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STUDY ON THE CHARACTERISTICS OF THE MITOCHONDRIAL GENOME OF SEVERAL PRIMATE SPECIES IN THE CENTRAL HIGHLANDS OF VIETNAM

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INTRODUCTION

1. Rational for the study

Primates are a mammalian group exhibiting a very high degree of genetic similarity to humans and play an important role in medical, ecological, and evolutionary research. Vietnam is one of the countries with high biodiversity, particularly with respect to primates. The Central Highlands, characterized by unique geographical, climatic, and habitat conditions, are home to several rare and endemic primate species.

However, under the strong impacts of deforestation, illegal hunting, habitat loss, and environmental changes, primate populations in this region have been rapidly declining, leading to an increased risk of extinction in the wild. In this context, the study of genetic structure and phylogenetic relationships is essential for understanding evolutionary origins and relationships among primate species distributed in the Central Highlands of Vietnam.

The present study focuses on five primate species collected from the Central Highlands, including the grey-shanked douc langur (*Pygathrix cinerea*), black-shanked douc langur (*Pygathrix nigripes*), red-shanked douc langur (*Pygathrix nigripes*), rhesus macaque (*Macaca mulatta*), and stump-tailed macaque (*Macaca arctoides*). These species represent both endemic and widely distributed primates in Vietnam and are of high scientific value for phylogenetic and genetic studies.

Globally, studies on genetic structure and phylogenetic relationships based on mitochondrial DNA have achieved significant progress. Mitochondrial gene regions such as *16S rRNA*, *cytochrome b*, and the *D-loop* have been widely used to reconstruct phylogenetic relationships in primates. However, in Vietnam—especially in the Central Highlands—systematic and comparative mitochondrial genetic data for these primate species remain limited. The lack of comprehensive mitochondrial genome data has constrained the understanding of genetic diversity and evolutionary relationships among local primate populations.

Therefore, this study was conducted to analyze mitochondrial gene sequences of the five selected primate species distributed in the Central Highlands of Vietnam. By examining mitochondrial genetic characteristics and reconstructing phylogenetic relationships, the study aims to fill existing data gaps and provide a scientific basis for understanding primate evolution and supporting future research and conservation efforts in Vietnam.

2. Specific objectives

(1) Sequence and annotate the complete or major portions of mitochondrial DNA (mtDNA) of selected primate species from the Central Highlands of Vietnam.

(2) Assess genetic diversity among primate individuals based on mitochondrial gene sequences.

(3) Reconstruct phylogenetic relationships of primates using mitochondrial gene sequences in order to clarify evolutionary relationships among species and populations.

3. Research Contents

The research was conducted with the following main contents:

(1) Analysis of mitochondrial DNA sequences, including the determination of nucleotide positions, nucleotide composition, base

composition, and genetic indices of selected primate species distributed in the Central Highlands of Vietnam.

(2) Reconstruction of phylogenetic trees based on mitochondrial gene sequence data in order to examine phylogenetic relationships among primate species and individuals.

(3) Comparative analysis between mitochondrial gene sequences obtained in this study and previously published sequences to determine phylogenetic

4. Scientific and Practical Significance

This study is grounded in the principles of molecular genetics, in which mitochondrial DNA is an effective tool for evolutionary and phylogenetic research due to its maternal inheritance, lack of recombination, and high mutation rate. By analyzing mitochondrial gene sequences of selected primate species from the Central Highlands of Vietnam, this study provides additional genetic data for understanding genetic diversity and phylogenetic relationships. The results contribute to clarifying evolutionary relationships among Vietnamese primates based on mitochondrial DNA. From a practical perspective, the generated genetic data support taxonomic studies and comparative analyses of primate species in Vietnam. The findings also provide reference information for future studies related to primate conservation and genetic resource management.

Chapter 1. RESEARCH OVERVIEW

1.1. Background and Necessity of Primate Conservation Research

This section outlines the scientific and conservation context of primate research. Primates play an important role in ecosystems and possess high scientific value due to their close genetic relationship with humans. However, primates are among the most threatened mammalian groups worldwide, facing severe population declines caused by habitat loss, hunting, and environmental change. These challenges highlight the urgent need for conservation-oriented research on primates.

