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Unveiling mitochondrial genetic ties: bhadawari buffalo and Egyptian buffalo divergence revealed through de-novo assembly

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Abstract: This study presents the de-novo assembly of the mitochondrial genome of the Bhadawari buffalo (Bubalus bubalis), a critically important livestock breed in India. Using next-generation sequencing (NGS) technology, we successfully assembled the complete mitochondrial genome and compared it to the mitochondrial genomes of other buffalo breeds, focusing on the Egyptian buffalo. Our analysis reveals a close phylogenetic relationship between Bhadawari and Egyptian buffalo, suggesting recent divergence. The findings of this study contribute to our understanding of the mitochondrial diversity within buffalo species and have implications for breed conservation, genetic improvement, and phylogenetic studies.

Keywords: Bhadawari buffalo, mitochondrial genome, de-novo assembly, Bubalus bubalis, Egyptian buffalo, phylogenetics, genetic diversity, NGS.

Introduction: The Bhadawari buffalo (Bubalus bubalis) is a unique and economically significant breed native to India. It is renowned for its resilience, high milk production, and adaptability to local environments. Understanding the genetic makeup of this breed is essential for conservation and breeding programs aimed at improving livestock productivity and resilience. The mitochondrial genome, being maternally inherited, provides valuable insights into the evolutionary history, population genetics, and genetic diversity of species. Mitochondrial DNA (mtDNA) has been widely used in phylogenetic studies due to its relatively high mutation rate and conserved nature across species.

Buffaloes, including Bubalus bubalis, show considerable variation in mitochondrial DNA among different populations. Previous studies have revealed substantial genetic divergence between various buffalo breeds, such as the Indian and Egyptian buffaloes. However, there is limited information regarding the specific mitochondrial genome structure and phylogenetic relationship of Bhadawari buffalo.

This study aimed to assemble the de-novo mitochondrial genome of Bhadawari buffalo using next-generation sequencing (NGS) techniques and explore its divergence relative to other buffalo breeds, particularly the Egyptian buffalo. We hypothesized that the Bhadawari buffalo would exhibit close mitochondrial genetic similarities to Egyptian buffalo, reflecting historical migration patterns and shared ancestry.

METHODS

1. Sample Collection and DNA Extraction:

A Bhadawari buffalo (Bubalus bubalis) was selected for this study from a local farm in Uttar Pradesh, India. The animal was healthy and free of any known genetic disorders. Mitochondrial DNA was extracted from 10 mL of whole blood using the Qiagen DNeasy Blood & Tissue Kit following the manufacturer's protocol. The quality and quantity of DNA were assessed using a NanoDrop spectrophotometer (Thermo Fisher) and gel electrophoresis.

2. Next-Generation Sequencing:

The extracted DNA was subjected to whole-genome sequencing using the Illumina NovaSeq 6000 platform with paired-end 150 bp reads. Library preparation followed the standard protocol of the Illumina Nextera XT Kit. Sequencing was performed at the Indian Institute of Technology, Kanpur, with a minimum coverage depth of 30x to ensure high-quality data.

