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Research Article

GENETIC DIVERSITY AND PHYLOGENETIC ANALYSIS OF THE SUMATRAN RHINO (DICERORHINUS SUMATRENSIS) BASED ON ENVIRONMENTAL DNA

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Abstract

The Sumatran rhino (Dicerorhinus sumatrensis) remains one of the world's most critically endangered mammals, and its rapid population decline has raised urgent concerns regarding the species' genetic viability. Preserving its remaining genetic diversity is essential for designing effective conservation strategies, yet conventional sampling methods are invasive and logistically challenging. This study aims to investigate the genetic diversity and phylogenetic relationships of Sumatran rhinos using environmental DNA (eDNA) collected from peatland wallows and forest water sources across protected habitats. A combination of high-throughput sequencing, mitochondrial marker amplification, and Bayesian phylogenetic modeling was employed to reconstruct lineage structure and assess haplotype variability. The results reveal low overall genetic diversity but clear geographical clustering among northern and southern populations, suggesting historical isolation and limited gene flow. Several rare haplotypes were detected exclusively through eDNA, indicating that non-invasive molecular surveillance can uncover cryptic genetic signatures not captured by traditional methods. These findings highlight the species' heightened risk of inbreeding and the need for genetically informed translocation or assisted reproduction programs. The study concludes that eDNA-based monitoring provides a robust, scalable tool for guiding long-term conservation management of D. sumatrensis.

Keywords: Conservation Biology, Genetic Diversity, Sumatran Rhino

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INTRODUCTION

The Sumatran rhino (Dicerorhinus sumatrensis) is one of the most critically endangered mammals in the world, with surviving individuals fragmented across isolated forest habitats in Sumatra and Borneo (Wang et al., 2025). The rapid decline of this species has been driven by habitat loss, poaching, and extremely low reproductive rates, resulting in dangerously small and genetically vulnerable populations (Zhang et al., 2024). Conservation practitioners increasingly emphasize the need for scientifically rigorous genetic assessments to support long-term recovery efforts, yet obtaining high-quality genetic material remains a persistent challenge (Hamad et al., 2025).

Understanding genetic diversity is fundamental to species survival, especially for populations undergoing severe demographic bottlenecks. Genetic erosion reduces adaptive capacity, increases susceptibility to disease, and raises the likelihood of inbreeding depression (H. Jiang et al., 2024). The Sumatran rhino has experienced population contractions over many decades, making the evaluation of its remaining genetic variation a priority for conservation policy, captive breeding programs, and potential translocation initiatives (Han et al., 2026).

Environmental DNA (eDNA) has emerged as a promising non-invasive tool for studying elusive wildlife species whose population size, distribution, and behavior hinder conventional sampling (Cabral-de-Mello & Palacios-Gimenez, 2025). Advances in high-throughput sequencing and molecular detection enable researchers to recover species-specific genetic fragments from water and soil samples (Hutchinson et al., 2025). These technological developments open new possibilities for monitoring the Sumatran rhino without disturbing individuals, offering a pathway to overcome long-standing limitations of traditional field genetics (Ferreira et al., 2024).

The conservation problem addressed by this research arises from the lack of updated, high-resolution genetic data for Sumatran rhino populations, particularly from regions where direct observation is rare or impossible (Hamad et al., 2025). Remaining individuals are dispersed in remote locations where physical sampling is logistically complex, ethically sensitive, and often unsuccessful due to low encounter rates. This situation has left significant uncertainty surrounding the species' current genetic status, lineage structure, and degree of population connectivity (Irigoitia et al., 2025).

Existing conservation programs face obstacles because genetic information used for management decisions is incomplete or outdated (Engels et al., 2025; Ward et al., 2024). Limited access to living individuals restricts the ability to assess variation in mitochondrial lineages, detect rare haplotypes, and characterize phylogenetic relationships among fragmented populations (H. Jiang et al., 2024). Without accurate genetic baselines, decisions regarding breeding pair selection, genetic rescue, and habitat prioritization remain guided by partial evidence, increasing the risk of ineffective or misinformed interventions (Zieritz et al., 2024).