1.1.1. Importance of Primates and Global Threat Status

Primates are a key mammalian group with significant ecological, evolutionary, and biomedical importance. Globally, a large proportion of primate species are currently classified as threatened or endangered due to deforestation, habitat fragmentation, illegal hunting, and climate change. Population declines and range contractions have been documented across Asia, Africa, and Madagascar, placing primates among the most threatened vertebrate groups worldwide.

1.1.2. Vietnam’s Position in the Global Primate Conservation Crisis

Vietnam is recognized as one of the world’s biodiversity hotspots for primates, harboring a high number of species, including several endemic and rare taxa. However, primate populations in Vietnam have experienced severe declines in recent decades as a result of forest loss, hunting pressure, and wildlife trade. Consequently, many Vietnamese primate species are currently listed in threatened categories, underscoring Vietnam’s critical role in global primate conservation efforts.

1.2. Role of Mitochondrial DNA in Phylogenetic Studies

This section introduces mitochondrial DNA as an important molecular marker for evolutionary and phylogenetic research. Due to its biological characteristics, mitochondrial DNA has been widely used to investigate evolutionary relationships among species and populations.

1.2.1. Structural and Genetic Characteristics of the Mitochondrial Genome

The mitochondrial genome is a small, circular DNA molecule characterized by maternal inheritance, lack of recombination, and a relatively high mutation rate. It contains protein-coding genes, ribosomal RNA genes, transfer RNA genes, and a control region. These features make mitochondrial DNA particularly suitable for phylogenetic and evolutionary analyses.

1.2.2. Applications of Mitochondrial DNA in Primate Research

Mitochondrial DNA has been extensively applied in primate studies to reconstruct phylogenetic relationships, assess genetic diversity, and clarify taxonomic relationships. Gene regions such as *cytochrome b*, *16S rRNA*, and the *D-loop* have been widely used to infer evolutionary relationships at different taxonomic levels in primates.

1.3. Overview of Mitochondrial DNA Research on Primates in Vietnam and Worldwide

1.3.1. Overview of Mitochondrial DNA Research on Primates in Vietnam

In Vietnam, studies on primate mitochondrial DNA have been conducted for several species; however, most research has focused on limited genetic markers or small sample sizes. Comprehensive analyses of mitochondrial genome characteristics and phylogenetic relationships remain scarce, particularly for primate species distributed in the Central Highlands.

1.3.2. Overview of Mitochondrial DNA Research on Primates Worldwide

Internationally, mitochondrial DNA has been widely used in primate phylogenetic and evolutionary studies. Numerous studies have successfully applied mitochondrial gene sequences and complete mitochondrial genomes to resolve phylogenetic relationships and investigate evolutionary history. These studies provide an important scientific foundation and methodological reference for mitochondrial DNA research on primates in Vietnam.

1.4. Research Objectives

This section presents the research objectives of the dissertation. The study aims to investigate mitochondrial genetic characteristics and phylogenetic relationships of selected primate species. Through mitochondrial DNA analysis, the research seeks to contribute genetic data and improve understanding of primate evolutionary relationships, particularly for species distributed in the Central Highlands of Vietnam.

Chapter 2. MATERIALS AND METHODS OF STUDY

2.1. Study Subjects

This study focuses on selected primate species distributed in the Central Highlands of Vietnam. The research subjects include five primate species representing both endemic and widely distributed taxa, namely the grey-shanked douc langur (*Pygathrix cinerea*), black-shanked douc langur (*Pygathrix nigripes*), red-shanked douc langur (*Pygathrix nigripes*), rhesus macaque (*Macaca mulatta*), and stump-tailed macaque (*Macaca arctoides*). Biological samples collected from these species served as the material for mitochondrial DNA analysis.

2.2. Research Methods

2.2.1. DNA Extraction and Sequencing

Genomic DNA was extracted from biological samples using standard molecular biology protocols. Mitochondrial DNA regions were amplified and sequenced to obtain genetic data for subsequent analyses. The quality and reliability of DNA sequences were ensured prior to further processing.

