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CDS Region of Uncoupling Protein 1 (UCP1) Gene in *Eothenomys miletus*: A Bioinformatics and Selection Pressure Analysis

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ABSTRACT

Background: The present study aimed to elucidate the fundamental composition of the CDS region sequence of *UCP1* gene in *Eothenomys miletus*, as well as the physicochemical properties, higher-order structure and structural domains of the UCP1 protein. Additionally, we analyzed the selection pressure on the *UCP1* gene in mammals inhabiting different latitudes, providing a data foundation for further exploration of the *UCP1* gene and its role in mammalian environmental adaptation.

Methods: The CDS region sequence of *UCP1* gene was cloned using PCR and the structure and function of the *UCP1* gene were characterized employing a bioinformatics platform. The *UCP1* gene of *Eothenomys miletus* was compared with other species for sequence similarity and phylogenetic tree construction. Furthermore, selection pressure on *UCP1* genes from mammals at different latitudes was analyzed.

Result: *UCP1* gene encodes a protein comprising 307 amino acids, located in the mitochondria, with a molecular formula of $C_{1498}H_{2402}N_{392}O_{434}S_{16}$. Threonine (Thr, T) is the most abundant amino acid, accounting for 11.1%. The protein is hydrophobic, positively charged, alkaline in nature and contains one potential N-glycosylation site and five O-glycosylation sites. It lacks a transmembrane region or signal peptide distribution and is a non-GPI-anchored protein. The secondary structure is predominantly composed of α-helices and random coils, featuring three Mito_carr structural domains. Homology comparisons revealed that the highest nucleotide sequence similarity exists between *Eothenomys miletus* and the species *Myodes glareolus*, *Microtus oregoni* and *Arvicola amphibius*. Selection pressure analysis indicated no positive selection sites for the *UCP1* gene in mammals at different latitudes. The *UCP1* gene in low-latitude, high-altitude mammals underwent purifying selection, forming a relatively stable function. These findings provided a theoretical basis for subsequent research on the mechanism of fat heat production and energy supply in the *Eothenomys miletus* *UCP1* gene and a data foundation for further exploration of the role of the *UCP1* gene in the adaptation of *Eothenomys miletus* to future environmental changes.

Key words: Bioinformatics, *Eothenomys miletus*, Latitude, Selection pressure, UCP1 gene.

INTRODUCTION

The mechanisms underlying thermogenesis are of critical importance for mammals in their ability to withstand low temperatures (Geng ang Zhu, 2024), which involves not only reducing heat loss through insulation mechanisms, but also enhancing heat production *via* elevated metabolism (Xia, 2021), primarily through facultative thermogenesis, specifically non-shivering thermogenesis (NST) (Alhamoud *et al*., 2024; Chen *et al*., 2021). NST is associated with mitochondria in brown adipose tissue (BAT), which is distributed around the scapulae, neck and aorta (Cannon and Nedergaard, 2004). These thermogenic mechanisms play a regulatory role in body temperature, body weight and body fat content (Wang *et al*., 2001). Mitochondria contain uncoupling proteins (UCPs), a family of tissue-specific mitochondrial inner membrane proteins belonging to the mitochondrial anion carrier family (MACF) (Luévano-Martínez, 2012). UCPs are distributed across diverse organisms including plants, animals, fish, fungi and protozoa(Adams, 2000).

Rodents, as commonly used models for studying cold adaptation and energy metabolism, exhibit physiological adjustments when exposed to prolonged cold environments: Their bodies cease shivering thermogenesis (ST) and elevate NST to maintain a higher metabolic rate (Carlson and Cottle, 1956). In this process, norepinephrine released from sympathetic nerve terminals activates BAT, leading to the upregulated expression of uncoupling protein 1 (UCP1) in mitochondria (Bartness *et al*., 2010). This mechanism

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suppresses adenosine triphosphate (ATP) synthesis on BAT mitochondrial membranes redirecting energy from substrate oxidation toward heat generation (Li *et al*., 2014; Matthias *et al*., 1999). The produced heat is then distributed to other body regions *via* blood circulation (Stier *et al*., 2014), ultimately enhancing survival rates in cold conditions (Ballinger and Andrews, 2018; Janský, 1973; Kang and Chen, 2023). Furthermore, studies have shown that in rodent adipocytes, UCP1 operates in parallel with creatine kinase b (CKB), highlighting a sophisticated regulatory mechanism that underscores the crucial role of UCP1 in body temperature regulation and energy homeostasis (Guan *et al*., 2022; Rahbani *et al*., 2024; Simon *et al*., 1986).

