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ANALYSIS OF THE COMPLETE MITOCHONDRIAL GENOME OF VIETNAMESE *Pygathrix nigripes*

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ABSTRACT

The black-shanked douc langur *Pygathrix nigripes* is a critically endangered primate species endemic to Vietnam, facing severe threats from habitat destruction, poaching, and climate change. This study presents the complete mitochondrial genome of *Pygathrix nigripes*, comprising 16,535 bp, which includes 37 genes: 13 protein-coding genes, 22 transfer RNAs, and 2 ribosomal RNAs. Mitochondrial genomes are crucial for cellular energy metabolism, and understanding their genetic structure is vital for effective conservation strategies. Our analysis reveals significant genetic diversity and an A-T rich nucleotide composition, characteristic of primate mitogenomes, indicating adaptive evolutionary strategies. The study also highlights the importance of mitochondrial protein-coding genes in energy production and metabolic efficiency, which are critical for the species' survival. We observed variations in nucleotide composition and codon usage bias that reflect historical population dynamics and evolutionary pressures. Comparative genomic analyses with other *Pygathrix* species suggest shared evolutionary pathways, emphasizing the need for targeted conservation efforts. The findings underscore the necessity of genetic monitoring in conservation planning, particularly as *Pygathrix nigripe* faces ongoing environmental challenges. This research aims to enhance the global mapping of the *Pygathrix nigripes* mitogenome, offering valuable insights that can support the development of effective conservation strategies. These strategies are essential for ensuring the survival of this unique and critically endangered primate species.

Keywords: AT skew, endangered, genetic diversity, mitochondrial, Vietnamese *Pygathrix nigripes*

INTRODUCTION

The black-shanked douc langur (*Pygathrix nigripes*) is a critically endangered primate species endemic to Vietnam (Ha *et al.,* 2023). This species faces significant threats from habitat destruction, poaching, and fragmentation, largely due to deforestation and agricultural expansion (Donnelly, 2024; Nadler, 2010). Recent research predicts that the distribution of the black-shanked douc could decline significantly, with an estimated loss of 66.19% of its current range by 2070 due to the climate change (Tran *et al.,* 2020). Understanding the genetic makeup of *P. nigripes* is essential for developing effective conservation strategies aimed at preserving this unique species (Freund *et al.,* 2021).

Mitochondrial genomes are vital in cellular energy metabolism, and their protein-coding genes play crucial roles in various biological functions, including oxidative phosphorylation and ATP production. The mitochondrial genome of *Pygathrix* contains 13 canonical protein-coding genes that are integral to its metabolic processes (Nguyen *et al.,* 2024). Analyzing these genes not only enhances our understanding of the mitochondrial genetic structure but also serves as a key indicator of the species' overall health and viability, particularly in the context of ongoing conservation efforts (Lynn *et al.,* 2016).

Moreover, the study of protein-coding genes within the mitochondrial genome can reveal important insights into the evolutionary history and genetic diversity of *P. nigripes*. The analysis of nucleotide composition, particularly the A-T ratio, provides valuable information about evolutionary pressures and mechanisms affecting the species. Variations in the A-T ratio may reflect historical population dynamics and adaptability, while the interplay between AT and GC content influences synonymous codon usage, impacting protein translation and selection at the amino acid level (Hooper *et al.* 2000; Pawlak *et al.,* 2023).

This article aims to comprehensively characterize the protein-coding genes in the mitochondrial genetic structure of Vietnamese *P. nigripes* and compare it with data from other douc langur species to assess genetic diversity and evolutionary trends within this unique group. Through this analysis, we hope to contribute valuable insights into the genetic landscape of *P. nigripes*, aiding in the development of effective conservation strategies to ensure the survival of this endangered primate.

Importance of mitochondrial genomes in evolutionary biology

Mitochondrial genomes are critical for understanding evolutionary biology and conservation genetics, especially in primates (Liedigk *et al.,* 2014; Roos *et al.,* 2020). Their relatively small size, maternal inheritance, and rapid mutation rates make them ideal for studies of genetic diversity and evolutionary dynamics. Mitochondrial DNA (mtDNA) provides insights into phylogenetic relationships and population structure, facilitating the investigation of evolutionary adaptations to specific environments (Shao *et al.,* 2023).

Protein-coding genes and their functions

The protein-coding genes in the mitochondrial genome are essential for various cellular functions, primarily energy production through the oxidative phosphorylation pathway. In primates, these genes include those coding for subunits of NADH dehydrogenase (ND), cytochrome c oxidase (COX), and ATP synthase, all of which are crucial for ATP synthesis. Variations in these genes can affect metabolic efficiency and overall fitness, making them vital for understanding species' adaptations, particularly in response to environmental pressures (Anderson *et al.*, 1981).

Genetic diversity in *Pygathrix nigripes*

Research on *P. nigripes* has highlighted its critical conservation status and the importance of understanding its genetic diversity for effective management strategies. Studies by Ha *et al.* 2023 have shown that genetic variation within populations can influence resilience to environmental changes and disease, making it essential to monitor mitochondrial genetic structures to inform conservation practices (Błażej *et al.*, 2017).