2.2.2. Mitochondrial Genome Assembly

Mitochondrial genome sequences were assembled from sequencing data using appropriate assembly approaches. The assembled mitochondrial genomes were examined to determine gene structure, gene order, and genome organization.

2.2.3. Bioinformatic Analyses

Bioinformatic analyses were conducted to process and analyze mitochondrial DNA sequences. These analyses included sequence editing, alignment, determination of nucleotide composition, and assessment of genetic characteristics of the mitochondrial genome.

2.2.4. Phylogenetic Analysis

Phylogenetic trees were reconstructed based on mitochondrial gene sequences to examine evolutionary relationships among the studied primate species. The resulting phylogenetic relationships were used to clarify genetic divergence and evolutionary positions of primates distributed in the Central Highlands of Vietnam.

Chapter 3. RESULTS AND DISCUSSION

3.1. Gene Sequencing Results

This section presents the results obtained from whole mitochondrial genome sequencing of primate individuals collected from the Central Highlands of Vietnam. The sequencing workflow began with the successful preparation of sequencing libraries, ensuring that mitochondrial DNA was sufficiently represented and suitable for high-throughput sequencing platforms. Quality control assessments demonstrated that all libraries met the required standards in terms of DNA integrity and library complexity.

Whole mitochondrial genome sequencing generated high-quality sequencing reads with adequate depth and coverage for downstream analyses. The sequencing output enabled reliable assembly of complete or nearly complete mitochondrial genomes for all studied individuals. The availability of high-quality mitochondrial genome sequences formed a solid foundation for subsequent analyses of genome organization, nucleotide composition, gene structure, and phylogenetic relationships among primate species.

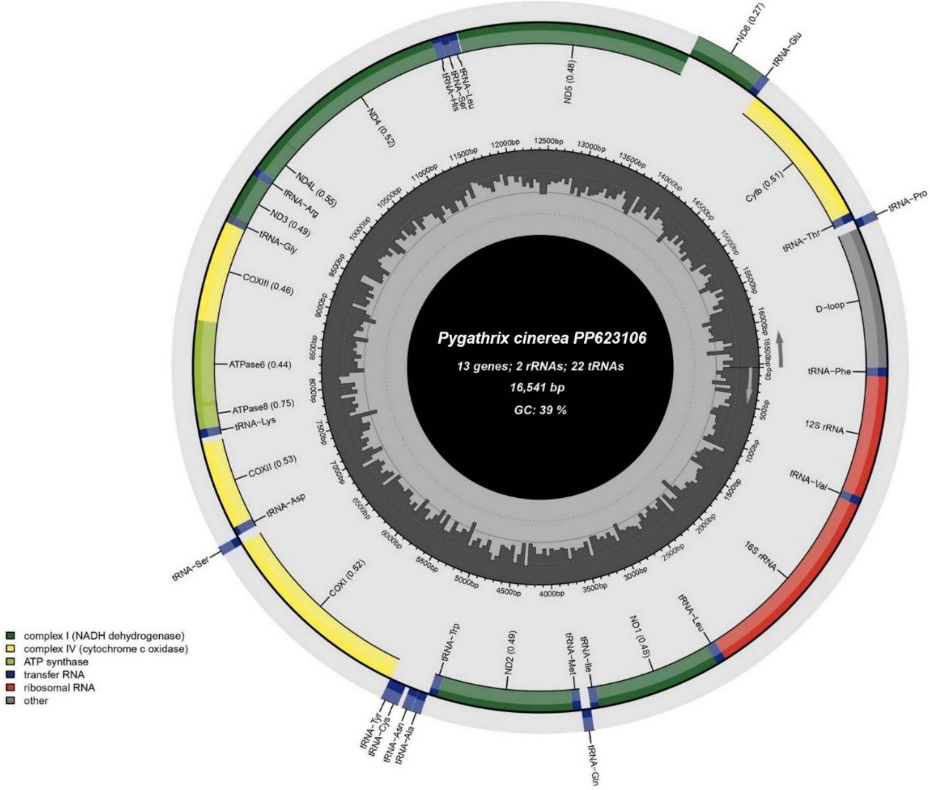

Figure 1. Complete mitochondrial gene structure of the grey-shanked douc langur from the Central Highlands of Vietnam.