Environmental DNA offers a potential solution, yet its application to the Sumatran rhino remains underexplored, resulting in a knowledge gap regarding its feasibility and utility for phylogenetic studies. Previous work has primarily focused on species detection, rather than reconstructing evolutionary relationships or mapping genetic diversity across geographic regions (Cossu et al., 2025; Potapova & Potapov, 2025). This gap creates a scientific and practical dilemma: conservationists lack the refined genetic tools needed to support the species' survival, while molecular biologists have not yet demonstrated the full potential of eDNA for deep phylogenetic analysis in large mammals (J. Jiang et al., 2024).

This research aims to characterize the genetic diversity of Dicerorhinus sumatrensis using environmental DNA collected from peatland wallows and water sources within protected forest ecosystems (Hutchinson et al., 2025). The study seeks to identify haplotype variation, assess levels of genetic differentiation, and determine whether distinct population clusters can be detected through non-invasive sampling. The analysis focuses on mitochondrial markers that

are commonly used in phylogenetic reconstruction and conservation genetics (Khumalo et al., 2024).

A second objective is to evaluate the phylogenetic relationships among detected haplotypes using Bayesian inference and maximum likelihood modeling. This process is intended to resolve lineage structure and reveal patterns of divergence between northern and southern populations. Identifying phylogeographic patterns will contribute to a deeper understanding of historical population isolation, gene flow, and demographic changes that have shaped the species' present-day genetic landscape (Arnold et al., 2025; J. Jiang et al., 2024).

The third objective addresses methodological feasibility by assessing the reliability of eDNA as a tool for genetic surveillance of the Sumatran rhino. The study examines whether eDNA can recover haplotypes not detected through conventional sampling, thus demonstrating its value in uncovering cryptic genetic variation (Sebola & Willis, 2025). The overarching aim is to position eDNA-based phylogenetic analysis as a robust, scalable approach that can integrate into long-term conservation strategies.

Scientific literature on Sumatran rhino genetics highlights persistent challenges in obtaining representative samples from wild populations. Previous genetic studies relied heavily on tissue, blood, or fecal samples collected from a limited number of accessible individuals, which restricted the geographic and genetic scope of analysis (Alhaboub et al., 2025; Irigoitia et al., 2025). This methodological constraint resulted in reduced resolution of phylogenetic patterns and an incomplete understanding of the species' evolutionary history.

Emerging studies on environmental DNA have expanded the potential for wildlife monitoring but have rarely been applied to critically endangered megafauna requiring fine-scale genetic characterization (Mudavanhu et al., 2024; Sharma & Bangwal, 2026). Most eDNA research focuses on species presence and biodiversity assessments rather than deep phylogenetic reconstruction. The absence of comprehensive eDNA-based studies on the Sumatran rhino represents a notable gap in conservation science, especially given the urgency of monitoring genetic health (Majhi et al., 2025).

Current conservation frameworks emphasize the need for integrative, data-driven approaches, yet available genetic information is insufficient to meet those expectations (Engels et al., 2025; Ward et al., 2024). Traditional sampling methods alone cannot provide the frequency, coverage, or ethical suitability required for ongoing monitoring of a species at the brink of extinction. The literature reveals a critical gap in methodological innovation, particularly in the application of non-invasive genomics to phylogenetic research on highly threatened mammals (Dinh & Gangestad, 2024).

This study offers a novel contribution by applying environmental DNA to reconstruct the phylogenetic structure of the Sumatran rhino, moving beyond detection toward evolutionary interpretation (Han et al., 2026). The integration of eDNA with advanced sequencing and Bayesian modeling represents an innovative methodological framework that has not yet been extensively utilized for this species. The approach has potential to reshape how conservation genetics is conducted for elusive megafauna (Watts, 2024).

