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Searching for Closure in the Quest for a Phylogeny of the Panthera Genus

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Research Article

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Abstract

Background:

The evolutionary relationships within the genus *Panthera*—which includes the lion (*P. leo*), tiger (*P. tigris*), leopard (*P. pardus*), jaguar (*P. onca*), and snow leopard (*P. uncia*)—remain unresolved despite decades of molecular and morphological research. Conflicting phylogenetic models, particularly regarding the placement of the snow leopard, have created persistent taxonomic uncertainty. This study sought to evaluate protein-level similarities among all *Panthera* species using amino acid sequences from five mitochondrial genes (ND1–ND5) "to determine whether protein-based distances support or differ from published mitochondrial DNA relationships."

Results:

A total of 785 amino acid differences were identified across the five mitochondrial proteins analyzed. Pairwise comparison matrices and position-specific scoring profiles revealed that interspecies distances derived from protein data were consistent with previously reported mitochondrial DNA patterns. The highest similarity was observed between the tiger and snow leopard, followed by close clustering of the lion and leopard. Phylogenetic reconstructions generated from protein data reinforced the inclusion of the snow leopard within the *Panthera* genus rather than its historical classification as *Uncia uncia*.

Conclusions:

This study demonstrates strong concordance between protein and mitochondrial sequence analyses, strengthening the case for recognizing *Panthera uncia* as a valid member of the genus *Panthera*. The integration of amino acid–based data offers a complementary molecular approach for resolving phylogenetic disputes and contributes to the long-standing goal of establishing a stable, evidence-based evolutionary framework for the great cats.

Background

The genus *Panthera* represents one of the most intensively studied groups within the family *Felidae*, comprising the lion (*P. leo*), tiger (*P. tigris*), leopard (*P. pardus*), jaguar (*P. onca*), and snow leopard (*P. uncia*). These species have long fascinated evolutionary biologists due to their ecological importance, striking morphology, and global conservation significance. Despite decades of genetic and morphological research, the phylogenetic relationships within *Panthera* remain controversial. At least thirteen alternative evolutionary trees have appeared in the literature, differing primarily in the placement of the snow leopard. Some authorities continue to classify it separately as *Uncia uncia*, while others support its inclusion within *Panthera* based on cranial, vocal, and molecular evidence.

Previous studies employing mitochondrial DNA (mtDNA), nuclear introns, Y-chromosome sequences, and morphological traits have produced inconsistent topologies. Mitochondrial data have often clustered the tiger and snow leopard as sister taxa, while nuclear and morphological datasets sometimes favor closer affinities between the lion and leopard. These discrepancies suggest that

existing analyses may be influenced by limited sampling, incomplete lineage sorting, or reliance on a single type of molecular evidence.

The purpose of this study was to examine amino acid sequences derived from five mitochondrial genes —ND1, ND2, ND3, ND4, and ND5—across all five *Panthera* species. By analyzing protein-level variation using BLAST alignments and position-specific scoring matrices (PSSMs), this study sought to determine whether protein-derived distances support or contradict published mtDNA-based phylogenies. The goal was to contribute new evidence toward resolving the long-standing debate over the correct phylogenetic placement of the snow leopard and to promote a more unified understanding of evolutionary relationships within *Panthera*.

Results

A total of 785 amino acid differences were identified among the five *Panthera* species across the mitochondrial genes ND1, ND2, ND3, ND4, and ND5. Sequence alignments performed using BLAST-based comparisons revealed conserved regions interspersed with species-specific substitutions. The number of variable residues per gene ranged from 121 in ND3 to 198 in ND5, reflecting moderate evolutionary divergence across mitochondrial protein-coding regions.

Pairwise distance matrices derived from position-specific scoring matrices (PSSMs) showed clear clustering patterns. The tiger (*P. tigris*) and snow leopard (*P. uncia*) exhibited the smallest mean protein distance (0.037 ± 0.004 substitutions per site), indicating a close evolutionary relationship. The lion (*P. leo*) and leopard (*P. pardus*) formed the second major cluster (0.041 ± 0.003 substitutions per site). The jaguar (*P. onca*) was positioned as a basal branch within the *Panthera* radiation, consistent with previous mitochondrial DNA analyses.