3.1.1. Results of Library Preparation for Whole-Genome Sequencing

Sequencing libraries were constructed using standardized protocols optimized for mitochondrial genome analysis. The prepared libraries exhibited appropriate fragment size distributions and quality indicators, confirming their suitability for sequencing. The success of library preparation ensured consistency across samples and minimized technical variation, thereby improving the reliability of comparative genomic analyses conducted in this study.

3.1.2. Results of Whole-Genome Sequencing

Whole mitochondrial genome sequencing produced high-quality reads that were effectively used for genome assembly and annotation. The sequencing results enabled the identification of all major mitochondrial genes and non-coding regions, allowing comprehensive characterization of mitochondrial genome structure. These results provided the basis for comparative analyses among primate species and for reconstructing phylogenetic relationships using mitochondrial genome data.

3.2.1. Characteristics of the Complete Mitochondrial Genome of the Grey-shanked Douc Langur (*Pygathrix cinerea*) from the Central Highlands of Vietnam

3.2.1.1. Nucleotide Composition

The complete mitochondrial genome of the grey-shanked douc langur (*Pygathrix cinerea*) exhibited a clear nucleotide bias toward adenine (A) and thymine (T), which is a typical feature of primate mitochondrial genomes. Nucleotide composition differed among functional regions, including protein-coding genes, RNA genes, and the control region, reflecting differences in functional constraints. Overall, the nucleotide composition of *P. cinerea* was consistent with that reported for other colobine primates.

3.2.1.2. Protein-Coding Genes

The mitochondrial genome of *Pygathrix cinerea* contains the standard set of protein-coding genes involved in oxidative phosphorylation. Gene order and orientation were conserved and similar to those of other primates. Codon usage patterns reflected the AT-rich nature of the mitochondrial genome. Comparative analysis indicated high similarity with other *Pygathrix* species, together with species-specific sequence variations contributing to genetic differentiation.

3.2.1.3. AT-Rich Region

The AT-rich region was identified as a non-coding region with a high proportion of adenine and thymine, playing an important role in mitochondrial DNA replication and transcription. This region showed higher sequence variability than coding regions and exhibited characteristics comparable to those observed in other primate species.

3.2.1.4. Transfer RNA (*tRNA*) and Ribosomal RNA (*rRNA*) Genes

The mitochondrial genome of *Pygathrix cinerea* includes a complete set of *tRNA* genes and two *rRNA* genes. Most *tRNA* genes were predicted to form typical cloverleaf secondary structures, while the *rRNA* genes showed conserved sequence features consistent with strong functional constraints.

3.2.1.5. Overlapping and Intergenic Regions

Analysis of overlapping and intergenic regions revealed a compact mitochondrial genome organization. Several genes overlapped or were separated by short intergenic spacers, a feature commonly observed in primate mitochondrial genomes.

3.2.1.6. Phylogenetic Position of *Pygathrix cinerea*

Phylogenetic analysis based on the complete mitochondrial genome placed *P. cinerea* within the genus *Pygathrix*, forming a distinct lineage consistent with its species status. The phylogenetic position inferred from whole mitochondrial genome data was consistent with results obtained from analyses using individual mitochondrial gene regions.

3.2.1.7. Discussion

The mitochondrial genome of *Pygathrix cinerea* from the Central Highlands exhibits conserved structural and genetic characteristics typical of primate mitochondrial DNA. Comparative analyses indicate close relationships with other *Pygathrix* species, while species-specific features support genetic differentiation and confirm the evolutionary distinctiveness of *P. cinerea*. These results provide valuable genetic information for further phylogenetic and comparative studies of douc langurs in Vietnam

3.2.2. Characteristics of the Complete Mitochondrial Genome of the Stump-tailed Macaque (*Macaca arctoides*) from the Central Highlands of Vietnam

3.2.2.1. Nucleotide Composition

The complete mitochondrial genome of the stump-tailed macaque (*Macaca arctoides*) exhibited a clear nucleotide bias toward adenine (A) and thymine (T), which is typical of primate mitochondrial genomes. Nucleotide composition varied among different functional regions, including protein-coding genes, RNA genes, and non-coding regions, reflecting differences in functional constraints. Overall, the nucleotide composition of *M. arctoides* was consistent with that reported for other macaque species.