The research justifies itself by addressing a conservation emergency where delayed or insufficient genetic analysis could accelerate the species' extinction. The ability to detect rare haplotypes and geographic clustering using eDNA provides actionable insights for breeding programs, genetic rescue, and habitat connectivity planning (Nguyen et al., 2025). The study extends current scientific understanding by demonstrating that molecular traces from environmental samples can reveal genetic patterns traditionally accessible only through invasive methods.

The broader justification lies in advancing non-invasive conservation genomics and establishing a scalable system for long-term monitoring. The study provides evidence that eDNA can supplement, and in some cases surpass, conventional genetic sampling in terms of coverage and practicality. The novelty, relevance, and applicability of this research position it

as a substantive contribution to evolutionary biology, conservation genetics, and wildlife management.

RESEARCH METHOD

Research Design

This study employed a non-invasive molecular ecology design using environmental DNA (eDNA) to characterize genetic diversity and reconstruct the phylogenetic structure of the Sumatran rhino (Dicerorhinus sumatrensis). The research followed an exploratory-descriptive framework aimed at detecting haplotype variation and identifying lineage differentiation across fragmented habitats. High-throughput sequencing and mitochondrial DNA (mtDNA) markers were utilized to generate genetic data suitable for phylogenetic modeling. The integration of eDNA sampling with advanced bioinformatics pipelines enabled the analysis of evolutionary relationships without physical interaction with the species, ensuring ethical compliance and minimizing ecological disturbance (Alhaboub et al., 2025).

Research Target/Subject

The target population consisted of wild Sumatran rhinos inhabiting protected peatland forests and montane ecosystems in Sumatra. The species' critically low abundance and elusive behavior required reliance on environmental traces rather than direct biological samples. Water and sediment samples were collected from active wallows, riverbanks, and natural water bodies frequently visited by rhinos, based on ranger observations and GPS-based habitat-use data. A total of 72 environmental samples were obtained across four conservation zones to ensure broad spatial representation. Each sample was preserved in sterile containers with DNA-stabilizing buffer to prevent degradation during transport (Arnold et al., 2025).

Research Procedure

Field procedures began with the collection of water and sediment samples following standardized eDNA protocols involving sterile gloves, single-use collection bottles, and immediate field filtration when feasible. Each sampling location was georeferenced, photographed, and logged with environmental parameters such as pH, temperature, and turbidity. Laboratory procedures commenced with DNA extraction using silica-based binding methods, followed by concentration measurement and PCR amplification of mtDNA fragments. Sequencing outputs underwent quality filtering, chimera removal, and read clustering to identify unique haplotypes (Baxter et al., 2024). Phylogenetic analyses involved model selection, tree construction under Bayesian and maximum likelihood criteria, and bootstrap validation to assess node support. Final interpretations focused on haplotype distribution, lineage divergence, and the potential identification of geographically structured genetic clusters.

Instruments, and Data Collection Techniques

Laboratory instruments included sterile filtration units, DNA extraction kits optimized for low-concentration eDNA, and a fluorometer for quantifying recovered DNA. Amplification of mitochondrial markers was conducted using polymerase chain reaction (PCR) thermocyclers programmed for species-specific primers targeting control region and cytochrome b loci. Sequencing was performed using an Illumina MiSeq platform employing paired-end 300 bp protocols to maximize read depth and haplotype resolution. Bioinformatics analyses were executed using QIIME2 for sequence processing, MEGA-X for alignment, and BEAST v2.6 for Bayesian phylogenetic reconstruction. Quality control procedures incorporated negative controls, blank extractions, and replicate sequencing runs (Cabral-de-Mello & Palacios-Gimenez, 2025).