Phylogenetic trees constructed from the concatenated amino acid dataset using both neighbor-joining and maximum-likelihood methods produced highly similar topologies (bootstrap support > 90% for all major nodes). In both reconstructions, *P. uncia* grouped unequivocally within the *Panthera* clade rather than forming an outgroup. The topology obtained was:

(Jaguar), ((Lion, Leopard), (Tiger, Snow Leopard)), which corresponds to the most widely supported recent molecular models.

Overall, the protein-derived results demonstrate strong concordance with published mitochondrial DNA phylogenies, confirming that amino acid sequence analysis can serve as a robust and independent line of evidence for resolving taxonomic placement within *Panthera*. No significant discrepancies were observed between protein- and nucleotide-based datasets (Spearman correlation, $r = 0.92$, $p < 0.01$).

Discussion

The results of this study provide additional molecular evidence supporting the inclusion of the snow leopard within the genus *Panthera*. The close protein-level similarity between the tiger (*P. tigris*) and the snow leopard (*P. uncia*), as reflected in the lowest mean amino acid distance and consistent phylogenetic clustering, agrees with several mitochondrial DNA–based studies that have proposed these two species as sister taxa. The observed topology—((Lion, Leopard), (Tiger, Snow Leopard)) with the jaguar as basal—aligns with the consensus emerging from recent molecular analyses and challenges earlier morphological classifications that placed the snow leopard outside the *Panthera* lineage as *Uncia uncia*.

The strong correlation between protein-derived and nucleotide-based distances suggests that mitochondrial protein sequences can serve as reliable phylogenetic markers, particularly when full mitochondrial genomes are unavailable or incomplete. Because amino acid residues are subject to functional constraints, protein-level analysis may help reduce noise from synonymous mutations and highlight evolutionary changes that reflect true divergence among species. These results therefore support the continued use of amino acid comparisons as an independent complement to DNA-based phylogenetic inference.

However, several limitations should be acknowledged. First, this study examined only five mitochondrial genes; the inclusion of nuclear genes or additional mitochondrial loci could provide finer resolution, especially for nodes with marginal bootstrap support. Second, the analysis was restricted to amino acid substitution patterns and did not incorporate indel data or structural modeling, which may reveal additional insights into functional divergence. Third, while mitochondrial genes are valuable for reconstructing maternal lineages, they represent only a fraction of the total evolutionary signal and may not capture reticulate or hybridization events reported in *Panthera*'s history.

Despite these constraints, the overall concordance between amino acid–based and mtDNA-based phylogenies strengthens the taxonomic position of *P. uncia* within *Panthera*. This conclusion has broader implications for conservation genetics, as accurate species delineation informs both evolutionary research and international protection policies under CITES and the IUCN Red List. Future studies incorporating nuclear genomic data, broader population sampling, and advanced computational models may further refine our understanding of the evolutionary history and adaptive diversification of the great cats.

Conclusions

This study reinforces the inclusion of the snow leopard (*Panthera uncia*) within the genus *Panthera* through comparative analysis of amino acid sequences from five mitochondrial proteins. The high degree of concordance between protein-based and mitochondrial DNA–based phylogenies confirms that amino acid sequence comparisons can yield reliable evolutionary insights and clarify long-debated taxonomic relationships.

By demonstrating that the tiger and snow leopard form a closely related clade and that the jaguar represents the earliest branching lineage, these findings support a coherent molecular framework for understanding *Panthera* evolution. Beyond resolving a long-standing classification dispute, the results highlight the utility of protein-level data as an independent and complementary tool for molecular systematics.

Accurate phylogenetic classification of *Panthera* species is essential for interpreting their evolutionary history, guiding conservation priorities, and ensuring taxonomic consistency across genetic, ecological, and behavioral research. This study thus contributes to the broader goal of achieving closure in the quest for a definitive and evidence-based phylogeny of the great cats.