The adaptation of animals to their environment and adaptive evolution have long been hotspots in research. For instance, Wang *et al*. (2022) revealed that the accelerated evolution of the FSHR gene contributes to the reproductive adaptation of "high-quality parenting'' in mammals. Kan *et al*. (2024) discovered that the rapid evolutionary rate of the FOXP2 gene in songbirds is associated with their exceptional vocal learning abilities. Wang *et al*. (2023) demonstrated that strong purifying selection on the BMPR1B gene in pigs correlates with their high fecundity traits. Studies on the evolutionary system of the cold-related *UCP1* gene can effectively and conveniently reveal its potential biological functions and environmental adaptations. Therefore, this study selected the *UCP1* gene from mammals distributed in both high- and low-latitude regions to conduct selection pressure analysis, investigate its evolutionary trends and employed the widely accepted maximum likelihood (ML) method to test selection pressure (Wang *et al*., 2022).

Additionally, Wei *et al*. (2023) cloned and conducted bioinformatics analyses of the *UCP1* gene in Nubian goats, tree shrews (Zhu, 2003), Yanbian cattle (Sun *et al*., 2021) and yaks (Zhao *et al*., 2021). However, research on *UCP1* genes in species inhabiting the Hengduan Mountains-a global biodiversity hotspot-remains limited. *Eothenomys miletus* belonging to the subfamily Arvicolinae and genus *Eothenomys*, is an endemic species to the Hengduan Mountains (Gong *et al*., 2021; Luo *et al*., 2000). Characterized by its low-latitude, high-altitude environment, the Hengduan region features small annual temperature variations, large diurnal temperature fluctuations, distinct dry and wet seasons and relatively abundant food resources (Gong *et al*., 2009). These conditions may drive unique physiological and ecological adaptations in *Eothenomys miletus*. Therefore, this study sequenced the *UCP1* gene of *Eothenomys miletus*, a rodent species endemic to the Hengduan Mountains and further investigated the biological characteristics of its *UCP1* gene to gain a more comprehensive and in-depth understanding of it. This reference facilitates the study of the biological functions of the gene. Furthermore, the present study investigated the evolutionary relationship of this gene in mammals to verify its potential association with the latitude-altitude relationship and to provide fundamental data for further research on its *UCP1* gene.

MATERIALS AND METHODS

Animal collection and processing

In the winter of 2024, field sampling was conducted in the Hengduan Mountains of Dali (100°42′49″E, 24°90′30″N). All experimental subjects were healthy adult *Eothenomys miletus* captured during non-reproductive periods. After disinfection and flea removal, the voles were transported to the animal facility of the School of Life Sciences, Yunnan Normal University. The voles were euthanized *via* CO_2 anesthesia and BAT from the interscapular and cervical regions was dissected using sterile scissors, while white adipose tissue (WAT) was removed. The BAT samples were weighed, placed in cryotubes and immediately stored at -80°C for further analysis.

Cloning of UCP1 gene in *Eothenomys miletus*

The *UCP1* gene sequence of the *Alexandromys fortis* (GenBank accession: XM_050161578.1) was retrieved from the NCBI database. Polymerase Chain Reaction (PCR) primers were designed using Primer 5.0 software, with forward primer F: CCAGAGCCCGACAACT and reverse primer R: CACCAAGAACACGACCTC, targeting an amplified product of approximately 970 bp. Total RNA was extracted from BAT cells using the Trizol method and RNA concentration and purity were measured. RNA integrity was verified *via* 1.0% agarose gel electrophoresis. cDNA was synthesized using the PrimeScript™ RT Reagent Kit with gDNA Eraser and stored at -20°C.PCR amplification was performed using BAT-derived cDNA as the template. The 10 µL reaction mixture included 5 µL 2 × Taq PCR MasterMix, 0.5 µL each of forward and reverse primers, 1 µL cDNA and 3 µL RNase-free dd H^2O. The PCR protocol comprised: 94°C pre-denaturation for 3 min; 35 cycles of 94°C denaturation (15 s), 62°C annealing (15 s) and 72°C extension (30 s); followed by a final extension at 72°C for 5 min. PCR products were electrophoresed on a 1.5% agarose gel, purified using a gel recovery kit and sequenced by Kunming Shuoyang Technology Co., Ltd.

Bioinformatics analysis

Probing the physicochemical properties of *Eothenomys miletus* UCP1 sequence based on various bioinformatics software and further predicting its protein high-level structure (Choudhary *et al*., 2016) (Table 1).

The *UCP1* gene sequence of *Eothenomys miletus* was subjected to sequence similarity analysis using the BLAST program (https://blast.ncbi.nlm.nih.gov/Blast.cgi) available in the NCBI database. Following this, a phylogenetic tree was reconstructed using the ML method implemented in MEGA X software, incorporating the following species: *Myodes glareolus* (XM_048447499.1), *Microtus oregoni* (XM_041663724.1), *Arvicola amphibius* (XM_038313207.1), *Peromyscus eremicus* (XM_059262645.1), *Meriones*

unguiculatus (XM_021632264.2), *Mus musculus* (NM_009463.3), *Ochotona curzoniae* (XM_040967137.1) and *Bos mutus* (XM_005895812.2). Nucleotide sequences were aligned using default parameters and bootstrap analysis (1,000 replicates) was performed to assess node support. The resulting tree topology was visualized and annotated to elucidate evolutionary relationships among taxa.