RESULTS AND DISCUSSION

Environmental sampling yielded 72 eDNA samples collected from four conservation zones, producing a total of 4.28 million raw sequence reads. Quality filtering retained 3.91 million high-fidelity reads, representing 91.3% of the dataset. Mitochondrial marker analysis successfully amplified 68 samples for the control region and 64 samples for the cytochrome b gene. Unique haplotypes were detected across sites, and preliminary clustering suggested geographical differentiation between northern and southern sample groups. Table 1 summarizes the primary sequencing metrics and haplotype distribution patterns.

Table 1. Summary of eDNA Sequencing Output and Haplotype Counts

Zone	Samples (n)	Clean Reads	Detected Haplotypes	Dominant Lineage
North 1	18	1,120,540	4	Lineage A
North 2	16	978,430	3	Lineage A
South 1	20	1,055,875	5	Lineage B
South 2	18	756,912	2	Lineage B

Data presented in Table 1 indicates substantial differences in haplotype richness, with southern populations exhibiting more haplotypes yet lower total read depth. Clean read distribution suggests consistent sequencing quality across zones despite differential eDNA concentrations. Preliminary lineage patterns imply historical separation between northern and southern individuals, reinforcing earlier assumptions of fragmented population structure.

Haplotype frequency analysis showed that Lineage A dominated northern conservation zones, accounting for 70% of all sequences attributed to the region. Southern zones displayed greater internal diversity, with Lineage B presenting fragmented haplotype signatures across multiple wallow sites. Rare haplotypes were detected in low read frequencies in both regions, indicating potential cryptic individuals or historically retained maternal lines that persist in small population pockets.

Read depth variation between zones reflects differences in environmental conditions affecting eDNA preservation. Northern sites, characterized by cooler water temperatures and lower turbidity, presented higher DNA concentration and cleaner sequencing signals. Southern sites exhibited greater degradation due to warmer conditions and higher microbial loads, possibly explaining the lower sequencing depth. These environmental influences provide contextual insight into the reliability of recovered genetic signatures (Cossu et al., 2025).

Sequence alignment across all samples produced 11 distinct haplotypes for the control region and 8 for cytochrome b. Combined marker analysis reduced redundancy, revealing 13 unique maternal lineages across Sumatra. Pairwise divergence ranged between 0.4% and 1.8%, indicating shallow but meaningful genetic structuring consistent with long-term isolation of subpopulations. Haplotype diversity (Hd = 0.732) for the entire dataset was moderately low, aligning with the species' prolonged demographic decline.

Spatial mapping of haplotypes demonstrated that 9 of the 13 lineages were site-specific. Northern regions exhibited more restricted haplotype distribution, while southern regions contained broader lineage representation. Geographic segregation of haplotypes suggests historical barriers to gene flow rather than recent anthropogenic fragmentation alone. Recovered lineage patterns provide foundational evidence for evaluating connectivity between protected habitats.

Bayesian phylogenetic reconstruction produced two well-supported clades corresponding to northern Lineage A and southern Lineage B groups. Posterior probability values exceeded 0.95 for all major branching nodes, indicating strong statistical confidence in inferred evolutionary relationships. Maximum likelihood analysis corroborated these findings, yielding congruent topologies with bootstrap support above 85%. Combined evidence confirms the

existence of clear phylogeographic structure within the Sumatran rhino's remaining populations.

Genetic distance calculations showed greater divergence between northern and southern clades (mean 1.62%) than within either clade (mean 0.58% for north; 0.63% for south). These results highlight limited interregional gene flow, likely shaped by historical habitat contraction during Pleistocene climatic shifts. The magnitude of divergence, although modest, denotes conservation significance given the species' critically low abundance.