3.2.2.2. Protein-Coding Genes

The mitochondrial genome of *Macaca arctoides* contains the standard set of protein-coding genes involved in oxidative phosphorylation. Gene order and orientation were conserved and similar to those of other primates. Codon usage patterns reflected the AT-rich nature of the mitochondrial genome. Comparative analysis indicated high similarity with other species within the genus *Macaca*, together with species-specific sequence variations contributing to genetic differentiation.

3.2.2.3. AT-Rich Region

The AT-rich region was identified as a non-coding region with a high proportion of adenine and thymine, playing an important role in mitochondrial DNA replication and transcription. This region showed higher sequence variability than coding regions and exhibited characteristics comparable to those reported in other macaque species.

3.2.2.4. Transfer RNA (*tRNA*) and Ribosomal RNA (*rRNA*) Genes

The mitochondrial genome of *Macaca arctoides* includes a complete set of *tRNA* genes and two *rRNA* genes. Most *tRNA* genes were predicted to form typical cloverleaf secondary structures, while the *rRNA* genes showed conserved sequence features consistent with strong functional constraints.

3.2.2.5. Overlapping and Intergenic Regions

Analysis of overlapping and intergenic regions revealed a compact mitochondrial genome organization. Several genes overlapped or were separated by short intergenic spacers, a feature commonly observed in primate mitochondrial genomes.

3.2.2.6. Discussion

The mitochondrial genome of *Macaca arctoides* from the Central Highlands exhibits conserved structural and genetic characteristics typical of primate mitochondrial DNA. Comparative analyses indicate close relationships with other macaque species, while species-specific features support genetic differentiation and confirm the evolutionary distinctiveness of *M. arctoides*. These results provide valuable genetic information for further comparative and phylogenetic studies of macaques in Vietnam.

3.2.3. Characteristics of the Complete Mitochondrial Genome of the Red-shanked Douc Langur (*Pygathrix nigripes*) from the Central Highlands of Vietnam

3.2.3.1. Nucleotide Composition

The complete mitochondrial genome of the red-shanked douc langur (*Pygathrix nigripes*) exhibited a clear nucleotide bias toward adenine (A) and thymine (T), which is characteristic of primate mitochondrial genomes. Nucleotide composition differed among protein-coding genes, RNA genes, and non-coding regions, reflecting differences in functional constraints.

Overall, the nucleotide composition of *P. nigripes* was consistent with that reported for other species within the genus *Pygathrix*.

3.2.3.2. Protein-Coding Genes

The mitochondrial genome of *Pygathrix nigripes* contains the standard set of protein-coding genes involved in oxidative phosphorylation. Gene order and orientation were conserved and similar to those of other colobine primates. Codon usage patterns reflected the AT-rich nature of the mitochondrial genome. Comparative analysis indicated close similarity with other *Pygathrix* species, together with species-specific sequence variations contributing to genetic differentiation.

3.2.3.3. AT-Rich Region

The AT-rich region was identified as a non-coding region with a high proportion of adenine and thymine and plays an important role in mitochondrial DNA replication and transcription. This region exhibited higher sequence variability than coding regions and showed characteristics comparable to those observed in other douc langur species.

3.2.3.4. Transfer RNA (*tRNA*) and Ribosomal RNA (*rRNA*) Genes

The mitochondrial genome of *Pygathrix nigripes* includes a complete set of *tRNA* genes and two *rRNA* genes. Most *tRNA* genes were predicted to form typical cloverleaf secondary structures, while the *rRNA* genes showed conserved sequence features consistent with strong functional constraints.

3.2.3.5. Overlapping and Intergenic Regions

Analysis of overlapping and intergenic regions revealed a compact mitochondrial genome organization. Several genes overlapped or were separated by short intergenic spacers, a feature commonly observed in primate mitochondrial genomes.