To investigate the adaptive evolution of the *UCP1* gene in mammals inhabiting distinct latitudinal and altitudinal environments, we retrieved 18 mammalian *UCP1* gene sequences from the NCBI database (Table 2). A phylogenetic tree was first reconstructed using the maximum likelihood (ML) method in MEGA X, followed by selection pressure analysis implemented in PAML 4.9a (codeml module) using site and branch models (Bin *et al*., 2021). The selection coefficient ω (nonsynonymous/ synonymous substitution rate ratio) was calculated to quantify adaptive evolutionary trends at codon levels. We employed the following nested models to detect codon-level positive selection: M0 (single-ratio model: uniform ω across all sites), M1 (nearly neutral model: two site classes-conserved sites ($0 < \omega < 1$) and neutral sites ($\omega = 1$), M2 (positive selection model: adds a third class of sites with $\omega > 1$), M3 (discrete model: ω varies across sites under a discrete distribution), M7 (β-distribution model: ω varies between 0 and 1), M8 (β+ω > 1 model: extends M7 by allowing a class of positively selected sites). Likelihood ratio tests (LRTs) were performed to compare nested models: M1 vs. M2, M0 vs. M3 and M7 vs. M8. Sites with posterior probabilities >0.95 under Bayesian empirical Bayes (BEB) analysis were considered under positive selection. To detect lineage-specific accelerated evolution, branch models were applied under two scenarios: Low-latitude, high-altitude mammals labeled as foreground branches. High-latitude, low-altitude mammals labeled as foreground branches. For each scenario, models M0 (single ω for all branches) and M2 (separate ω for foreground vs. background branches) were compared using LRTs. A significantly higher ω in foreground branches ($p<0.05$) indicated accelerated evolution driven by positive selection.

RESULTS AND DISCUSSION

Homology comparison of UCP1 in *Eothenomys miletus* with other species

The nucleotide sequences of UCP1 in *Eothenomys miletus* showed the highest similarity with those of *Myodes glareolus*, *Microtus oregoni* and *Arvicola amphibius*, while lower similarity was observed with *Ochotona curzoniae* (81.696%) and *Bos mutus* (80.022%) (Table 3). Phylogenetic tree analysis aligned with sequence comparisons (Fig 1). Consequently, it is hypothesized that the *UCP1* gene is relatively conserved in rodents, yet it also exhibits specificity among different species (Liu *et al*., 2016 and Fu *et al*., 2006). This finding is analogous to the

Table 1: Bioinformatics software.

Software brand	Uniform resource locator	Function
Cell-PLoc 2.0	http://www.csbio.sjtu.edu.cn/bioinf/Cell-PLoc-2/	Protein Subcellular Localization
ExPASy	http://www.expasy.Org/tools/	Protein Physical and Chemical Properties
TMHMM 2.0	https://services.healthtech.dtu.dk/services/TMHMM-2.0/	Transmembrane Structure
ProtScale	https://web.expasy.org/protscale/	Protein hydrophilicity
CBS NetPhos 3.1	http://www.cbs.dtu.dk/services/NetPhos/	Protein phosphorylation potential
SignalP 4.1 Server	https://services.healthtech.dtu.dk/services/SignalP-4.1/	Protein Signal Peptide Prediction
		TBtools-II
PredGPI	http://gpcr.biocomp.unibo.it/predgpi/	Prediction of potential protein GPI anchor sites
NetNGlyc 1.0	https://services.healthtech.dtu.dk/services/NetNGlyc-1.0/	Protein Glycosylation Prediction
NetOGlyc 4.0	https://services.healthtech.dtu.dk/services/NetOGlyc-4.0/	Protein Glycosylation Prediction

Table. 2: Mammalian UCP1 gene information.

Species	Genbank No.	Species	Genbank No.
Eumetopias jubatus	XM_028102302	*Panthera leo*	XM_042933917
Lynx canadensis	XM_032593040	*Panthera tigris*	XM_007091783
Lynx rufus	XM_047091459	*Hyaena hyaena*	XM_039249162
Mirounga leonina	XM_035023261	*Ursus arctos*	XM_026499089
Neomonachus schauinslandi	XM_021679332	*Ursus maritimus*	XM_008685422
Apodemus sylvaticus	XM_052166745	*Psammomys obesus*	XM_055621496
Arvicanthis niloticus	XM_034522636	*Peromyscus californicus insignis*	XM_052729196
Myodes glareolus	XM_048447499	*Onychomys torridus*	XM_036189172
Mirounga angustirostris	XM_045881734	*Mesocricetus auratus*	NM_001281332

results of Gao (2014)'s analysis of partial sequences of the *UCP1* gene in the Chinese-Myanmar tree shrew and also supports the view of Beáta *et al*. (2024) that the *UCP1* gene exhibits both conservation and species specificity between humans and rodents.

