees have poor resolution and the interrelationships are controversial (Martínková and Moravec, 2012; Fabre *et al*., 2012; Wang *et al*., 2022). Notably, even the phylogeny of Cricetidae has been paid more and more attention, including species, genera, tribes and subfamilies (Teta *et al*., 2017; Liu *et al*., 2018; Castañeda Rico *et al*., 2023), it still remains controversial. Therefore, there was an urgent need for more effective molecular markers to further investigate the phylogenetic and evolutionary relationships of the genus *Eothenomys* and its Cricetidae.

[1]Key Laboratory of Ecological Adaptive Evolution and Conservation on Animals-Plants in Southwest Mountain Ecosystem of Yunnan Province Higher Institutes College, School of Life Sciences, Yunnan Normal University, Kunming-650500, China.
[2]Institute of Eastern-Himalaya Biodiversity Research, Dali University, Dali-671003, China.

Corresponding Author: Wanlong Zhu, Key Laboratory of Ecological Adaptive Evolution and Conservation on Animals-Plants in Southwest Mountain Ecosystem of Yunnan Province Higher Institutes College, School of Life Sciences, Yunnan Normal University, Kunming-650500, China. Email: zwl_8307@163.com

How to cite this article: Zhang, J., Yang, C., Luo, C., Zhu, W. and Mu, Y. (2024). Phylogenetic Insights from the Mitochondrial Genome of *Eothenomys olitor* (Thomas, 1911): A Cricetidae Perspective. Indian Journal of Animal Research. 58(12): 2023-2031. doi: 10.18805/IJAR.BF-1833.

Submitted: 20-06-2024 **Accepted:** 16-08-2024 **Online:** 06-09-2024

Mitochondrial DNA (mtDNA) was the most efficient and sensitive phylogenetic marker (Ding *et al*., 2016; Kiraz *et al*., 2024). Specifically, mtDNA evolved separately from nuclear DNA and has the characteristics of maternal inheritance, conservative organization and high evolutionary rate, thus, it has always been used for phylogenetic construction and population genetic differentiation and other related studies (Xin *et al*., 2017; Wang *et al*., 2018). Additionally, complete mitochondrial genome datasets provide robust insights into phylogenetic relationships (Xin *et al*., 2015; Wang *et al*., 2021).

In this research, we successfully conducted the entire mitochondrial genome analysis of *E. olitor* and subsequently utilized the 13 PCGs to elucidate the phylogenetic relationships within the Cricetidae. Furthermore, we have conducted a detailed examination of the evolutionary rates across these PCGs. The objectives of our research are two-fold: (1) decipher the mitogenomic structure of *E. olitor* and (2) investigate its phylogenetic position and adaptive traits within Cricetidae.

MATERIALS AND METHODS

Sample collection, DNA extraction and sequencing

The research samples were collected in May 2023 at the Dashanbao Black-necked Crane National Nature Reserve in China (Fig 1) and then sent to Yunnan Normal University for a one-year study. The liver samples were preserved in 100% ethanol before being dispatched to Shanghai Personalbio Technology Co. for the genome extraction and sequencing. The general steps were as follows: Firstly, the total DNA of the liver was extracted by the improved CTAB method and then its quality and concentration were detected using the Thermo Scientific NanoDrop 2000 and agarose gel electrophoresis. Secondly, the qualified DNA was used to construct a library with various insert fragments of 400 bp utilizing the Whole Genome Shotgun (WGS) approach, followed by Paired-end sequencing (PE, 2×150 bp) using Illumina NovaSeq Next-generation sequencing (NGS). Thirdly, data quality control was conducted using Fastp (Chen *et al*., 2018) to obtain high-quality sequences. Fourthly, the sequence was assembled and identified using a suite of bioinformatics tools: A5-miseq v20150522 (David *et al*., 2014), SPAdes v3.9.0 (Bankevich *et al*., 2012), Mummer v3.1 (Delcher *et al*., 2003) and Pilon v1.18 (Walker *et al*., 2017) to deduce the definitive mitochondrial sequence. Subsequently, submit the sequence to NCBI (GenBank accession number: NC_086591).

Sequence annotation and analyses

Following the splicing process, the refined sequence was deposited to the MITOS (http://mitos.bioinf.uni-leipzig.de/) for functional annotation and download tRNA to check its predicted secondary structures (Matthias *et al*., 2012). Additionally, we conducted an analysis of the base composition and skewness across the whole genome, PCGs and rRNAs utilizing the PhyloSuite v1.2.3 (Zhang *et al*., 2020). The strand asymmetry indices were derived using established formula (Nicole and Thomas, 1995; Hassan *et al*., 2023). The entire mitochondrial genome′s structure was visually represented utilizing the CGView (Paul and David, 2005).