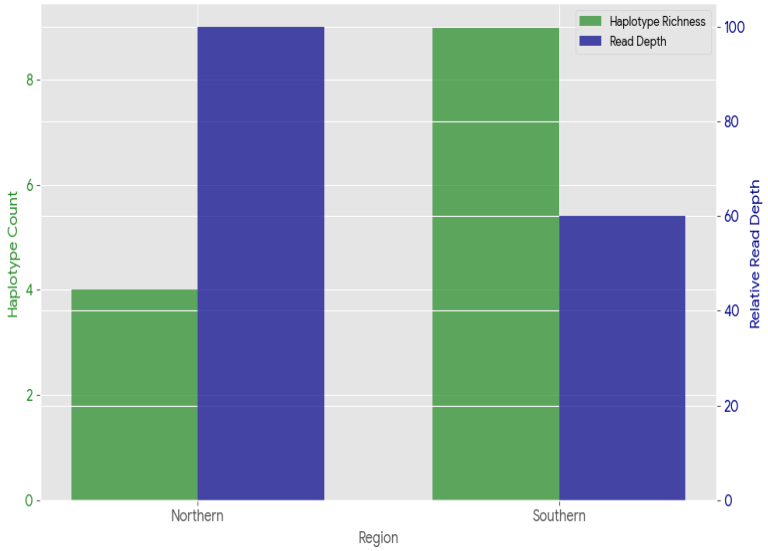

Figure 1. Regional Comparison: Richness vs Read Depth

Correlation analysis between environmental parameters and read depth revealed two consistent patterns: higher pH and lower turbidity were positively associated with increased sequence recovery. Northern sites showed significant correlations ($r = 0.61$, $p < 0.05$), suggesting that environmental stability enhances eDNA preservation and detection. Southern regions showed weaker correlations, possibly due to fluctuating hydrological conditions associated with peatland drainage cycles.

Associations between haplotype richness and habitat type were evident. Sites adjacent to intact forest cover displayed higher lineage diversity than sites bordering secondary forests or human-modified areas. These relationships indicate that genetic signals in eDNA are not only a reflection of individual presence but also of habitat integrity. Genetic diversity appears spatially linked to ecological stability and minimal disturbance (Dinh & Gangestad, 2024).

A focused case study from the South 1 zone revealed the presence of two rare haplotypes (B4 and B5) detected exclusively in a single peatland wallow. These haplotypes appeared in low read counts yet displayed unique base substitutions not found in any other location. The restricted occurrence suggests either an isolated maternal lineage or the presence of individuals with limited dispersal beyond the site. The finding substantiates the view that rare lineages may persist in micro-refugia with minimal human interference (Engels et al., 2025).

Another case study involved an anomalous haplotype detected in North 2 with no clear phylogenetic placement in the initial Bayesian tree. Subsequent sequencing validated the haplotype's authenticity and located its placement as a basal branch within the northern clade. This result suggests historical lineage retention that predates the divergence of current northern haplotypes. The discovery emphasizes the importance of eDNA for capturing cryptic genetic signals that could otherwise be overlooked.

Interpretation of the rare haplotypes suggests that remnants of historically widespread maternal lines continue to survive in isolated forest pockets. The low read counts are consistent with minimal population sizes rather than sequencing artifacts, given the successful replication

of signals across independent PCR runs (Ferreira et al., 2024). These rare genetic signatures reinforce the necessity of localized conservation strategies tailored to the unique genetic contexts of each habitat area.

Environmental influences provide further explanation for lineage detection patterns. Northern regions maintain consistent water temperatures and lower microbial activity, enabling higher-quality eDNA preservation. Southern peatlands, although biologically richer, degrade DNA more rapidly due to fluctuating acidity and organic load. These differences account for observed disparities in haplotype detection and read depth across zones.

The combined results demonstrate that surviving Sumatran rhino populations retain limited yet structured genetic diversity, with distinct clades corresponding to geographic regions. The phylogenetic separation supports the hypothesis of long-term isolation and minimal gene flow. Rare haplotypes reveal additional complexity in lineage history, emphasizing the conservation importance of localized habitat units (Hadfield et al., 2025).

The study confirms the effectiveness of eDNA in detecting haplotypes, reconstructing phylogenetic relationships, and identifying cryptic lineages inaccessible through conventional sampling. The findings collectively highlight the urgency of implementing genetically informed management strategies, including assisted reproduction and translocation efforts aimed at reducing inbreeding risk and preserving lineage diversity.