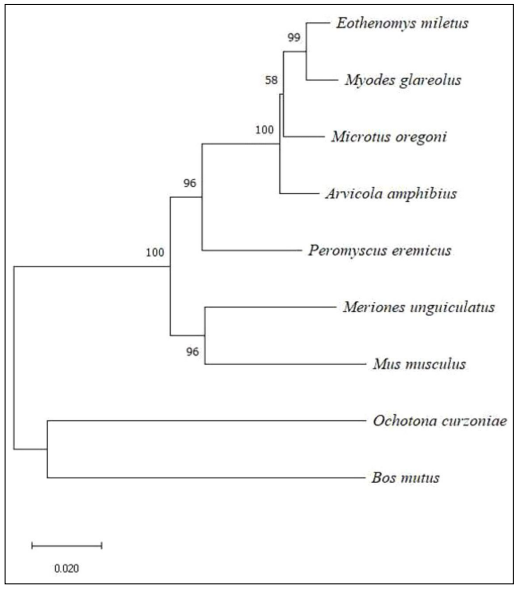

Fig 1: Phylogenetic tree of UCP1 gene.

Table 3: BLAST comparison of species information.

Species	Nucleotide	
	Homology/%	Genbank No.
Myodes glareolus	98.377	XM_048447499.1
Microtus oregoni	97.511	XM_041663724.1
Arvicola amphibius	97.294	XM_038313207.1
Peromyscus eremicus	93.398	XM_059262645.1
Meriones unguiculatus	91.135	XM_021632264.2
Mus musculus	89.827	NM_009463.3
Ochotona curzoniae	81.696	XM_040967137.1
Bos mutus	80.022	XM_005895812.2

Bioinformatics analysis of *UCP1* gene sequence

The *UCP1* gene in *Eothenomys miletus* encodes 307 amino acids (Table 4), with a molecular weight of 33 kDa, theoretical isoelectric point (pI) of 9.23, molecular formula $C_{1498}H_{2402}N_{392}O_{434}S_{16}$, total atom count of 4,742, instability index of 32.61, extinction coefficient of 24,785, grand average of hydropathicity (GRAVY) of 0.157 and a half-life of 30 hours. The protein is alkaline, with threonine (Thr, T) being the most abundant amino acid (11.1%) (Fig 2). It contains 19 negatively charged and 28 positively charged residues. Post-translational modifications (PTMs) include 33 phosphorylation sites (15 serine [S], 17 threonine [T] and 1 tyrosine [Y]) (Fig 3), 1 potential N-glycosylation site at position 188 (Fig 4) and 5 O-glycosylation sites at positions 3, 5, 6, 7 and 12. *Eothenomys miletus* is an endemic species of the Hengduan Mountains in China (Luo *et al*., 2000). In order to adapt to environmental changes at different altitudes in this region, the species enhances its thermogenic capacity by upregulating UCP1 expression in brown adipose tissue, thereby better coping with cold environments (Han *et al*., 2022). Furthermore, post-translational modifications (PTMs), including phosphorylation and glycosylation, have been demonstrated to play a pivotal regulatory role in the process of mRNA translation into proteins and are implicated in a variety of biological processes (Wang and Zhang, 2019). Therefore, the present study conducted a bioinformatics analysis of the UCP1 protein in *Eothenomys miletus*, systematically elucidating its basic characteristics, including amino acid composition, molecular weight, hydrophilic/hydrophobic amino acid ratio and phosphorylation and glycosylation sites. This study provides important data for further exploration of the functional role of the *UCP1* gene.

Subcellular localization and structural features

UCP1 is localized to mitochondria, lacks a GPI anchor or transmembrane domains and contains ~11.20 amino acids embedded in transmembrane regions (Fig 5). The first 60 amino acids include 5.52 transmembrane helices,

Table 4: Amino acid composition of UCP1 protein.