Phylogenetic and selection analyses

To investigate the position of *E. olitor* and the phylogenetic relationships of Cricetidae, mitochondrial genomes from 22 genera of Cricetidae were obtained, complemented by outgroup representatives from Muridae and Spalacidae sourced from NCBI (Table 1). The phylogenetic tree was then constructed based on 13 PCGs from 98 species employing Maximum Likelihood (ML) methods in PhyloSuite v1.2.3, resulting in a single topology with robust support (Zhang *et al*., 2020). This tree wasconducted in IQ-TREE

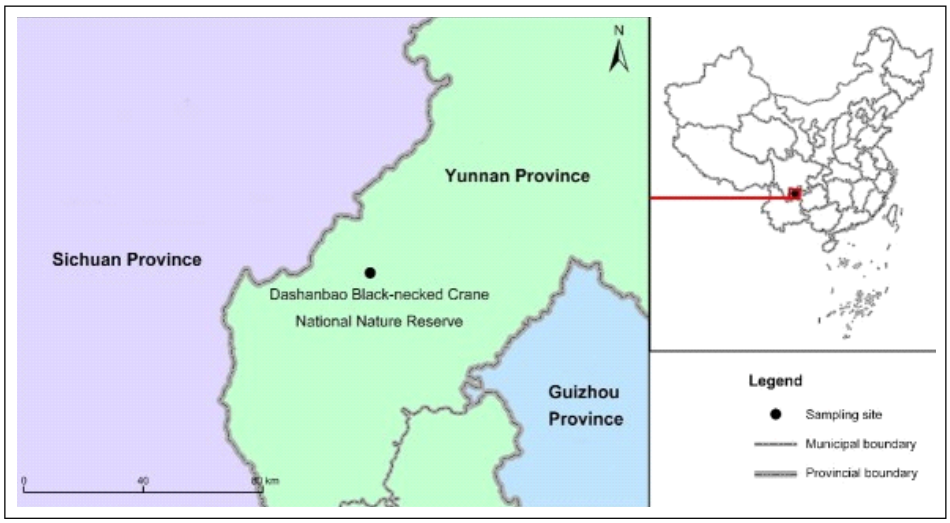

Fig 1: Sampling site of *E. olitor*.

Table 1: The species name and ID number retrieved from NCBI.

Species	ID	Species	ID
Alexandromys_fortis	NC_015243	*Eothenomys_chinensis*	NC_013571
Alexandromys_fortis_fortis	NC_015241	*Eothenomys_olitor*	NC_086591
Alexandromys_limnophilus	MN487090	*Caryomys_eva*	MN487084
Alexandromys_kikuchii	NC_003041	*Caryomys_inez*	KU200225
Alexandromys_montebelli	NC_059928	*Dicrostonyx_groenlandicus*	NC_034313
Lasiopodomys_brandtii	NC_057534	*Dicrostonyx_hudsonius*	NC_034307
Lasiopodomys_mandarinus	NC_025283	*Dicrostonyx_torquatus*	NC_034646
Lasiopodomys_gregalis	MT381937	*Ondatra_zibethicus*	NC_036035
Lasiopodomys_raddei	MT381929	*Myopus_schisticolor*	MT381931
Neodon_clarkei	MN487089	*Arvicola_amphibius*	MN122834
Neodon_medogensis	MN487087	*Peromyscus_eremicus*	NC_047188
Neodon_leucurus	MN487079	*Peromyscus_pectoralis*	NC_035598
Neodon_sikimensis	NC_035503	*Peromyscus_attwateri*	NC_035593
Neodon_forresti	MN487103	*Peromyscus_aztecus*	NC_035596
Neodon_irene	NC_016055	*Peromyscus_boylii*	MZ433362
Neodon_linzhiensis	MN487081	*Peromyscus_megalops*	NC_035613
Neodon_fuscus	NC_040138	*Peromyscus_melanophrys*	NC_035611
Microtus_levis	NC_008064	*Peromyscus_californicus*	OP524493
Microtus_rossiaemeridionalis	DQ015676	*Peromyscus_crinitus*	NC_035614
Microtus_arvalis	NC_038176	*Peromyscus_maniculatus*	NC_039921
Microtus_guentheri	MT381935	*Peromyscus_polionotus*	NC_035571
Microtus_socialis	MT381932	*Peromyscus_leucopus*	NC_037180
Microtus_daghestanicus	MT381933	*Cricetulus_barabensis*	NC_053822
Microtus_thomasi	NC_057558	*Cricetulus_griseus*	NC_007936
Microtus_agrestis	NC_041250	*Cricetulus_sokolovi*	NC_056331
Microtus_cabrerae	NC_057556	*Cricetulus_longicaudatus*	NC_025330
Microtus_californicus	MT381939	*Cricetulus_migratorius*	KT918407
Microtus_ochrogaster	NC_027945	*Cricetus_cricetus*	NC_037888
Microtus_montanus	NC_068211	*Tscherskia_triton*	NC_013068
Microtus_richardsoni	NC_049220	*Mesocricetus_auratus*	NC_013276
Microtus_chrotorrhinus	NC_057557	*Cricetulus_kamensis*	NC_024592
Microtus_miurus	MT381943	*Phodopus_roborovskii*	NC_031809
Proedromys_liangshanensis	NC_013563	*Phodopus_sungorus*	NC_042823
Ellobius_fuscocapillus	MT483991	*Eospalax_baileyi*	NC_018098
Ellobius_lutescens	MT483992	*Eospalax_cansus*	NC_021129
Ellobius_talpinus	NC_054160	*Eospalax_rothschildi*	NC_018535
Aschizomys_macrotis	MT381923	*Eospalax_rufescens*	NC_047427
Myodes_centralis	MT381940	*Eospalax_smithii*	NC_048987
Myodes_glareolus	NC_024538	*Eospalax_fontanierii*	NC_045904
Myodes_rutilus	MK482363	*Myospalax_aspalax*	NC_026915
Aschizomys_lemminus	MT381922	*Myospalax_psilurus*	NC_026034
Alticola_olchonensis	MT381924	*Meriones_meridianus*	NC_027684
Alticola_strelzovi	MT381925	*Meriones_unguiculatus*	NC_023263
Crazeomys_regulus	NC_016427	*Meriones_libycus*	NC_027683
Crazeomys_rufocanus	NC_029477	*Brachiones_przewalskii*	KT834972
Eothenomys_eleusis	NC_065401	*Psammomys_obesus*	NC_037509
Eothenomys_miletus	NC_030330	*Rhombomys_opimus*	MK359635
Eothenomys_cachinus	MN487067	*Meriones_tamariscinus*	NC_034314
Eothenomys_melanogaster	NC_027418	*Mus_spretus*	NC_025952