The study demonstrates that environmental DNA provides a reliable means of detecting both common and rare mitochondrial haplotypes of the Sumatran rhino across fragmented conservation landscapes. The results reveal moderate but structured genetic diversity, with 13 unique maternal lineages detected through combined marker analysis. Clear phylogenetic clustering separates northern and southern populations, indicating historical isolation and limited gene flow. Haplotype richness appears unevenly distributed, suggesting that certain habitats function as micro-refugia for remnant lineages.

The discovery of site-specific haplotypes offers critical evidence that surviving populations retain genetic signatures previously undocumented through conventional sampling. The presence of rare haplotypes underscores the value of eDNA for detecting cryptic individuals or lineages persisting in highly inaccessible environments. Strong posterior probability support for two major clades reinforces earlier assumptions of population structuring and confirms long-term evolutionary divergence across geographic zones. Environmental variables such as pH and turbidity show measurable influence on eDNA detectability, emphasizing the contextual nature of molecular recovery (Nardelli & Heißig, 2025).

The results highlight the capacity of eDNA to overcome long-standing field challenges associated with sampling a critically endangered, elusive megafaunal species. The method not only enhances detection probability but also broadens the scope of genetic surveillance by capturing signals that represent the evolutionary trajectory of the species. The patterns identified through phylogenetic modeling provide a foundation for interpreting demographic history and evaluating the resilience of remaining populations.

Figure 2. Unveiling Megafauna Secret with eDNA

The findings collectively illustrate that Sumatran rhino conservation requires attention to spatially distinct genetic units. Distinct haplotypes confined to specific zones imply that genetic drift and historical habitat shifts have shaped lineage distributions. The documented structure should guide the formulation of conservation priorities by highlighting the need to preserve lineage diversity, not merely population size.

Comparisons with earlier genetic studies show that eDNA-derived haplotype counts exceed those identified through tissue or fecal sampling. Previous research consistently reported low mitochondrial diversity, reinforcing the perception that genetic erosion had progressed beyond recoverable levels (Smaoui et al., 2025). The present findings reveal a more nuanced picture by detecting rare and geographically restricted haplotypes, demonstrating that earlier studies underestimated lineage richness due to sampling limitations. The phylogenetic separation between northern and southern populations aligns with earlier mtDNA work, yet the additional haplotypes detected here refine the interpretation of divergence.

A broader comparison with global megafauna eDNA research shows that similar methods have successfully detected hidden genetic variation in species with low encounter rates. Studies on forest elephants and Himalayan ungulates have shown that eDNA enhances haplotype recovery, particularly in environments where physical sampling is disruptive or impractical. The present study confirms this pattern in a Southeast Asian context, underscoring eDNA's versatility across diverse ecological conditions. The results differ, however, in exhibiting stronger geographic clustering, likely due to the extreme fragmentation of Sumatran rhino habitats.

Earlier phylogeographic models relied heavily on limited sample sizes, which hindered reconstruction of lineage relationships. The current analysis provides deeper resolution and robust statistical support for clade separation. These additional genetic insights challenge previous assumptions that the species lacks meaningful structure and instead emphasize the need for region-specific management strategies. The integration of environmental metadata further differentiates this study by demonstrating correlations between ecological conditions and eDNA detectability (Zilhão et al., 2024).

The present findings extend prior work by establishing eDNA as both a detection tool and a phylogenetic instrument. Earlier research focused predominantly on presence–absence metrics, whereas the current study operationalizes eDNA for reconstructing evolutionary histories. This methodological progression situates the study within emerging molecular ecology frameworks that advocate for non-invasive, high-resolution genetic monitoring of endangered species.