Aminoacids	Quantity/number	Proportion %	Aminoacids	Quantity/number	Proportion %
Ala (A)	21	6.8	Leu (L)	31	10.1
Arg (R)	12	3.9	Lys (K)	16	5.2
Asn (N)	10	3.3	Met (M)	9	2.9
Asp (D)	8	2.6	Phe (F)	14	4.6
Cys (C)	7	2.3	Pro (P)	17	5.5
Gln (Q)	13	4.2	Ser (S)	22	7.2
Glu (E)	11	3.6	Thr (T)	34	11.1
Gly (G)	24	7.8	Trp (W)	2	0.7
His (H)	4	1.3	Tyr (Y)	9	2.9
Ile (I)	20	6.5	Val (V)	23	7.5

Note: Ala: Alanine; Arg: Arginine; Asn: Asparagine; Asp: Aspartic acid; Cys: Cysteine; Gln: Glutamine;Glu: Glutamic acid; Gly: Glycine; His: Histidine; Ile: Isoleucine; Leu: Leucine; Lys: Lysine; Met: Methionine; Phe: Phenylalanine; Pro: Proline; Ser: Serine; Thr: Threonine; Trp: Tryptophan; Tyr: Tyrosine; Val: Valine.

with a 21.93% probability of cytoplasmic orientation. The protein exhibits strong hydrophobicity (peak value: 2.311) and weak hydrophilicity (minimum: -2.033), with dominant hydrophobic regions at positions 4-7 (Fig 6). Signal peptide analysis (D=0.214; cutoff=0.450) confirmed UCP1 as a non-secretory protein (Fig 7). Secondary structure comprises 51.79% α-helices, 37.13% random coils and 11.07% extended strands (Fig 8). Three Mito_carr domains span residues 10–107, 109–206 and 209–300 (Fig 9). Tertiary modeling (SWISS-MODEL) yielded a high-quality structure (GMQE=0.87, 82.27% sequence identity) (Fig 10). The first identification of UCP1 was in the fat mitochondria of hamsters, rats and guinea pigs in the mid-1970s, with particularly high activity in BAT (Rodríguez-Cuenca *et al*., 2010). Subsequent studies revealed that UCP1 is also expressed in the thymus of mice (Adams *et al*., 2008). As a pivotal mitochondrial inner membrane protein, UCP1 exerts a pivotal role in temperature regulation, energy metabolism and obesity control in animals (Zhou *et al*., 2022). This study shows that the UCP1 protein in *E. miletus* is localized in mitochondria. The secondary and tertiary structure prediction results of the UCP1 protein are consistent, indicating significant structural stability and functionality. This provides a foundation of data for a better understanding of UCP1.

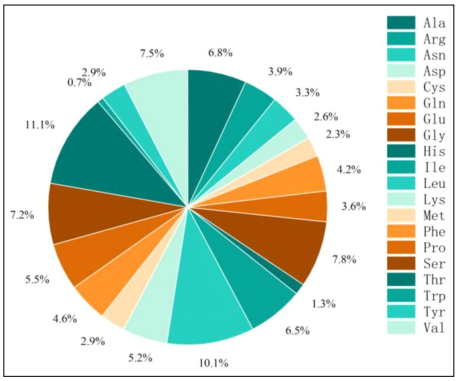

Fig 2: Amino acid proportion of UCP1 protein.

Selection pressure analysis

In this study, the ML method in MEGA X was used to construct the phylogenetic tree of the *UCP1* gene (Fig 11). The topology of the phylogenetic tree constructed by MEGA X was basically consistent with the traditional classification and the bootstrap values were also basically consistent, with most above 90 and a few below 70. It is inferred that the evolutionary rate of the *UCP1* gene is moderate and has basically formed a relatively stable function.

Likelihood ratio tests (2ΔlnL) revealed significant differences between models M0 and M3 (2ΔlnL = 92.3228, $p<0.05$) but not between M7 and M8 ($p = 0.99985601$). M3 indicated three site classes (p0=0.02029, p1=0.02341, p2=0.58008; ω=0, 0, 0.05405), while M1 showed two classes (p0=0.82334, p1=0.17666; ω=0.11383, 1.00000). No positive selection signals were detected. During the course of a long-term evolutionary process, selective pressure does not invariably act on the entirety of a gene;