3.2.3.6. Discussion

The mitochondrial genome of *Pygathrix nigripes* from the Central Highlands exhibits conserved structural and genetic characteristics typical of primate mitochondrial DNA. Comparative analyses indicate close relationships with other *Pygathrix* species, while species-specific features support genetic differentiation and confirm the evolutionary distinctiveness of *P. nigripes*. These results provide valuable genetic information for comparative and phylogenetic studies of douc langurs in Vietnam.

3.2.4. Characteristics of the Complete Mitochondrial Genome of the Black-shanked Douc Langur (*Pygathrix nigripes*) from the Central Highlands of Vietnam

3.2.4.1. Nucleotide Composition

The complete mitochondrial genome of the black-shanked douc langur (*Pygathrix nigripes*) exhibited a pronounced bias toward adenine (A) and thymine (T), which is typical of primate mitochondrial genomes. Differences in nucleotide composition were observed among protein-coding genes, RNA genes, and non-coding regions, reflecting functional and evolutionary constraints. Overall, the nucleotide composition of *P. nigripes* was consistent with that reported for other species within the genus *Pygathrix*.

3.2.4.2. Protein-Coding Genes

The mitochondrial genome of *Pygathrix nigripes* contains the standard set of protein-coding genes involved in oxidative phosphorylation. Gene order and orientation were conserved and similar to those of other colobine primates. Codon usage patterns reflected the AT-rich nature of the mitochondrial genome. Comparative analyses showed high similarity with other *Pygathrix* species, together with species-specific sequence variations contributing to genetic differentiation.

3.2.4.3. AT-Rich Region

The AT-rich region was identified as a non-coding region with a high proportion of adenine and thymine and plays an important role in mitochondrial DNA replication and transcription. This region exhibited higher sequence variability than coding regions and showed characteristics comparable to those observed in other douc langur species.

3.2.4.4. Amino Acid Composition

Analysis of amino acid composition derived from mitochondrial protein-coding genes revealed patterns typical of primate mitochondrial genomes. The distribution of amino acids reflected codon usage bias associated with the AT-rich nature of mitochondrial DNA. Overall, the amino acid composition of *P. nigripes* was generally similar to that of other *Pygathrix* species, with minor differences likely reflecting species-specific evolutionary divergence.

3.2.4.5. Overlapping and Intergenic Regions

The mitochondrial genome of *Pygathrix nigripes* exhibited a compact organization, with several overlapping genes and short intergenic spacers. These features are characteristic of primate mitochondrial genomes and contribute to efficient genome organization. The pattern of overlapping and intergenic regions was comparable to that observed in other *Pygathrix* species.

3.2.4.6. Discussion

The mitochondrial genome of *Pygathrix nigripes* from the Central Highlands exhibits conserved structural and genetic characteristics typical of primate mitochondrial DNA. Comparative analyses indicate close relationships with other *Pygathrix* species, while species-specific features support genetic differentiation and confirm the evolutionary distinctiveness of *P. nigripes*. The analysis of amino acid composition complements nucleotide-based results and provides additional insight into mitochondrial protein characteristics.

3.2.5. Characteristics of the Complete Mitochondrial Genome of the Rhesus Macaque (*Macaca mulatta*) from the Central Highlands of Vietnam

3.2.5.1. Nucleotide Composition

The complete mitochondrial genome of the rhesus macaque (*Macaca mulatta*) exhibited a clear nucleotide bias toward adenine (A) and thymine (T), which is typical of primate mitochondrial genomes. Variation in nucleotide composition was observed among protein-coding genes, RNA genes, and non-coding regions, reflecting differences in functional constraints. Overall, the nucleotide composition of *M. mulatta* from the

Central Highlands was consistent with that reported for other populations of this species.

3.2.5.2. Protein-Coding Genes

The mitochondrial genome of *Macaca mulatta* contains the standard complement of protein-coding genes involved in oxidative phosphorylation. Gene order and orientation were conserved and similar to those of other primates. Codon usage patterns reflected the AT-rich nature of the mitochondrial genome. Comparative analysis indicated high similarity with other *M. mulatta* mitochondrial genomes, together with minor sequence variations.