The A-T ratio in mitochondrial genomes

The relative abundance of adenine and thymine (A-T) versus guanine and cytosine (G-C) in genomic sequences plays a crucial role in evolutionary processes, influencing factors such as mutation rates and codon usage bias. Variations in A-T and G-C content can affect protein translation efficiency and adaptability, shaping the evolutionary trajectories of species in response to environmental pressures. The A-T ratio, which measures the relative abundances of adenine (A) and thymine (T) in the mitochondrial genome, serves as an important indicator of evolutionary pressures. Variations in the A-T ratio can reflect historical population dynamics and adaptations to ecological niches. Research indicates that species with high A-T content may experience different selection pressures, influencing their evolutionary trajectories (Błażej *et al.*, 2017).

Codon usage bias and its evolutionary implications

Codon usage bias, which refers to the preferential use of certain codons over others in protein-coding genes, is influenced by factors such as mutational pressures and natural selection. Studies show that biased nucleotide substitutions can have significant implications for protein translation efficiency and overall fitness (Anderson *et al.*, 1981). In *P. nigripes*, understanding codon usage bias is crucial for elucidating how genetic composition impacts adaptation and evolutionary success.

Comparative studies across *Pygathrix* species

Comparative genomic studies across different species of *Pygathrix* have revealed distinct patterns in mitochondrial genetic structures. For instance, research by Liedigk *et al.*, (2014) indicates that variations in the A-T skew among *Pygathrix* species correlate with ecological niches and evolutionary adaptations. Such comparative analyses can provide valuable insights into the evolutionary relationships among species within this genus and their responses to environmental challenges.

Conservation implications of mitochondrial genetics

Understanding the genetic diversity represented by mitochondrial protein-coding genes is vital for developing targeted conservation strategies. Tran *et al.,* (2020) emphasize that populations with greater genetic diversity are better equipped to adapt to changing environments, thus reducing their risk of extinction. Genetic monitoring, incorporating mitochondrial genomic data, should be an integral part of conservation planning for critically endangered species like *P. nigripes*.

MATERIAL AND METHODS

DNA extraction and sequencing

DNA Extraction: Tissue samples from *P. nigripes* in the Vietnamese Central Highlands were obtained at the Biological Museum of the Tay Nguyen Institute for Scientific Research, Vietnam Academy of Science and Technology. The mitochondrial genome (mitogenome) was extracted using the QIAamp DNA Mini Kit (Qiagen, Germantown, MD, USA), following the manufacturer's protocols.

The quality of the DNA samples was assessed using multiple methods: Concentration Measurement: DNA concentration was determined using the fluorometric method (Qubit). Purity Assessment: The OD260/OD280 ratio was calculated through absorbance measurements. Size Determination: DNA size was analyzed via agarose gel electrophoresis. Samples were considered suitable for further experiments if they met the following criteria: DNA concentration ≥ 2 ng/µL; total amount ≥ 90 ng; OD260/OD280 ratio ≥ 1.70 (Nguyen *et al.*, 2024).

Samples with a DNA size less than 1000 bp were flagged for exclusion

Whole genome sequencing libraries were prepared using the NEBNext Ultra II DNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, MA, USA) in accordance with the manufacturer's guidelines. The concentration of the library was measured using the fluorometric method, while the average library size was determined using a Bioanalyzer (Agilent, Santa Clara, CA, USA). Samples were deemed suitable for sequencing if their concentration was ≥ 0.50 ng/µL for genomes less than 1 Gb or ≥ 2 ng/µL for genomes greater than 1 Gb. Sequencing was conducted using the 150 PE Next Generation Sequencing method on MiniSeq, MiSeq, or NovaSeq instruments (Illumina, San Diego, CA, USA).

Bioinformatics analysis

The raw sequencing data underwent purification using the fastp tool (v0.23.1), during which low-quality nucleotides and unreliable N nucleotides were eliminated based on their Phred scores. After purification, the reads were assembled de novo using the GetOrganelle tool (v1.7.7.0). The processed data, including optimization, assembly, and genome annotation, were saved in FASTQ format, which contains both the read sequences and their corresponding quality scores. To evaluate the amino acid and nucleotide compositions, a comparison was made across 55 primate mitogenomes using MEGA 11, employing the formulas for AT-skew = $(A - T)/(A + T)$ and GC-skew = $(G - T)/(G + T)$. Additionally, tRNA sequences were aligned with homologous sequences from related species, while protein-coding genes (PCGs) were identified through comparisons with other *Pygathrix* mitogenomes.