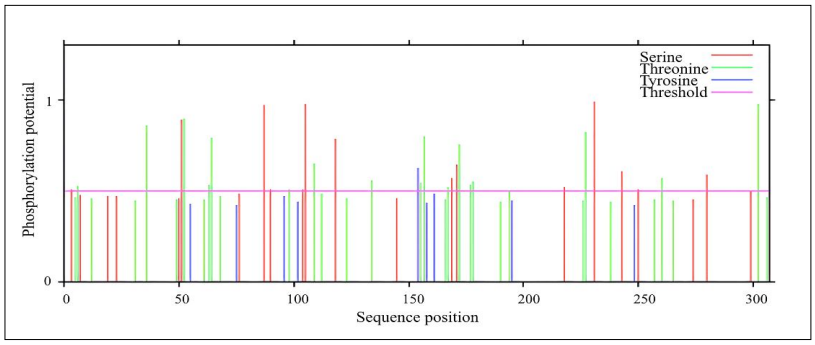

Fig 3: Prediction of UCP1 protein phosphorylation site in *Eothenomys miletus.*

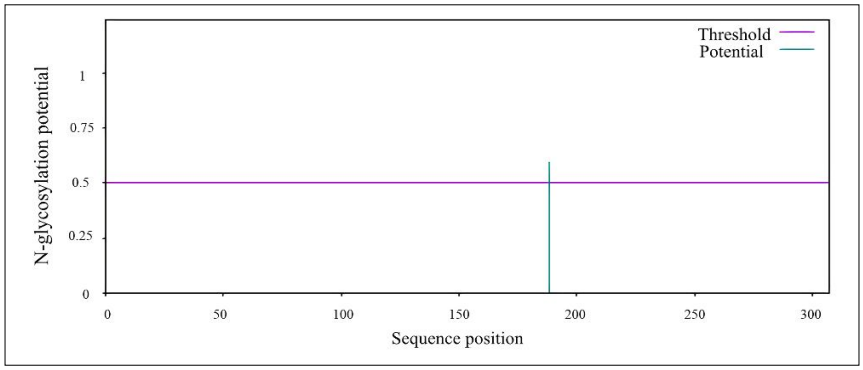

Fig 4: Prediction of N-glycosylation site of UCP1 protein in *Eothenomys miletus.*

rather, it acts on specific functional sites, such as particular sites within the coding region of a gene (Kamath and Getz, 2011). In this study, no positive selection sites were identified in the *UCP1* gene across the selected species, indicating that UCP1 is a relatively conserved gene.

A comparison of the high-latitude, low-altitude (foreground branch) model (M0 vs. M2: 2ΔlnL = 0.23402, p>0.05) with the low-latitude, high-altitude model (2ΔlnL = 7.17748, p<0.05, dN/dS = 0.15496) indicates that UCP1 in low-latitude, high-altitude rodents tends toward purifying selection (Table 5). This evolutionary phenomenon can be inferred from the environmental adaptation mechanisms of *Eothenomys miletus*, a species endemic to the Hengduan Mountains. The low-latitude, high-altitude regions of the Hengduan Mountains possess unique environmental characteristics, including small annual temperature fluctuations and relatively abundant food resources (Gong *et al*., 2001). This environmental stability may have reduced the selective pressure on the UCP1 gene. Mu (2015) observed that this species employs metabolic, regulatory and hormonal mechanisms to mitigate the effects of environmental fluctuations on the organism. Research has demonstrated that regulatory processes, encompassing metabolic and hormonal regulation, have been shown to provide adaptive contributions in response to environmental stress (Scherbarth *et al*., 2010; Kuzmenko *et al*., 2024; Kuti *et al*., 2022). Consequently, these regulatory adaptive mechanisms can be employed to mitigate selection pressure on the UCP1 gene. In relatively stable environments, natural selection tends to preserve the existing functional conservatism of the UCP1 gene rather than promote new adaptive mutations. Animals have been shown to optimize the thermogenic efficiency of brown adipose tissue by maintaining the stability of the UCP1 gene. This gene may confer evolutionary advantages more effectively than

Table 5: Results of the likelihood ratio test and germline selection pressure of the branch model.

Branch model	np	lnL	p-value
M0	37	5675.390262	0.0074*
M2	38	5671.801523	

Note: * indicates P<0.05.