3.2.5.3. Transfer RNA (*tRNA*) and Ribosomal RNA (*rRNA*) Genes

The mitochondrial genome of *Macaca mulatta* includes a complete set of *tRNA* genes and two *rRNA* genes. Most *tRNA* genes were predicted to form typical cloverleaf secondary structures, while the *rRNA* genes showed conserved sequence characteristics consistent with strong functional constraints.

3.2.5.4. Overlapping and Intergenic Regions

Analysis of overlapping and intergenic regions revealed a compact mitochondrial genome organization. Several genes overlapped or were separated by short intergenic spacers, a feature commonly observed in primate mitochondrial genomes.

3.2.5.5. Phylogenetic Position of *Macaca mulatta*

Phylogenetic analysis based on mitochondrial genome data placed *Macaca mulatta* within the genus *Macaca*, clearly separated from colobine primates. The inferred phylogenetic position was consistent with established taxonomic classifications and supported the evolutionary relationship of *M. mulatta* with other macaque species.

3.2.5.6. Discussion

The mitochondrial genome of *Macaca mulatta* from the Central Highlands exhibits conserved structural and genetic characteristics typical of primate mitochondrial DNA. Comparative analyses confirm close relationships with other *M. mulatta* populations, while minor genetic variations may reflect geographic differentiation. These results provide useful genetic information for comparative and phylogenetic studies of macaques in Vietnam.

3.3. Analysis of Mitochondrial Sequence Variation for Phylogenetic Studies

This part of the study examines mitochondrial DNA sequence variation to clarify phylogenetic relationships among several primate species distributed in the Central Highlands of Vietnam. Analyses were conducted using commonly employed mitochondrial markers, including the *16S rRNA* gene, *cytochrome b*, and the *D-loop* region, together with complete mitochondrial genome data, in order to assess genetic divergence and phylogenetic resolution at different taxonomic levels.

The results show that the *16S rRNA* gene is highly conserved, exhibiting only limited sequence variation among individuals of the same species, and is therefore appropriate for resolving higher-level phylogenetic relationships. In contrast, the *cytochrome b* gene displays greater sequence variability, enabling clearer discrimination among closely related species. The *D-loop* region presents the highest level of sequence divergence, reflecting its rapid evolutionary rate and sensitivity to recent genetic differentiation.

The phylogenetic tree reconstructed from the complete mitochondrial genome resolves the evolutionary relationships among the studied primates with high clarity and strong statistical support. The analyzed individuals from the Central Highlands of Vietnam are consistently grouped into two major clades corresponding to the genera *Pygathrix* and *Macaca*, reflecting deep evolutionary divergence between colobine and cercopithecine primates.

Within the *Pygathrix* clade, individuals of *Pygathrix cinerea*, *P. nigripes*, and *P. nigripes* form distinct, well-supported subclades, confirming their taxonomic independence while also indicating close evolutionary relationships among the douc langurs. The relatively short branch lengths separating these species suggest recent divergence events within the genus, consistent with their overlapping geographic distributions in Indochina.

Within the *Macaca* clade, the Central Highlands individuals of *Macaca arctoides* and *Macaca mulatta* cluster with conspecific sequences from other geographic regions, forming species-specific lineages with high bootstrap support. This pattern indicates genetic continuity across their distribution ranges and supports the current species-level classification within the genus *Macaca*. The deeper branching structure within *Macaca* reflects greater genetic divergence compared with *Pygathrix*, consistent with the broader ecological and geographic diversity of macaques.

Overall, the complete mitogenome-based phylogeny shows strong concordance with established primate taxonomy and previously published phylogenetic frameworks. The clear separation of genera and species, together with high node support and stable topology, demonstrates the effectiveness of whole mitochondrial genome data for resolving evolutionary relationships among primates and provides robust genetic evidence for the phylogenetic placement of primate species from the Central Highlands of Vietnam.

Conclusions and Recommendations

Conclusions

This study provides comprehensive mitochondrial genetic data for five primate individuals distributed in the Central Highlands of Vietnam, including *Pygathrix cinerea*, *Pygathrix nigripes*, *Pygathrix nigripes*, *Macaca arctoides*, and *Macaca mulatta*. Through the analysis of whole mitochondrial genome sequences and individual mitochondrial gene regions, the study successfully characterized mitochondrial genome structure, genetic composition, and phylogenetic relationships of these primates.