RESULTS

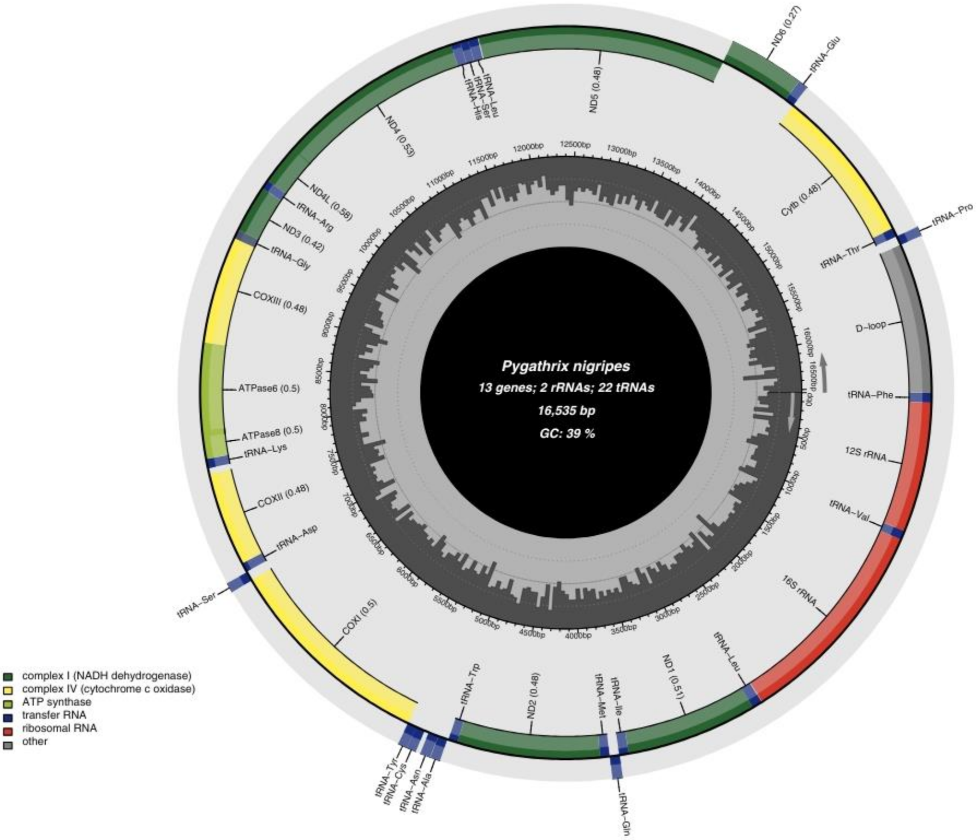

Figure 1. The circular mitochondrial genome organization of Vietnamese *Pygathrix nigripes*. This genome encodes 37 genes, including transfer RNAs (tRNA) represented in indigo, which correspond to their respective amino acids. The subunits of the cytochrome c oxidase gene (COXI, COXII, COXIII) are highlighted in yellow. Cytochrome b is abbreviated as cytb. ATPase 6 and ATPase 8 refer to specific classes of ATP synthase, while the ribosomal RNAs, 12S rRNA and 16S rRNA, are marked in red. The components of NADH dehydrogenase are labeled from ND1 to ND6.

This paper reports the first complete mitochondrial genome of the Vietnam *P. nigripes*, consisting of 16,535 bp (Figure 1), which is bigger in size than the mitogenome of two other studied *P. nigripes* collected in Nanning, China, 16534 bp (Su *et al*., 2018), and smaller than *P. nigripes* in Myanmar (16536 bp).

The complete mitogenome of *P. nigripes*

encodes a total of 37 genes, out of which 13 were protein-coding genes (PCGs) with 2 PCGs encoding NAD6_a and NAD6_b, 22 transfer RNA, 2 ribosomal RNA genes and an A+T rich region (Table 1), which is typically observed in vertebrates. The novel mitogenome sequence of *M. indica* was deposited in GenBank with the accession number KY290452.

Table 1: The organization and characterization of the complete mitochondrial genome of *Pygathrix nigripes*.

Name	Start	Stop	Strand	Length
nad6_a	22	276	L	255
trnE(gaa)	277	345	L	69
cob	350	1483	H	1134
trnT(aca)	1491	1554	H	64
trnP(cca)	1557	1623	L	67
A + T rich region	1624	2716	-	1093
trnF(ttc)	2717	2787	H	71
rrnS	2788	3736	H	949
trnV(gta)	3737	3802	H	66
rrnL	3801	5362	H	1562
trnL2(tta)	5363	5437	H	75
nad1	5440	6390	H	951
trnI(atc)	6395	6464	H	70
trnQ(caa)	6462	6533	L	72
trnM(atg)	6534	6601	H	68
nad2	6602	7639	H	1038
trnW(tga)	7644	7710	H	67
trnA(gca)	7719	7787	L	69
trnN(aac)	7789	7861	L	73
trnC(tgc)	7894	7959	L	66
trnY(tac)	7960	8024	L	65
cox1	8026	9567	H	1542
trnS2(tca)	9568	9636	L	69
trnD(gac)	9640	9707	H	68
cox2	9709	10368	H	660
trnK(aaa)	10436	10503	H	68
atp8	10505	10687	H	183
atp6	10666	11340	H	675

cox3	11346	12128	H	783
trnG(gga)	12130	12196	H	67
nad3	12197	12541	H	345
trnR(cga)	12543	12607	H	65
nad4l	12608	12901	H	294
nad4	12898	14265	H	1368
trnH(cac)	14276	14344	H	69
trnS1(agc)	14345	14403	H	59
trnL1(cta)	14404	14474	H	71
nad5	14478	16277	H	1800
nad6_b	16290	16535	L	246