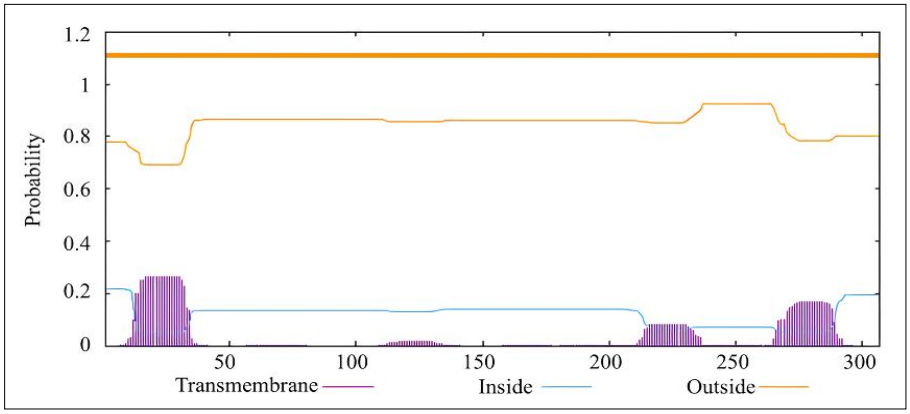

Fig 5: Prediction of UCP1 protein transmembrane region in *Eothenomys miletus.*

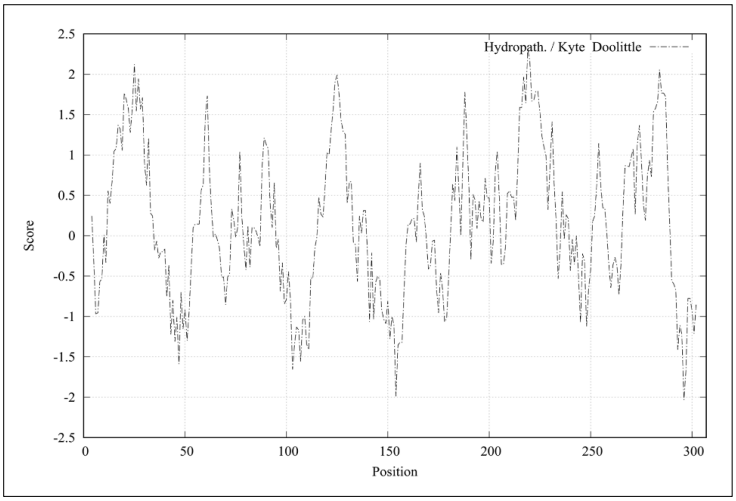

Fig 6: Prediction of hydrophilicity/hydrophobicity of UCP1 protein in *Eothenomys miletus*.

developing new metabolic pathways (Kamath and Getz, 2011).

From a functional perspective, the UCP1 gene primarily participates in energy metabolism and body temperature regulation processes, which are crucial for the survival and reproduction of the species. During the course of long-term evolution, it is imperative for this gene to preserve relatively stable functions, thereby ensuring the normal physiological activities of the organism. For instance, in cold environments, the UCP1 protein converts chemical energy into thermal energy through uncoupling oxidative phosphorylation to maintain body temperature (Dieckmann *et al*., 2022). This fundamental and critical function subjects the gene to strong purifying selection, continuously eliminating harmful mutations and making it difficult for adaptive mutations represented by positive selection sites to accumulate. Consequently, this process maintains functional conservation. Furthermore, UCP1 demonstrates adaptive differentiation in thermal environments: high-latitude mammals exhibit higher evolutionary rates (Su *et al*., 2025), while low-latitude species, such as *Heterocephalus glaber*, exhibit loss-of-function mutations (Kim *et al*., 2011). The UCP1 gene, located in low-latitude, high-altitude regions, functions as an evolutionary "hub," balancing ancestral thermogenesis (cold adaptation) with regulatory flexibility (environmental variability). The coexistence of thermal stress and hypoxia in high-altitude regions may drive a trade-off between thermogenesis efficiency and energy conservation, providing a natural model for studying metabolic adaptation thresholds in cross-latitude dispersal. However, further research is needed in subsequent studies to elucidate the precise function of the *UCP1* gene and its regulatory mechanisms.

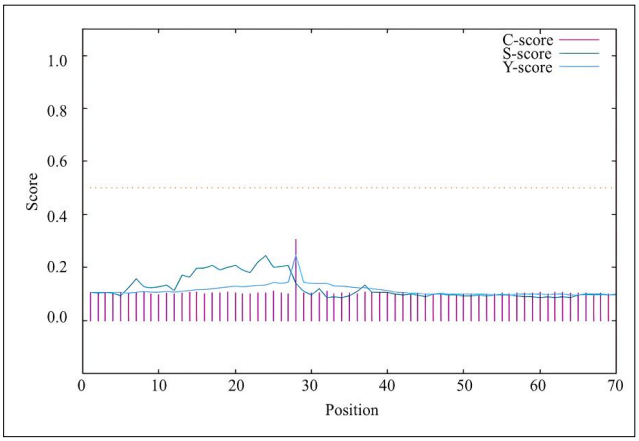

Fig 7: UCP1 protein signal peptide prediction in *Eothenomys miletus*.

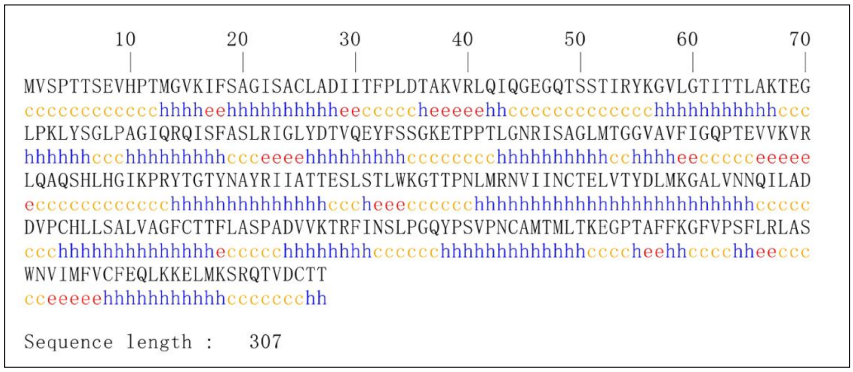

Fig 8: Prediction of UCP1 protein secondary structure in *Eothenomys miletus*.