(Nguyen *et al*., 2015), with bootstrap values (BS) above 75% considered significant, indicating higher reliability the topology (Wang *et al*., 2023). Subsequently, the definitive trees were rendered and refined with the assistance of FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/Figtree/).

To evaluate selective pressures acting on 13 PCGs across 98 species, we utilized Code ML in PAML v4.9 (Yang, 2007) to determine the evolutionary rates via the free-ratio model (dN/dS). To statistically test for variations in evolutionary rates among the 13 PCGs, we extracted the dN/dS ratios for all species. Subsequently, a one-way ANOVA analysis using the R software (https://www.r-project.org/) to identify significant differences in it. Notably, to ensure the validity of the statistical analysis, data points where dN or dS was zero and where dN/dS was greater than or equal to one, were specifically excluded.

RESULTS AND DISCUSSION

Genome structure, composition and skewness

The mitochondrial genome of *E. olitor* was fully sequenced, measuring 16,351 bp, following a typical rodent structure.

Table 2: Correlation analysis of mitochondrial genome features in *E. olitor*.

Feature	Strand	Position (Start-end)	Length (bp)	Initiationcodon	Stopcodon	Anticodon	Intergenicnucleotide
trnF	N	1-66	66			GAA	2
rrnS	N	69-1,016	948				
trnV	N	1,017-1,087	71			TAC	
rrnL	N	1,088-2,655	1,568				1
trnL2	N	2,657-2,731	75			TAA	-3
nad1	N	2,729-3,688	960	ATA	TAG		-2
trnI	N	3,687-3,755	69			GAT	-3
trnQ	J	3,753-3,824	72			TTG	
trnM	N	3,825-3,893	69			CAT	
nad2	N	3,894-4,928	1,035	ATC	TAG		2
trnW	N	4,931-4,997	67			TCA	1
trnA	J	4,999-5,067	69			TGC	2
trnN	J	5,070-5,139	70			GTT	2
OL	N	5,142-5,171	30				-1
trnC	J	5,171-5,238	68			GCA	
trnY	J	5,239-5,305	67			GTA	1
cox1	N	5,307-6,851	1,545	ATG	TAA		-3
trnS2	J	6,849-6,917	69			TGA	3
trnD	N	6,921-6,988	68			GTC	1
cox2	N	6,990-7,673	684	ATG	TAA		3
trnK	N	7,677-7,740	64			TTT	
atp8	N	7,741-7,944	204	ATG	TAA		-43
atp6	N	7,902-8,582	681	ATG	TAA		-1
cox3	N	8,582-9,366	785	ATG	TA(A)		-1
trnG	N	9,366-9,434	69			TCC	
nad3	N	9,435-9,782	348	ATT	TAA		1
trnR	N	9,784-9,851	68			TCG	2
nad4l	N	9,854-10,150	297	ATG	TAA		-7
nad4	N	10,144-11,521	1,378	ATG	T(AA)		
trnH	N	11,522-11,589	68			GTG	
trnS1	N	11,590-11,648	59			GCT	-1
trnL1	N	11,648-11,717	70			TAG	
nad5	N	11,718-13,529	1,812	ATA	TAA		-4
nad6	J	13,526-14,050	525	ATG	TAA		
trnE	J	14,051-14,119	69			TTC	5
cytb	N	14,125-15,267	1,143	ATG	TAA		2
trnT	N	15,270-15,336	67			TGT	
trnP	J	15,337-15,404	68			TGG	247
OH	N	15,652-15,934	283				416

In strand, J is the major strand and N is the minor strand.

It consists of 13 PCGs, 22 tRNAs, 2 rRNAs and 1 D-loop (Table 2, Fig 2). Notably,1 PCG (*nad6*) and 8 tRNAs (*trnQ*, *trnA*, *trnN*, *trnC*,*trnY*, *trnS2*, *trnE*, *trnP*) were positioned on the J-strand, while the majority of genetic elements were on the N-strand (Table 2). The genome's nucleotide composition showed A + T content at 59.09% and G + C content at 40.91%, the former was significantly higher than the later. Except *nad4l*, *nad6* and OH, the AT_skew was predominantly positive. Conversely, except OL and *nad6*, the GC_skew was predominantly negative. The smallest AT_skewwas *nad4l* (-0.06) and the largest was OL (0.273). The smallest GC_skew was nad2 (-0.593) and the largest was *nad6* (0.538) (Table 3).