Sequence and nucleotide composition of protein-coding genes

The mitochondrial genome of the Vietnamese *P. nigripes* was sequenced, revealing diverse protein-coding genes with varying lengths. Notably, in Table 2, the longest gene identified was cytochrome b (cob) at 1,134 bp, while the shortest was ATP8 at 183 bp. The overall nucleotide composition showed a higher abundance of adenine (A) and thymine (T) compared to guanine (G) and cytosine (C). This A-T richness is characteristic of many primates' mitochondrial genomes and suggests adaptive evolutionary strategies.

Table 2. Protein coding gene in the mitochondrial genome of the Vietnamese *Pygathrix nigripes*

Name	Start	Stop	Strand	Length
nad6_a	22	276	-	255
cob	350	1483	+	1134
nad1	5440	6390	+	951
nad2	6602	7639	+	1038
cox1	8026	9567	+	1542
cox2	9709	10368	+	660
atp8	10505	10687	+	183
atp6	10666	11340	+	675
cox3	11346	12128	+	783
nad3	12197	12541	+	345
nad4l	12608	12901	+	294
nad4	12898	14265	+	1368
nad5	14478	16277	+	1800

nad6_b	16290	16535	-	246

The nucleotide composition (T, C, A, G) of 13 protein-coding genes in the mitochondrial genome of *Pygathrix nigripes*.

The Table 3 lists the percentages of each nucleotide for each gene, along with the total nucleotide count for that gene. The average values across all genes are: Thymine (T): 31.24%; Cytosine (C): 25.66%; Adenine (A): 30.73%; Guanine (G): 12.37%

This composition indicates a general A-T richness, which is characteristic of many mitochondrial genomes. Nad6_a (22-276): This gene shows a remarkably high T content (43.92%) and a low C content (9.41%). Such a high T proportion may suggest a role in regulatory functions or a specific adaptation that enhances its transcription efficiency. Nad1 (5440-6390): Displays a more balanced nucleotide composition, with T at 29.13%, A at 30.70%, and C at 29.65%. This balance may indicate functional stability and less evolutionary pressure. Nad2 (6602-7639): Has the highest A content (35.07%), which could signify its importance in metabolic processes, potentially enhancing ATP synthesis in mitochondria. Nad6_b (16290-16535): Exhibits a very low C content (5.28%) and a high G content (32.52%), suggesting unique evolutionary pressures. This unusual distribution could affect gene function and stability.

The average values indicate the overall trends in nucleotide usage: There is a preference for A and T (A-T richness) over C and G (G-C deficiency) and the low G content across all genes could impact the genome's stability and functionality.

The nucleotide composition can reflect the functional roles of these genes. Genes with higher T and A content may be more actively expressed or involved in critical metabolic pathways.

Variations in nucleotide composition can also provide insights into evolutionary adaptations, such as responses to environmental pressures or changes in habitat.

Understanding the genetic structure and diversity of *P. nigripes* is crucial for conservation efforts. Genes that show significant variations may require targeted management strategies to preserve genetic diversity and adaptability.

Table 3: The nucleotide composition (T, C, A, G) of 13 protein-coding genes in the mitochondrial genome of *Pygathrix nigripes*.

	T(U)	C	A	G	Total
circular) 22-276 - nad6_a	43.92157	9.411765	21.96078	24.70588	255
circular) 5440-6390 + nad1	29.12723	29.653	30.70452	10.51525	951
circular) 6602-7639 + nad2	28.42004	27.842	35.06744	8.67052	1038
circular) 8026-9567 + cox1	32.36057	24.44877	26.6537	16.53696	1542
circular) 9709-10368 + cox2	29.84848	24.84848	31.81818	13.48485	660
circular) 10505-10687 + atp8	28.96175	25.68306	38.79781	6.557377	183

circular) 10666-11340 + atp6	30.07407	27.25926	31.40741	11.25926	675
circular) 11346-12128 + cox3	31.41762	26.94764	27.96935	13.66539	783
circular) 12197-12541 + nad3	32.46377	25.50725	32.17391	9.855072	345
circular) 12608-12901 + nad4l	34.69388	25.17007	28.57143	11.56463	294
circular) 12898-14265 + nad4	31.21345	27.04678	31.72515	10.01462	1368
circular) 14478-16277 + nad5	30.27778	26.61111	33.27778	9.833333	1800
circular) 16290-16535 - nad6_b	40.65041	5.284553	21.54472	32.52033	246
Avg.	31.2426	25.66075	30.72978	12.36686	780

Genes like nad6_a, with a high T content, may be more actively transcribed, reflecting their critical roles in mitochondrial function and energy metabolism. The variations in nucleotide composition may indicate different evolutionary pressures and adaptations across *P. nigripes* and related species. For example, the low C content in nad6_b suggests unique evolutionary constraints that could affect its function.