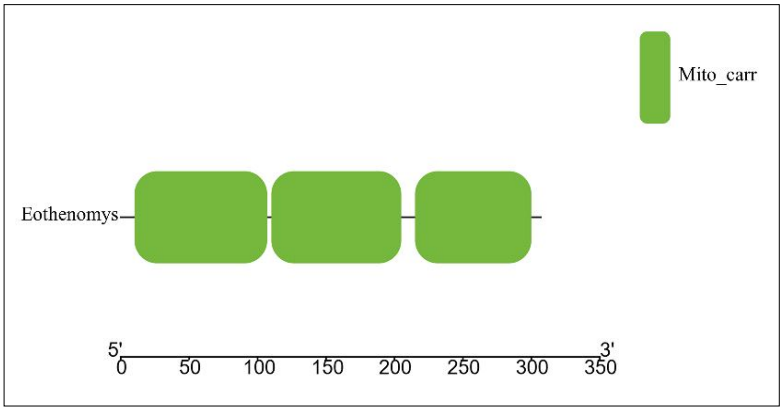

Fig 9: Prediction of UCP1 protein domain in *Eothenomys miletus*.

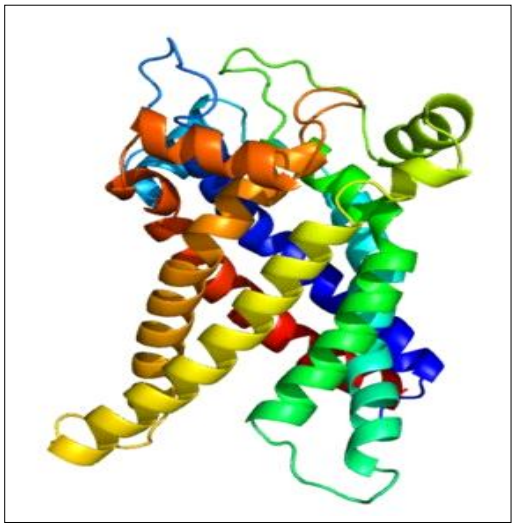

Fig 10: Prediction of tertiary structure of UCP1 protein in *Eothenomys miletus.*

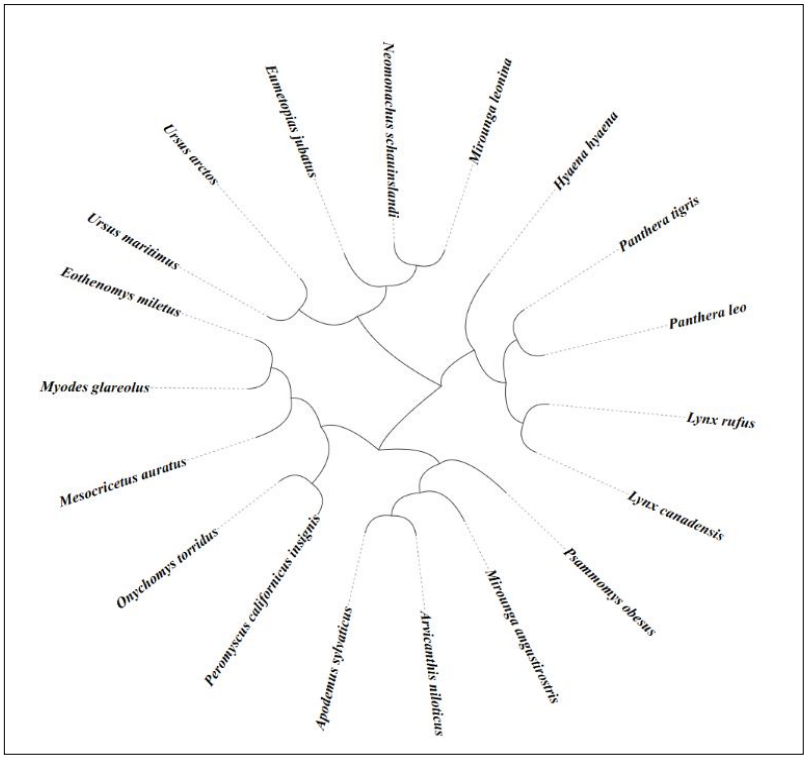

Fig 11: Phylogenetic tree based on mammalian UCP1 gene.

CONCLUSION

In conclusion, the UCP1 gene of *Eothenomys miletus* encodes a protein comprising 307 amino acids, characterized as hydrophobic and predominantly composed of α-helices, random coils and extended chains. Homology analysis revealed high sequence similarity between the UCP1 gene of *Eothenomys miletus* and that of the *Myodes glareolus*. Selective pressure analysis further indicates that the UCP1 gene is highly conserved in mammals. However, the UCP1 gene in mammals inhabiting low-latitude, high-altitude environments exhibits a propensity for purifying selection. Collectively, these findings contribute to a more comprehensive understanding of the functional data of the *Eothenomys miletus* UCP1 gene and provide a valuable foundation for future studies on its physiological functions and adaptive significance.

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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

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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