PCGs, tRNAs and rRNAs

The 13 PCGs in the *E. olitor* include *nad1-6* and *nad4l*, *atp6* and *atp8*, *cox1-3* and *cytb*. The size varies from 204 bp (*atp8*) to 1812 bp (*nad5*). PCGs typically begin with ATN codons (ATA, ATC, ATG and ATT) and with TAN codons (Table 2), consistent with other *Eothenomys* as documented in previous researches (Yang *et al*., 2012; Chen *et al*., 2015; Mu *et al*., 2019; Zhu *et al*., 2023). Except *nad4l* and *nad6*, the skewness of the majority of genetic elements predominantly favors As and Cs, reflecting a nucleotide compositional bias (Table 3). Meanwhile, *E. olitor* has 22 tRNAs, varying in size between 59 bp (*trnS1*) and 75 bp (*trnL2*) (Table 2). Notably, the *trnS1* lacks the dihydrouridine (DHU) arm, while other genetic elements can from the cloverleaf structure. In addition to the normal base pairing, G-U pairing exists in 10 tRNAs (*trnR*, *trnN*, *trnQ*, *etc*) (Fig 3). The *rrnS* and *rrnL* are positioned on the N-strand, separated by *trnV* (Table 2), with the highest A + T % content (Table 3).

Phylogenetic and selection analyses

This research examines 13 PCGs across 98 species utilizing ML methods to reconstruct phylogenetic relationships. The resulting tree had strong support, with most nodes having BS values over 75% (Fig 4). The analysis showed *E. olitor* as a sister of *E. chinensis* and the genus *Eothenomys* was monophyletic, divided into two main lineages: (*E. eleusis*, *E. miletus*, *E. cachinus*, *E. melanogaster*) and (*E. olitor*, *E. chinensis*). This classification aligns with previous studies by Liu *et al*. (2012, 2018) and Wang *et al*. (2022). Concurrently, *Neodon*, *Microtus*, *Peromyscus*, *etc*., were also monophyletic. However, some genera in Cricetidae were paraphyletic, especially *Myodes* and *Cricetulus*, aligning with findings by Liu *et al*. (2019), Tang *et al*. (2021) and Wang *et al*. (2022), but different from

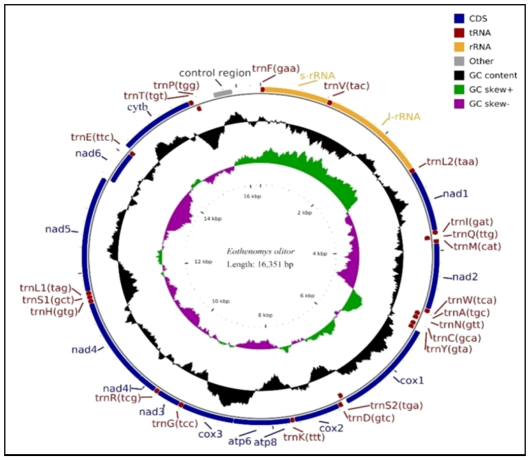

Fig 2: Mitochondrial genome structure loop map of *E. olitor*.

Table 3: The base composition and skewness of whole genome, PCGs, rRNAs of *E. olitor*.

Region	A %	C %	G %	T %	A + T %	G + C %	AT_skew	GC_skew
Whole genome	32.63	27.3	13.61	26.46	59.09	40.91	0.104	-0.335
rrnS	36.39	21.84	18.25	23.52	59.92	40.08	0.215	-0.089
rrnL	37.24	21.75	17.86	23.15	60.4	39.6	0.233	-0.098
nad1	31.04	31.25	11.98	25.73	56.77	43.23	0.094	-0.446
nad2	35.46	31.01	7.92	25.6	61.06	38.94	0.161	-0.593
OL	23.33	26.67	36.67	13.33	36.67	63.33	0.273	0.158
cox1	28.35	27.77	16.57	27.31	55.66	44.34	0.019	-0.253
cox2	32.89	27.49	13.3	26.32	59.21	40.79	0.111	-0.348
atp8	37.25	28.43	8.33	25.98	63.24	36.76	0.178	-0.547
atp6	30.25	30.1	10.87	28.78	59.03	40.97	0.025	-0.47
cox3	27.9	29.94	14.78	27.39	55.29	44.71	0.009	-0.339
nad3	32.76	29.6	11.78	25.86	58.62	41.38	0.118	-0.431
nad4l	26.6	30.3	13.13	29.97	56.57	43.43	-0.06	-0.395
nad4	33.16	29.54	10.89	26.42	59.58	40.42	0.113	-0.461
nad5	31.46	29.03	12.03	27.48	58.94	41.06	0.067	-0.414
nad6	19.62	9.33	31.05	40	59.62	40.38	-0.342	0.538
cytb	29.83	29.4	13.47	27.3	57.13	42.87	0.044	-0.371
OH	22.26	27.92	20.14	29.68	51.94	48.06	-0.143	-0.162