Understanding the nucleotide composition and its implications for gene function is crucial for conservation strategies. Genes that exhibit significant variation in nucleotide composition may reflect the adaptability of *P. nigripes* to its environment and the pressures it faces.

AT-skew and GC-skew of Vietnamese central highland *Pygathrix nigripes*

Most *Pygathrix* species, including *P. nigripes*, *P. cinerea*, and *P. nemaeus*, show a similar nucleotide composition with A and T being slightly more abundant than C and G (Table 4). *Macaca* species exhibit a notable decrease in A and T, with a corresponding increase in C, which suggests different evolutionary pressures or functional adaptations. The average GC content appears higher in *Macaca* species, reflected in their positive GC-skew, indicating a more balanced nucleotide composition compared to *Pygathrix* species.

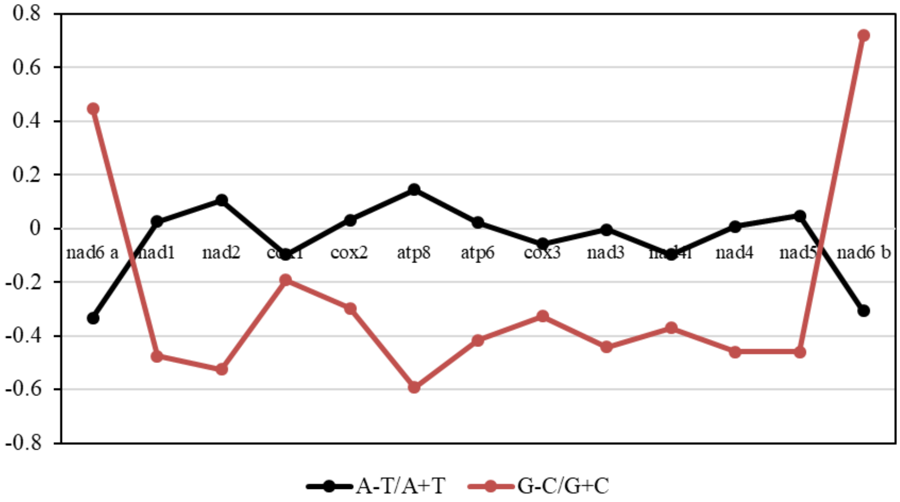

Figure 2. Graphical representation of AT and GC skew across all 13 protein-coding genes of the *Pygathrix nigripes* mitochondrial genome from Vietnam.

The AT-skew values for *Pygathrix* species are consistently negative, indicating a slight preference for Thymine over Adenine (Figure 2). For example, the Vietnamese *P. nigripes* has an AT-skew of -0.00827. In contrast, species like *Macaca leonina* and *M. arctoides* show positive AT-skew values (0.065732 and 0.063577, respectively), suggesting a higher preference for Adenine. This can influence gene expression and regulation. The GC-skew values are negative for all *Pygathrix* species listed, indicating a higher frequency of Cytosine over Guanine. The Vietnamese *P. nigripes* has a GC-skew of -0.34959. The more negative GC-skew values in *Pygathrix* species compared to other genera may indicate selective pressures that favor C in their mitochondrial genomes.

The nucleotide composition can influence the functionality of mitochondrial genes, affecting processes such as energy production and metabolic efficiency. The preference for T and A in *Pygathrix* species suggests adaptations that may optimize mitochondrial function in their specific habitats. The similarities in nucleotide composition among *Pygathrix* species suggest a shared evolutionary history and similar selective pressures. The differences observed in *Macaca* species may indicate divergent evolutionary paths and adaptations to different environmental conditions or ecological niches. Understanding the genetic structure and diversity of *P. nigripes* compared to related species is vital for conservation efforts. The data can inform strategies aimed at preserving genetic diversity and adaptability in changing environments.

The analysis of nucleotide composition and skews in Table 3 provides valuable insights into the evolutionary dynamics and functional adaptations of mitochondrial genomes in *P. nigripes* and related species. The consistent patterns of A-T richness and negative GC-skew across *Pygathrix* species highlight their shared evolutionary history, while the differences in *Macaca* species suggest distinct adaptive strategies. These findings are essential for developing effective conservation strategies to protect this critically endangered species and its relatives. Future research should continue to explore these genetic patterns and their implications for primate biology and conservation.