The results demonstrate that the complete mitochondrial genomes of the studied primates exhibit typical structural features of primate mitochondrial DNA, including conserved gene content, gene order, and overall genome organization. Analyses of nucleotide composition, protein-coding genes, RNA genes, AT-rich regions, and intergenic regions revealed both conserved characteristics and species-specific features among the studied primates.

Comparative analyses among the five primate individuals indicated clear genetic differentiation corresponding to species boundaries. Differences in mitochondrial genome characteristics and gene sequences reflect evolutionary divergence among primate taxa distributed in the Central Highlands. These findings contribute to a clearer understanding of mitochondrial genome variation and genetic diversity among primates in this region.

Phylogenetic analyses based on multiple mitochondrial markers, including *16S rRNA*, *cytochrome b*, *D-loop*, and complete mitochondrial genomes, consistently resolved evolutionary relationships among the studied primate species. Phylogenetic trees constructed using complete mitochondrial genome data provided the highest resolution and supported the robustness of phylogenetic relationships inferred from individual gene analyses. Overall, the results enhance understanding of primate evolutionary relationships in the Central Highlands of Vietnam.

In summary, this study supplements existing mitochondrial genetic data for Vietnamese primates, clarifies phylogenetic relationships among selected species, and provides a reliable genetic foundation for further evolutionary and genetic research on primates in Vietnam.

Recommendations

Based on the results of this study, several recommendations can be proposed for future research and application.

First, further studies should expand sampling to include a larger number of individuals and additional primate species from different geographic regions in Vietnam. Increased sampling coverage would allow a more comprehensive assessment of genetic diversity and population structure.

Second, future research should integrate mitochondrial DNA data with nuclear genetic markers to provide a more complete understanding of primate genetic diversity and evolutionary history. Combined analyses of mitochondrial and nuclear genomes would improve the resolution of phylogenetic relationships and population-level differentiation.

Third, the mitochondrial genome data generated in this study should be incorporated into national and international genetic databases to support comparative studies and facilitate broader phylogenetic analyses of primates in Southeast Asia.

Finally, the results of this study can serve as a scientific reference for subsequent research related to primate taxonomy, evolutionary biology, and conservation-oriented genetic studies in Vietnam. Continued molecular genetic research will contribute to improving knowledge of primate diversity and support long-term conservation efforts for primates in the Central Highlands.

LIST OF THE PUBLICATIONS RELATED TO THE DISSERTATION

1. Mai Thi Phuong Nguyen†& **Tram Thi Thuy Nguyen†,** Tung Thanh Ha, Chi Nguyen Quynh Ho, Cuong Phan Minh Le, Huy Nghia Quang Hoang, Quynh Thi Nhu Nguyen, Tao Thien Nguyen, Dung Tri Luu, Khoa Dang Dang, Quan Ke Thai and Long Thanh Le*. (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). Curr Issues Mol Biol 46(9): 9928-9947.https://doi.org/10.3390/cimb46090592
2. **Tram Thi Thuy Nguyen**, Chi Nguyen Quynh Ho, Cuong Phan Minh Le, Nhan Lu Chinh Phan, Loan Tung Dang, Quan Ke Thai, Mai Thi Phuong Nguyen, Phuc Bui Tran, Long Thanh Le, Vu Quang Manh. (2025). The complete mitochondrial genome of the Vietnamese *macaca mulatta*. Natural history 126(3): 2-20. https://doi.org/10.59879/1Ia6i
3. **Nguyen Thi Thuy Tram**, Le Thanh Long, Ho Nguyen Quynh Chi, Phan Lu Chinh Nhan, Ha Thanh Tung, Dang Thi Tung Loan, Nguyen Thi Phuong Mai (2025) Analysis of the complete mitochondrial genome of Vietnamese *Pygathrix nigripes*. Vietnam Journal of Biotechnology 23(1): 25-39. DOI: 10.15625/vjbt-21785