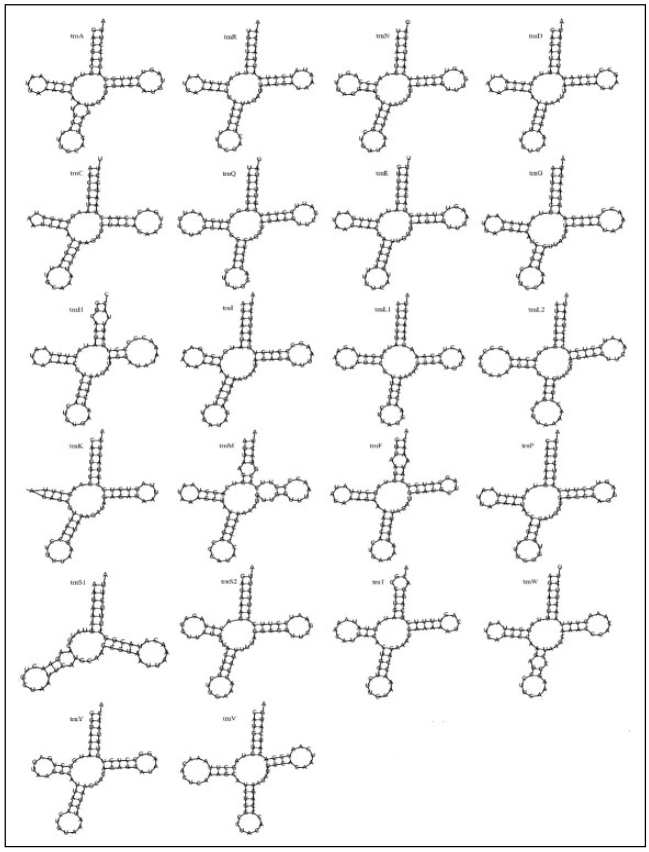

Fig 3: The secondary structure chart of 22 tRNAs in *E olitor*.

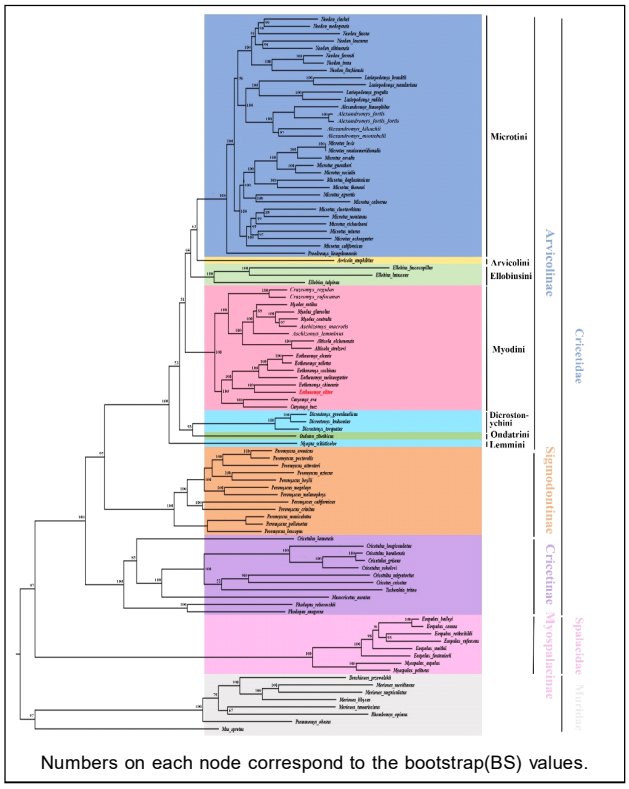

Fig 4: Phylogenetic tree inferred from 13 PCGs of 98 species by using ML.

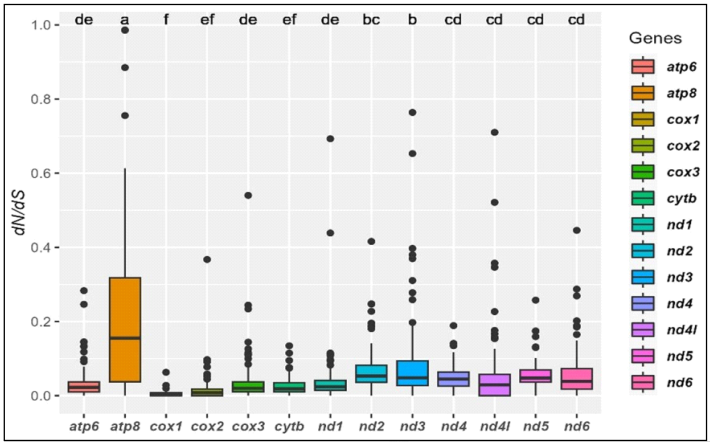

Fig 5: Boxplot of dN/dS of the 13 PCGs from 98 species. These data were calculated with the free-ratio model.

Martínková and Moravec (2012). Notably, the phylogeny of Cricetidae remains controversial due to adaptations to diverse environments, variations in evolutionary rates among lineages, inconsistencies in DNA markers and unresolved node. For example, the phylogeny proposed by Wang *et al*. (2022) (6517 nucleus genes, 122 taxa) supports the monophyletic status of Arvicolinae, including *Microtus*. Conversely, the phylogeny of Arvicolinae by Martínková and Moravec (2012) (mitochondrial and nuclear DNA) indicates the paraphyly of *Microtus*. The phylogenies reconstructed by Luo *et al*. (2004) and Elena *et al*. (2008) for Arvicolinae using *cytb* tentatively position *Ondatra* as a sister of *Arvicola*, contrasting with our research. Consequently, future research on Cricetidae necessitates the use of effective molecular markers and comprehensive sampling to enhance our understanding of their phylogenetic relationships.