Table 4. AT-skew and GC-skew of Vietnamese *Pygathrix nigripes*

	Accession Number	**T(U)**	**C**	**A**	**G**	**Total**	**AT-Skew**	**GC-Skew**
Vietnamese *Pygathrix nigripes*		31.2	25.7	30.7	12.4	780.0	-0.00827	-0.34959
Pygathrix nigripes	MH064177	31.2	25.9	30.6	12.3	807.4	−0.01002	−0.35463
Pygathrix cinerea	JQ821842	30.9	26.1	30.5	12.5	868.6	−0.0062	−0.35277
Pygathrix nemaeus	JF293096	30.9	26.1	30.5	12.5	868.6	−0.00678	−0.35155
Macaca leonina	KP330231	26.5	30.9	30.2	12.3	870	0.065732	−0.42945

Presbytis femoralis	KU899140	31.5	25.4	30.9	12.2	867.5	−0.00896	−0.34998
Nasalis larvatus	DQ355298	30.5	26.7	30.6	12.2	869.1	0.001593	−0.37082
Rhinopithecus roxellana	KM504390	31.4	25.6	30.8	12.2	868.6	−0.01054	−0.35253
Rhinopithecus brelichi	JQ821836	31.6	25.5	30.6	12.4	868.8	−0.01638	−0.34612
Rhinopithecus bieti	JQ821839	31.5	25.6	30.7	12.2	869.1	−0.01181	−0.35612
Rhinopithecus avunculus	JF293093	31.5	25.5	30.8	12.3	868.4	−0.01138	−0.34898
Macaca arctoides	KM360179	26.4	31	30	12.5	870.2	0.063577	−0.4244
Macaca mulatta	JQ821843	26.6	31	29.8	12.6	870	0.056444	−0.42052
Papio hamadryas	NC001992	26.2	31.2	29.7	12.9	868.6	0.061768	−0.41663
Callithrix jacchus	KM588314	28.3	27.8	31.1	12.9	867.7	0.046296	−0.36736
Homo sapiens neanderthalensis	OM062614	26.2	31.9	28.8	13.1	867.8	0.046422	−0.41631
Cebus albifrons	NC002763	29.9	26.7	31.3	12.1	866.8	0.02306	−0.37663

Amino acid composition

Table 5 and Figure 3 present the percentage frequency of amino acids in the mitochondrial genomes of three *Pygathrix* species: *P. nigripes* from Vietnam, *P. nigripes* from China, and *P. nemaeus* from China. The amino acid composition shows that Leucine (Leu) is the most abundant amino acid, with a frequency of 13.0% in *P. nigripes* from Vietnam and 13.9% in *P. nigripes* from China, indicating a conserved role in mitochondrial function. Additionally, Isoleucine (Ile) has the highest frequency in *P. nigripes* from Vietnam at 9.5%, suggesting unique adaptations in metabolic pathways, while amino acids such as Serine (Ser) and Threonine (Thr) also exhibit high frequencies, emphasizing their importance in mitochondrial function.

When comparing the populations, it is evident that the amino acid profiles of the two *P. nigripes* populations are quite similar, indicating strong genetic conservation. The minor differences observed may reflect environmental adaptations rather than significant evolutionary divergence. In contrast, when compared to *P. nemaeus*, the amino acid frequencies in *P. nigripes* are generally higher, which may suggest differences in mitochondrial function or evolutionary pressures between the species.

Understanding these amino acid compositions can significantly impact mitochondrial functionality, including energy production and metabolic pathways. The high levels of Leu and Ile may be particularly important for energy metabolism, indicating that these species have adapted to optimize their mitochondrial efficiency in their respective environments. These findings provide valuable insights into the genetics and functional aspects of *Pygathrix* species, highlighting the importance of preserving genetic diversity to maintain the adaptability of these species in the future.

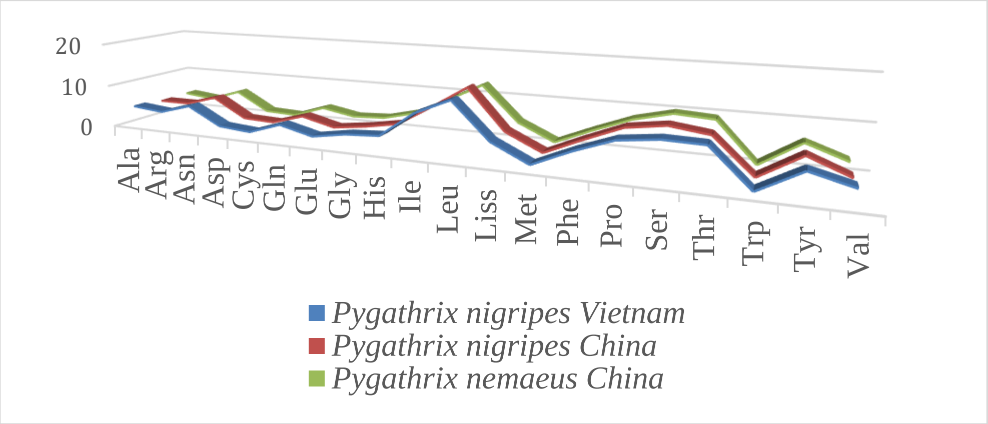

Figure 3. Amino acid composition and their relative frequency (%) in the complete mitogenome of Vietnamese *P. nigripes*; Chinese *P. nigripes* and Chinese *P. nemaeus*

Table 5. Percentage frequency of amino acid composition in the whole mitogenome of *P.* species: Vietnamese *P. nigripes*, Chinese *P. nigripes*, Chinese *P. nemaeus*.