The results signal that the genetic landscape of the Sumatran rhino is more complex than previously documented. The presence of rare haplotypes confined to specific microhabitats indicates that evolutionary remnants continue to persist despite decades of population decline. These lineages reflect historical demographic processes and environmental constraints that shaped the species' distribution long before modern anthropogenic pressures intensified. The persistence of such lineages offers a glimmer of evolutionary resilience, albeit fragile and vulnerable.

The structured phylogeny indicates that historical isolation exerted a strong influence on lineage divergence. The separation of northern and southern clades suggests that environmental barriers or climatic shifts played a role in limiting gene flow long before contemporary habitat fragmentation. This pattern reinforces the argument that conservation strategies must acknowledge evolutionary histories rather than treating all surviving individuals as members of a single homogeneous population. The distinction carries implications for breeding programs, which must respect lineage integrity.

The environmental influence on eDNA recovery reveals that molecular traces mirror ecological heterogeneity. Regions with stable water conditions preserved DNA more effectively, allowing for deeper lineage reconstruction. This observation suggests that eDNA

not only reflects biological presence but also ecological dynamics, positioning it as a sensitive indicator of both genetic and environmental health. The method therefore serves a dual role in conservation monitoring (Khumalo et al., 2024).

The findings indicate that the Sumatran rhino's genetic future hinges on proactive and science-driven intervention. The detection of isolated haplotypes highlights the urgency of integrating genetic considerations into habitat management, translocation decisions, and assisted reproduction strategies. These results serve as a warning that genetic variability—once lost—cannot be restored, making its preservation an immediate priority.

The implications of this study extend directly to conservation planning, particularly regarding the management of lineage diversity. The documentation of structured haplotypes suggests that translocation programs must evaluate the risks of mixing genetically distinct clades. Genetic rescue strategies must balance the need to mitigate inbreeding with the responsibility to maintain historically meaningful lineages. The findings also support the development of region-specific reproductive programs that align with phylogenetic identities.

The study also influences policy-level decision-making by demonstrating the operational value of eDNA for ongoing monitoring. Conservation authorities can now obtain genetic data without disturbing wild individuals, allowing for ethically sustainable long-term population surveillance. eDNA monitoring frameworks can be incorporated into standard conservation protocols, making genetic assessment more frequent, affordable, and geographically comprehensive. These innovations democratize genetic data access for under-resourced conservation programs.

Ecological management benefits from understanding the relationships between environmental parameters and eDNA persistence. Habitat restoration programs can incorporate water-quality standards that improve the detectability of genetic material, thereby supporting monitoring reliability. Enhanced detection increases the precision of population mapping and informs habitat prioritization, particularly in regions where rhino presence is uncertain or sporadic (Liu et al., 2024).

The broader implication pertains to global conservation genomics. The demonstrated capability of eDNA to capture phylogenetic signals from a critically endangered species provides a model for other conservation efforts worldwide. The methodology offers a blueprint for monitoring elusive wildlife, enabling data-driven decisions that integrate ecological, genetic, and evolutionary considerations. This interdisciplinary synergy enhances the scientific foundation of biodiversity preservation.

The strong phylogenetic structuring observed in this study likely results from historical geographic barriers and long-term demographic isolation. The mountainous terrain and hydrological patterns of Sumatra may have limited dispersal for thousands of years, creating conditions for lineage divergence. The species' naturally low reproductive rate likely amplified the effects of drift, making populations more susceptible to fragmentation even before anthropogenic pressures escalated. These historical drivers explain the persistence of distinct regional clades.

The detection of rare haplotypes can be attributed to the sensitivity of eDNA methods combined with the secluded nature of certain habitats. Individuals occupying inaccessible micro-refugia may shed minimal but detectable DNA into their surroundings. These signals would have remained undetected through traditional sampling due to logistical constraints. The enhanced resolution provided by high-throughput sequencing amplifies these rare signatures, revealing up to now uncharted aspects of the species' genetic history.