Organisms frequently evolve adaptive mechanisms to survive, focusing on energy metabolism crucial in various environments. Mitochondrial genes have attracted much attention for their selective role in adaptive evolution because of its crucial function on energy metabolism (Wu *et al*., 2022). The selection analysis showed *atp8* has high dN/dS average values, indicating accelerated evolution or relaxed selective constraint, which is consistent with research on other species like *Orthoptera* insects (Chang *et al*., 2020) and *Lamprologus* (Wang *et al*., 2023). In contrast, *cox1* has low dN/dS average values, suggesting stronger evolutionary constraints (Li *et al*., 2021) (Fig 5). Therefore, *cox1* and genes with similar constraints are commonly used for phylogenetic reconstructions. Additionally, there were significant differences ($P<0.05$) in the evolutionary rates of the genes (Fig 5).

CONCLUSION

The current research represents the initial comprehensive sequencing and in-depth analysis of the entire mitochondrial genome of *E. olitor* and the phylogenetic position was assessed using ML methods, offering a fresh perspective for the phylogenetic and adaptive studies within the genus *Eothenomys* and Cricetidae. However, disagreements persist regarding the classification and placement of genera like *Myodes*, *Cricetulus* and *Ondatra* within Cricetidae. Therefore, there were critical needs for more comprehensive and efficient data to enhance understanding of the phylogenetic and evolutionary relationships among Cricetidae genera.

ACKNOWLEDGEMENT

This work was financially supported by the National Natural Scientific Foundation of China (32160254, 32360119), Yunnan Fundamental Research Projects (202401AS070039), Yunnan Ten Thousand Talents Plan Young and Elite Talents Project (YNWR-QNRC-2019-047).

Ethical approval

All animal procedures were within the rules of Animals Care and Use Committee of School of Life Sciences, Yunnan Normal University. This study was approved by the committee (13-0901-011).

Conflict of interest

All authors declare that they have no conflicts of interest.

REFERENCES

Abramson, N.I., Bodrov, S.Y., Bondareva, O.V., GeneltYanovskiy, E.A., Petrova, T.V. (2021). A mitochondrial genome phylogeny of voles and lemmings (Rodentia: Arvicolinae): Evolutionary and taxonomic implications. Plos One. 16: 0248198-0248198.

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A.A., Dvorkin, M., Kulikov, A.S., Lesin, V.M., Nikolenko, S.I., Pham, S., Prjibelski, A.D. (2012). SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. Journal of Computational Biology: A Journal of Computational Molecular Cell Biology. 19: 455-477.

CastañedaRico, S., Parker, L.D., Sánchez, E., RivasTrasvina, S., Hawkins, M.T.R. Edwards, C.W., Maldonado, J.E. (2023). Novel genomic resources contribute to the systematics of threatened arboreal deer mice of the genus *Habromys* Hooper Musser, 1964 (Cricetidae, Neotominae) within a Neotomine-Peromyscine phylogeny. Zookeys. 1179: 157-168.

Chang, H.H., Qiu, Z.Y., Yuan, H., Wang, X.Y., Li, X.J. Sun, H.M., Guo, X.Q., Lu, Y.C., Feng, X.L., Majid, M., Huang, Y. (2020). Evolutionary rates of and selective constraints on the mitochondrial genomes of Orthoptera insects with different wing types. Molecular Phylogenetics and Evolution. 145: 106734.

Chen, S.D., Chen, G.Y., Wei, H.X., Wang, Q. (2015). Complete mitochondrial genome of the Père David's Vole, *Eothenomys melanogaster* (Rodentia: Arvicolinae). Mitochondrial DNA Part A. 27: 2496-2497.

Chen, S.D., Zhou, Y.Q., Chen, Y.R., Gu, J. (2018). Fastp: An ultra-fast all-in-one FASTQ preprocessor. Bioinformatics (Oxford, England). 34: i884-i890.

David, C., Guillaume, J., Aaron, E.D. (2014). A5-miseq: An updated pipeline to assemble microbial genomes from Illumina MiSeq data. Bioinformatics (Oxford, England). 31: 587-589.

Delcher, A.L., Salzberg, S.L., Phillippy, A.M. (2003). Using MUMmer to identify similar regions in large sequence sets. Current Protocols in Bioinformatics. 10: 1-18.

Ding, L. (2017). Characteristics of the Mitochondrial Genomes and Phylogenetic Relationship of *Cricetulus* Species. MaD Thesis, Lanzhou University.

Ding, L., Li, W.J., Liao, J.C. (2016). Characterization of the complete mitochondrial genome of *Phodopus roborovskii* (Rodentia: Cricetidae) and systematic implications for Cricetinae phylogenetics. Biochemical Systematics and Ecology. 69: 226-235.

Elena, V.B., Boris, K., Bernd, H., William, F.H. (2008). Mitochondrial phylogeny of arvicolinae using comprehensive taxonomic sampling yields new insights. Biological Journal of the Linnean Society. 94: 825-835.

Fabre, P.H., Hautier, L., Dimitrov, D., Douzery-Emmanuel, J.P. (2012). A glimpse on the pattern of rodent diversification: A phylogenetic approach. BMC Evolutionary Biology. 12: 1-19.