	Vietnamese *Pygathrix nigripes*	**Chinese *Pygathrix nigripes***	**Chinese *Pygathrix nemaeus***
Ala	4.7	4.2	4.4
Arg	4	4.2	3.6
Asn	6.1	6.3	6
Asp	2	1.9	1.7
Cys	1.4	1.6	1.4
Gln	3.9	3.8	3.9
Glu	2	1.9	2.5
Gly	3.3	2.9	2.9
His	3.8	4.3	4.8
Ile	9.5	9	8.7
Leu	13	13.9	12.7
Liss	5	4.9	4.8
Met	1.1	1.1	1.1
Phe	4.8	4.8	4.6
Pro	7.9	8.2	7.8
Ser	8.9	9.4	9.8
Thr	8.7	8.3	9.3
Trp	0.8	0.7	0.6
Tyr	5.7	6	6.1
Val	3.5	2.6	3.1

DISCUSSION

The complete mitochondrial genome analysis of *Pygathrix nigripes* has revealed significant insights into its genetic structure, evolutionary adaptations, and conservation needs. The mitochondrial genome is pivotal for understanding species evolution due to its maternal inheritance and relatively rapid mutation rates, making it a valuable tool for phylogenetic studies (Duminil and Di Michele, 2009; Zhang *et al.*, 2016).

This A-T skew may reflect adaptive evolutionary strategies that enhance the efficiency of mitochondrial functions, particularly in energy metabolism (González-Gómez *et al.*, 2023). The implications of this genetic diversity are profound; populations with higher genetic variability are typically more resilient to environmental changes and diseases (Frankham, 2005).

Research has shown that mitochondrial genetic structures can serve as indicators of population health, especially in endangered species such as *P. nigripes* (Ha *et al.*, 2023). Understanding the genetic diversity within this species is crucial for developing effective conservation strategies, particularly as habitat destruction and climate change pose significant threats to its survival (Sterling *et al.*, 2013).

Comparative genomics and evolutionary Insights

Comparative analyses with other *Pygathrix* species suggest shared evolutionary pathways, while also highlighting unique adaptations that may enhance resilience to habitat changes. For example, variations in the nucleotide composition and codon usage across different species may indicate differing evolutionary pressures and ecological adaptations (Liedigk *et al.*, 2014). Such studies are essential not only for understanding the evolutionary dynamics within the genus but also for informing conservation efforts.

The negative GC-skew observed in *P. nigripes* compared to other genera, such as *Macaca*, indicates selective pressures that favor cytosine over guanine, which could influence gene expression and regulatory mechanisms (Pawlak *et al.*, 2023). This highlights the potential for distinct evolutionary adaptations among primate lineages and underscores the importance of examining mitochondrial genomes in conservation genetics.

Future research should focus on expanding the sampling of mitochondrial genomes across *Pygathrix* species and related genera to further elucidate the evolutionary relationships and diversification patterns. Integrating genomic data with ecological and behavioral studies will provide a comprehensive understanding of how these species adapt to their environments. Long-term genetic monitoring, incorporating mitochondrial genomic data, should be an integral part of conservation planning for critically endangered species like *P. nigripes*.

CONCLUSIONS

This study provides a detailed analysis of the protein-coding genes in the mitochondrial genome of the Vietnamese *P. nigripes*, highlighting its unique genetic characteristics and evolutionary significance. The characterization of these genes revealed a distinct nucleotide composition, with a notable A-T richness that suggests specific adaptations to the species' ecological niche.

The presence of key amino acids, particularly Leu and Ile, indicates their critical roles in mitochondrial function and energy metabolism, reflecting evolutionary pressures that favor metabolic efficiency in response to environmental challenges. The variations in the nucleotide composition and codon usage among the protein-coding genes may provide insights into the evolutionary history of *P. nigripes*, revealing how this species has adapted to its habitat over time.

Furthermore, comparative analyses with other *Pygathrix* species suggest shared evolutionary pathways, while also highlighting unique adaptations that may enhance resilience to habitat changes. Understanding these specific characteristics is essential for developing effective conservation strategies aimed at preserving the genetic diversity and evolutionary potential of *P. nigripes*. Future research should continue to explore these genetic patterns and their implications for the species' adaptability and survival in a rapidly changing environment.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

Anderson, S., Bankier, A. T., Barrell, B. G., de Bruijn, M. H., Coulson, A. R., Drouin, J., *et al.*, (1981). Sequence and organization of the human mitochondrial genome. *Nature*, *290*, 457-465. https://doi.org/10.1038/290457a0.

Błażej, P., Mackiewicz, D., Wnętrzak, M. and Mackiewicz, P. (2017). The impact of selection at the amino acid level on the usage of synonymous codons. G3: Genes, Genomes. *Genetics*, *7*, 967-981. https://doi.org/10.1534/g3.116.038125.