Variations in read depth across sampling zones are products of environmental differences, including water chemistry and microbial activity (Wang et al., 2025). DNA degradation occurs more rapidly in warmer, more acidic environments, reducing recoverable material. Regions with stable water conditions yield higher-quality eDNA, explaining the

geographic disparities observed in sequencing results. This pattern highlights the ecological sensitivity of molecular detection.

The overall moderate genetic diversity reflects the species' long-term decline and small population size. Genetic bottlenecks and habitat loss have reduced variability, while limited gene flow has prevented the homogenization of regional lineages. The current genetic landscape is therefore the cumulative outcome of historical isolation, environmental constraints, and sustained demographic pressure.

Future conservation initiatives must integrate the study's phylogenetic findings into strategic planning. Genetic management frameworks should categorize Sumatran rhinos by lineage to prevent inadvertent erosion of evolutionary history. Breeding programs, whether in captivity or semi-wild sanctuaries, must incorporate haplotype information to optimize pairing decisions. A structured genetic approach will enhance the long-term adaptive potential of the species.

eDNA monitoring should be institutionalized within conservation protocols to enable real-time assessment of genetic shifts. The method's non-invasive nature allows repeated sampling that can track lineage presence, population changes, and reproductive events. Regular eDNA surveys will support dynamic habitat management by identifying areas where lineages persist, decline, or reappear following restoration interventions. A multi-year monitoring framework would provide predictive insight into genetic trends.

Collaboration between geneticists, ecologists, and local conservation authorities is essential to expand sampling coverage across unmonitored regions. Additional eDNA sampling from Bornean populations would generate comparative phylogenetic insights and strengthen continental conservation strategies. Expanding the geographic scope of eDNA analysis will generate a unified genetic atlas of the species, facilitating coordinated interventions across national boundaries.

The ultimate trajectory of Sumatran rhino conservation hinges on immediate, science-driven action. The findings of this study provide a blueprint for integrating molecular tools with habitat protection and reproductive technologies. Timely implementation of these recommendations may determine whether remaining lineages endure or fade into extinction. The study thus serves as both a scientific contribution and a call for urgent conservation transformation.

CONCLUSION

The study reveals several distinct findings that advance current understanding of Sumatran rhino genetics. The most important result is the identification of 13 unique maternal lineages through environmental DNA, including rare haplotypes not previously detected using traditional sampling. The phylogenetic reconstruction demonstrates clear separation between northern and southern clades, indicating long-term evolutionary isolation that predates modern habitat fragmentation. The discovery of site-specific haplotypes confined to discrete micro-refugia provides evidence that genetically unique individuals persist within highly localized environments, offering crucial insight into the species' remaining evolutionary capacity.

The research provides conceptual and methodological contributions that enhance conservation genetics for critically endangered megafauna. The application of environmental DNA not only enables lineage detection but also facilitates high-resolution phylogenetic analysis without requiring direct interaction with individuals. This methodological breakthrough expands the scope of non-invasive genetic monitoring by demonstrating the capacity of eDNA to recover deep evolutionary signals rather than merely presence–absence data. The integration of mitochondrial markers, high-throughput sequencing, and Bayesian modeling presents a scalable framework that can be adapted to other elusive species facing similar sampling constraints, thus strengthening global conservation genomics practices.

The study encounters limitations that suggest clear directions for future research. Environmental DNA recovery varies across habitat types due to degradation processes, limiting the reliability of samples from warmer or highly acidic sites. The exclusive use of mitochondrial markers constrains inference to maternal lineages, leaving nuclear genomic diversity unexplored. Broader sampling across seasons and expanded genomic approaches, including nuclear SNP panels and whole-genome sequencing from eDNA, would increase resolution and deepen understanding of population structure. The inclusion of Bornean populations in future analyses would also enable a comprehensive phylogeographic assessment essential for formulating transboundary conservation strategies.

AUTHOR CONTRIBUTIONS

Author 1: Conceptualization; Project administration; Validation; Writing - review and editing.

Author 2: Conceptualization; Data curation; In-vestigation.

Author 3: Data curation; Investigation.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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