Hassan, M.A., Shen, R.R., Zhang, L., Sheikh, T., Xing, J.C. (2023). Mitogenomic phylogeny of nymphalid subfamilies confirms the basal clade position of Danainae (Insecta: Lepidoptera: Nymphalidae). Ecology and Evolution. 13: 10263-10263.

Kiraz, S., Koncagül, S., Vural, M.E., Koyun, H. (2024). A mitochondrial DNA-based molecular phylogenetics study of the Mahalli goat as a new animal genetic resource in Southern Anatolia in Turkey. Indian Journal of Animal Research. 58(11): 1846-1853. doi: 10.18805/IJAR.BF-1738.

Li, F., Lv, Y.Y., Wen, Z.Y., Bian, C., Zhang, X.H., Guo, S.T., Shi, Q., Li, D.Q. (2021). The complete mitochondrial genome of the intertidal spider (*Desis jiaxiangi*) provides novel insights into the adaptive evolution of the mitogenome and the evolution of spiders. BMC Ecology and Evolution. 21: 72-72.

Liu, S.Y., Chen, S.D., He, K., Tang, M.K., Liu, Y., Jin, W., Li, S., Li, Q., *et al*. (2018). Molecular phylogeny and taxonomy of subgenus *Eothenomys* (Cricetidae: Arvicolinae: *Eothenomys*) with the description of four new species from Sichuan, China. Zoological Journal of the Linnean Society. 186: 569-598.

Liu, S.Y., Jin, W., Liu, Y., Murphy, R.W., Lv, B., Hao H.B., Liao, R., Sun, Z.Y., Tang, M.K., Chen, W.C., Fu, J.R. (2017). Taxonomic position of chinese voles of the tribe arvicolini and the description of 2 new species from Xizang, China. Journal of Mammalogy. 98: 166-182.

Liu, S.Y., Jin, W., Tang, M.K. (2020). Review on the taxonomy of Microtini (Arvicolinae: Cricetidae) with a catalogue of species occurring in China. Acta TheriologicaSinica. 40: 290-301.

Liu, S.Y., Liu, Y., Guo, P., Sun, Z.Y., Murphy, R.W., Fan, Z.X., Fu, J.R., Zhang, Y.P. (2012). Phylogeny of oriental voles (Rodentia: Muridae: Arvicolinae): Molecular and Morphological Evidence. Zoological Science. 29: 610-622.

Liu, Z., Zhang, Q., Wen, Y.C., Min, J.Z., Song, Q.K. (2019). The complete mitochondrial genome of *Microtus fortis pelliceus* (Arvicolinae: Rodentia) from China and its phylogenetic analysis. Mitochondrial DNA Part B. 4: 2039-2041.

Luo, J., Yang, D.M., Suzuki, H., Wang, Y.X., Chen, W.J., Campbell, L.K., Zhang, Y.P. (2004). Molecular phylogeny and biogeography of oriental voles: Genus *Eothenomys* (Muridae, Mammalia). Molecular Phylogenetics and Evolution. 33: 349-362.

Luo, Z.X., Chen, W., Gao, W., Wang, Y.X., Li, C.Y., Li, H. (2000). Fauna sinica: Mammalia. Rodentia Part III: Cricetidae, Chinese academy of sciences. vol. 6. Beijing: Science Press. pp. 522.

Martínková, N. and Moravec, J. (2012). Multilocus phylogeny of Arvicoline voles (Arvicolini, Rodentia) shows small tree terrace size. Folia Zoologica. 61: 254-267.

Matthias, B., Alexander, D., Frank, J., Fabian, E., Catherine, F., Guido, F., Joern, P., Martin, M., Peter, F.S. (2012). MITOS: Improved de novo metazoan mitochondrial genome annotation. Molecular Phylogenetics and Evolution. 69: 313-319.

Mu, Y., Duan, Y.Q., Zhang, D., Wang, Z.K., Zhu, W.L. (2019). The complete mitochondrial genome of the yunnan red-backed vole *Eothenomysmiletus* (Rodentia: Cricetidae) and its phylogeny. Mitochondrial DNA Part B. 4: 1424-1425.

Nguyen, L.T., Schmidt, H.A., von Haeseler, A., Minh, B.Q. (2015). IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution. 32: 268-274.

Nicole, T.P. and Thomas, D.K. (1995). Patterns of nucleotide composition at fourfold degenerate sites of animal mitochondrial genomes. Journal of Molecular Evolution. 41: 353-358.

Paul, S. and David, S.W. (2005). Circular genome visualization and exploration using CGView. Bioinformatics. 21: 537-539.

Pavlinov, I.Y. and Lissovsky, A.A. (2012). The mammals of Russia: A taxonomic and geographic reference. Moscow: KMK Scientific Press Ltd. pp. 613.

Steppan, S.J. and Schenk, J.J. (2017). Muroid rodent phylogenetics: 900-species tree reveals increasing diversification rates. Plos One. 12: e0183070.

Tang, M.K., Chen, Z.H., Wang, X., Chen, Z.X., He, Z.Q., Liu, S.Y. (2021). A summary of phylogenetic systematics studies of Myodini in China (Rodentia: Cricetidae: Arvicolinae). Acta TheriologicaSinica. 41: 71-81.

Teta, P., Cañón, C., Patterson, D.B., Ulyses, J.F.P. (2017). Phylogeny of the tribe Abrotrichini (Cricetidae, Sigmodontinae): Integrating Morphological and Molecular Evidence Into a New Classification. Cladistics. 33: 153-182.