Donnelly D (2024). Endangered Species Conservation in Vietnam. https://www.academia.edu/download/114497316/Vietnam_SAR_ddonnelly_202405.pdf

Duminil, J. and Di Michele, M. (2009). Plant species delimitation: a comparison of morphological and molecular markers. *Plant Biosystems*, *143*, 528-542. https://doi.org/10.1080/11263500902722964.

Frankham, R. (2005). Genetics and extinction. *Biological Conservation*, *126*, 131-140. https://doi.org/10.1016/j.biocon.2005.05.002.

González-Gómez, J. P., Lozano-Aguirre, L. F., Medrano-Félix, J. A., Chaidez, C., Gerba, C. P., Betancourt, W. Q. *et al.*, (2023). Evaluation of nuclear and mitochondrial phylogenetics for the subtyping of *Cyclospora cayetanensis*. *Parasitology Research*, *122*, 2641-2650. https://doi.org/10.1007/s00436-023-07963-8.

Ha, N. H., Dam, V. M. and Dung, V. T. (2023). The population status of the black-shanked douc langurs (*Pygathrix nigripes*) in Chua Chan Mountain, Dong Nai Province, Vietnam. *Vietnamese Journal of Primatology*, *3*, 69-78. https://static1.1.sqspcdn.com/static/f/1200343/28627709/1702718352323/Vietnamese_J_Primatol_Vol_34_2023.pdf?token=o68RvH%2FKUX0Au%2FL9XMKvLmSsS9w%3D

Hooper, S. D. and Berg, O. G. (2000). Gradients in nucleotide and codon usage along *Escherichia coli* genes. *Nucleic Acids Research*, *28*, 3517-3523. http://doi.org/10.1093/nar/28.18.3517

Liedigk, R., Roos, C., Brameier, M. and Zinner, D. (2014). Mitogenomics of the Old World monkey tribe Papionini. *BMC Ecology and Evolution*, *14*, 1-12. http://www.biomedcentral.com/1471-2148/14/176.

Lynn, M. S., Sechi, P., Chikhi, L. and Goossens, B. (2016). Primate conservation genetics at the dawn of conservation genomics. *An introduction to primate conservation-Oxford Academy:* 19-53 http://doi.org/10.1093/acprof:oso/9780198703389.003.0005

Nadler, T. (2010). Status of Vietnamese primates-complements and revisions. *Conservation of primates in Indochina*: 3-16.

Nguyen, M. T. P., Nguyen, T. T. T., Ha, T. T., Ho, C. N. Q., Le, C. P. M., Hoang, *et al.*, (2024). Characterization of the Complete Mitochondrial Genome of the Central Highland Grey-Shanked Douc Langur (*Pygathrix cinerea*), a Critically Endangered Species Endemic to Vietnam (Mammalia: Primates). *Current Issues in Molecular Biology, 46,* 9928-9947. https://doi.org/10.3390/cimb46090592.

Pawlak, K., Błażej, P., Mackiewicz, D. and Mackiewicz, P. (2023). The influence of the selection at the amino acid level on synonymous codon usage from the viewpoint of alternative genetic codes. *International Journal of Molecular Sciences, 24,* 1185. https://doi.org/10.3390/ijms24021185.

Roos, C., Helgen, K. M., Miguez, R. P., Thant, N. M. L., Lwin, N., …Hein, Z. M. *et al.*, (2020). Mitogenomic phylogeny of the Asian colobine genus *Trachypithecus* with special focus on *Trachypithecus phayrei* (Blyth, 1847) and description of a new species. *Zoological Research, 41*: 656. http://doi.org/10.24272/j.issn.2095-8137.2020.254.

Shao, Y., Zhou, L., Li, F., Zhao, L., Zhang, B. L., X. L., *et al.*, (2023). Phylogenomic analyses provide insights into primate evolution. *Science, 380,* 913-924. http://doi.org/10.1126/science.abn6919.

Sterling, E. J., Bynum, N. and Blair, M. E. (2013). Conclusion: the future of studying primates in a changing world. *Oxford University Press, Oxford*.

Su, Q., Chen, C., Wei, D., Li, D., Xu, H., Zhu, G., *et al.*, (2018) . Complete mitochondrial genome of black-shanked douc langurs (*Pygathrix nigripes*) and its phylogenetic analysis. *Mitochondrial DNA Part B, 3,* 1085-1086. https://doi.org/10.1080/23802359.2018.1512386.

Tran, D. V., Vu, T. T., Tran, B. Q., Nguyen, M. D., Vu, P. T., Nguyen, T. C., *et al.*, (2020). Modelling the change in the distribution of the black-shanked douc, *Pygathrix nigripes* (Milne-Edwards) in the context of climate change: Implications for conservation. *Raffles Bulletin of Zoology, 68,* 769-778. http://doi.org/10.26107/RBZ-2020-0088

Zhang, Y., Liu, X., Bai, J., Tian, X., Zhao, X., Tong, C., *et al.*, (2016). Mitoguardin regulates mitochondrial fusion through MitoPLD and is required for neuronal homeostasis. *Molecular Cell, 61,* 111-124. http://dx.doi.org/10.1016/j.molcel.2015.11.017.