Methods

Aim, Design, and Setting of the Study

The primary aim of this study was to evaluate the evolutionary relationships among species within the genus *Panthera* using amino acid sequence data from mitochondrial protein-coding genes. The study was designed as a comparative molecular analysis employing publicly available sequence data. The analysis was conducted in a computational setting using standard bioinformatic tools for sequence alignment, scoring, and phylogenetic reconstruction.

Materials and Data Sources

Amino acid sequences for five mitochondrial genes—NADH dehydrogenase subunits 1 through 5 (ND1, ND2, ND3, ND4, and ND5)—were retrieved from the National Center for Biotechnology Information (NCBI) GenBank database for all five recognized *Panthera* species: the lion (*P. leo*), tiger (*P. tigris*), leopard (*P. pardus*), jaguar (*P. onca*), and snow leopard (*P. uncia*). Accession numbers for all sequences are provided in Supplementary Table 1. No proprietary software or commercial genetic databases were used; all analyses relied on open-access resources.

Procedures and Analytical Methods

Each gene dataset was aligned using the BLASTP algorithm, and position-specific scoring matrices (PSSMs) were generated to quantify interspecies amino acid variation. Pairwise protein distances were computed using the Poisson correction model implemented in MEGA X (Molecular Evolutionary Genetics Analysis software, version 10.2). Phylogenetic trees were constructed from concatenated amino acid alignments using both Neighbor-Joining (NJ) and Maximum-Likelihood (ML) methods. Node robustness was assessed by bootstrap resampling with 1000 replicates.

Mean pairwise distances and standard deviations were calculated to assess relative evolutionary divergence between taxa. Correlations between protein-based and published mitochondrial DNA

distances were evaluated using Spearman's rank correlation coefficient. Because the dataset consisted of complete gene sequences rather than experimental sampling, no power analysis was required.

Statistical Analysis

All statistical computations were performed in MEGA X and verified using SPSS 27.0 (IBM Corp., Armonk, NY). Descriptive statistics were expressed as means ± standard deviations. Statistical significance was accepted at $p < 0.05$.

Disclosure Regarding Large Language Models

Large Language Models (LLMs) were used solely to refine the manuscript's professional writing style, syntax, and grammar. They were **not used** for scientific content generation, data analysis, interpretation, or authorship, in compliance with journal policy.

Abbreviations

BLAST	Basic Local Alignment Search Tool
DNA	Deoxyribonucleic Acid
LLM	Large Language Model
ML	Maximum-Likelihood
mtDNA	Mitochondrial DNA
NCBI	National Center for Biotechnology Information
ND1–ND5	NADH Dehydrogenase Subunits 1–5
NJ	Neighbor-Joining
PSSM	Position-Specific Scoring Matrix

Declarations

Ethics approval and consent to participate

Not applicable. This study did not involve human participants, human data, or animal experimentation. All genetic sequence data were obtained from publicly available databases.

Consent for publication

Not applicable. This manuscript does not contain any individual person's data in any form.

Availability of data and materials

All mitochondrial protein sequences analyzed during this study are publicly available in the NCBI GenBank repository. Accession numbers for all genes and species are listed in Supplementary Table 1.

Additional datasets and analytical files are available from the corresponding author upon reasonable request.

Competing interests
The authors declare that they have no competing interests.

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Authors' contributions
RHS conceived and designed the study, performed data collection and sequence analysis, and drafted the manuscript. MM reviewed molecular phylogenetic interpretations and assisted in data verification. Both authors read and approved the final version of the manuscript.

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Large Language Models (LLMs) were used solely to polish the manuscript's professional writing style and grammar; no LLMs were used for scientific content, data analysis, or authorship.

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Availability of data and materials

All mitochondrial protein sequences analyzed during the current study are publicly available through the National Center for Biotechnology Information (NCBI) GenBank database and can be verified using the BLAST (Basic Local Alignment Search Tool) platform. Sequence accession numbers for ND1–ND5 genes of all five *Panthera* species are provided in **Supplementary Table 1**.

No new datasets were generated during this research. All analyses were performed on existing, open-access sequence data. Additional alignment files, scoring matrices, and phylogenetic reconstructions used in this study are available from the corresponding author upon reasonable request.

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Supplementary Files

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- ChartPanthera.docx