Walker, J.B., Abeel, T., Shea, T., Priest, M., Abouelliel, A., Sakthikumar, S., Cuomo, A.C., Sun, Z.Y., Tang, M.K., Chen, W.C., Fu, J.R. (2017). Pilon: An integrated tool for comprehensive microbial variant detection and genome assembly improvement. Plos One. 9: e112963.

Wang, H.D., Chen, Y., Shi, W., Guo, Y.Y., He, J.H., Chu, Z.J., Zhao, B. (2021). A comprehensive description and evolutionary analysis of testudines mitochondrial genomes. Indian Journal of Animal Research. 55: 1430-1438. doi: 10.18805/IJAR.B-1394.

Wang, J.C., Tai, J.Z., Zhang, W.W., He, K., Lan, H., Liu, H.Y. (2023). Comparison of seven complete mitochondrial genomes from *Lamprologus* and *Neolamprologus* (Chordata, Teleostei, Perciformes) and the phylogenetic implications for Cichlidae. Zookeys. 1184: 115-132.

Wang, X.Y., Liang, D., Wang, X.M., Tang, M.K., Liu, Y., Liu, S.Y., Zhang, P. (2022). Phylogenomics reveals the evolution, biogeography and diversification history of voles in the hengduan mountains. Communications Biology. 5: 1124-1124.

Wang, Z.F., Wang, Z.Q., Shi, X.J., Wu, Q., Tao, Y.T., Guo, H.Y., Ji, C.Y., Bai, Y.Z. (2018). Complete mitochondrial genome of *Parasesarma affine* (Brachyura: Sesarmidae): Gene rearrangements in Sesarmidae and phylogenetic analysis of the Brachyura. International Journal of Biological Macromolecules. 118: 31-40.

Wang, Z.F., Zheng, Y.Q., Zhao, X.Y., Xu, X.Y., Xu, Z.W., Cui, C. (2023). Molecular phylogeny and evolution of the Tuerkayana (Decapoda: Brachyura: Gecarcinidae) genus based on whole mitochondrial genome sequences. Biology. 12: 974.

Wilson, D.E. and Reeder, D.M. (2005). Mammal Species of the World: A Taxonomic and Geographic Reference (3rd ED.). Baltimore: Johns Hopkins University Press. pp. 1531.

Wilson, D.E., Lacber, T.E., Mittermeier, R.A. (2017). Handbook of the Mammals of the World. Vol. 7. Rodents II. Barcelona: Lynx Edicions. pp. 1008.

Wu, L., Tong, Y., AyiviSam, P.G., Storey, K.B., Zhang, J.Y., Yu, D.N. (2022). The complete mitochondrial genomes of three Sphenomorphinae species (Squamata: Scincidae) and the selective pressure analysis on mitochondrial genomes of limbless *Isopachys gyldenstolpei*. Animals. 12(16): 2015. https://doi.org/10.3390/ani12162015.

Xin, G.E., Huang, Y.F., Zhao, Y.J., Su, N.R., Zhao, Z.Q., Sun, Y.W., Chen, L.P., Qiu, X.Y. (2015). Comparative analysis the variability of a complete mitochondrial genome of polledness intersexual goat (PIS-) from China. Indian Journal of Animal Research. 49: 761-763. doi: 10.18805/ijar.5925.

Xin, Z.Z., Liu, Y., Zhang, D.Z., Chai, X.Y., Wang, Z.F., Zhang, H.B., Zhou, C.L., Tang, B.P., Liu, Q.N. (2017). Complete mitochondrial genome of *Clistocoeloma sinensis* (Brachyura: Grapsoidea): gene rearrangements and higher-level phylogeny of the Brachyura. Scientific Reports. 7: 4128. doi: 10.1038/s41598-017-04489-9.

Yang, C.Z., Hao, H.B., Liu, S.Y., Liu, Y., Yue, B.S., Zhang, X.Y. (2012). Complete mitochondrial genome of the chinese oriental vole *Eothenomys chinensis* (Rodentia: Arvicolinae). Mitochondrial DNA. 23: 131-133.

Yang, Z.H. (2007). PAML 4: phylogenetic analysis by maximum likelihood. Molecular Biology and Evolution. 24: 1586-1591.

Zeng, T., Jin, W., Sun, Z.Y., Liu, Y., Murphy, R.W., Fu J.R., Wang, X., Hou, Q.F., Tu, F.Y., Liao, R., Liu, S.Y., Yue, B.S. (2013). Taxonomic position of *Eothenomys wardi* (Arvicolinae: Cricetidae) based on morphological and molecular analyses with a detailed description of the species. Zootaxa. 3682: 85-104.

Zhang, D., Gao, F.L., Jakovlić, I., Zou, H., Zhang, J., Li, W.X., Wang, G.T. (2020). PhyloSuite: An integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. Molecular Ecology Resources. 20: 348-355.

Zhu, L., Bi, Z.D., Qian, H.A., Mei, X.F., Zhao, J.Y., Zhao, X.X., Chen, H., Zhang, J.S., Piao, Z.W. (2023). Sequencing and analysis of the complete mitochondrial genome of *Eothenomyseleusis* Thomas 1911 from China and its phylogenetic analysis. Mitochondrial DNA Part B: Resources. 8: 493-496.