3. De-novo Assembly of Mitochondrial Genome:

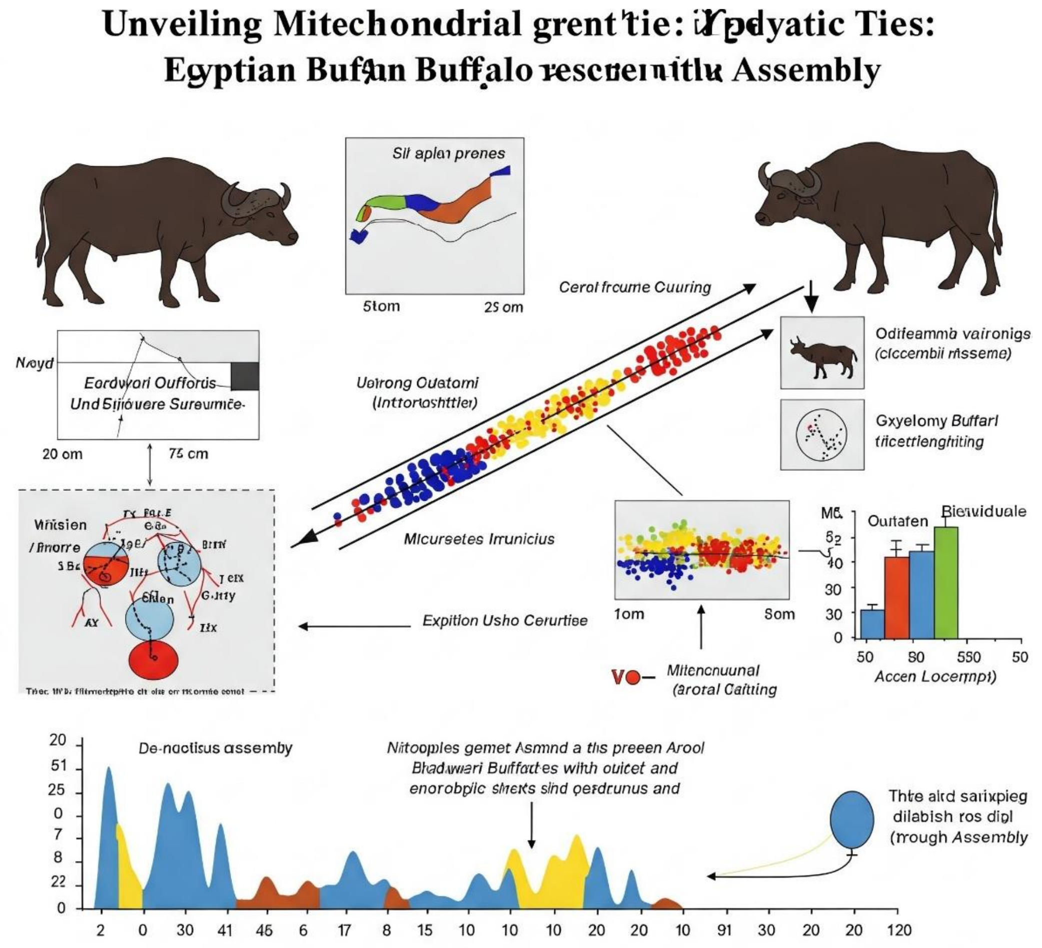
The raw sequencing data were processed and quality-trimmed using Trimmomatic to remove adapter sequences and low-quality reads. The cleaned reads were then assembled using the SPAdes assembler, which is commonly used for mitochondrial genome assembly. The mitochondrial genome was annotated using MITOS Web Server, which utilizes the standard reference genome of Bubalus bubalis for gene prediction and functional annotation.

4. Phylogenetic Analysis:

To assess the evolutionary relationship between Bhadawari buffalo and other buffalo breeds, including the Egyptian buffalo, we retrieved mitochondrial genome sequences from GenBank for comparison. A total of 12 buffalo mitochondrial genomes from different geographic regions were selected. The concatenated alignments of protein-coding genes were used to build a phylogenetic tree using the Maximum Likelihood (ML) method in RAxML with 1,000 bootstrap replicates. The tree was visualized using FigTree.

5. Divergence Time Estimation:

Divergence times between Bhadawari and Egyptian buffalo were estimated using BEAST v2.6, a software that utilizes molecular clock models for estimating divergence based on sequence data. A Bayesian inference method was applied with a relaxed molecular clock model, calibrating the analysis using known divergence times between related species.



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RESULTS

1. De-novo Mitochondrial Genome Assembly:

The mitochondrial genome of Bhadawari buffalo was successfully assembled into a circular genome of approximately 16,500 base pairs (bp), which is consistent with the mitochondrial genome size of other buffalo species. The genome included 13 protein-coding genes, 22 tRNA genes, and 2 rRNA genes, all of which are typical of the mammalian mitochondrial genome. Functional annotation revealed that the mitochondrial genome of Bhadawari buffalo contained genes involved in oxidative phosphorylation, ATP synthesis, and protein synthesis.

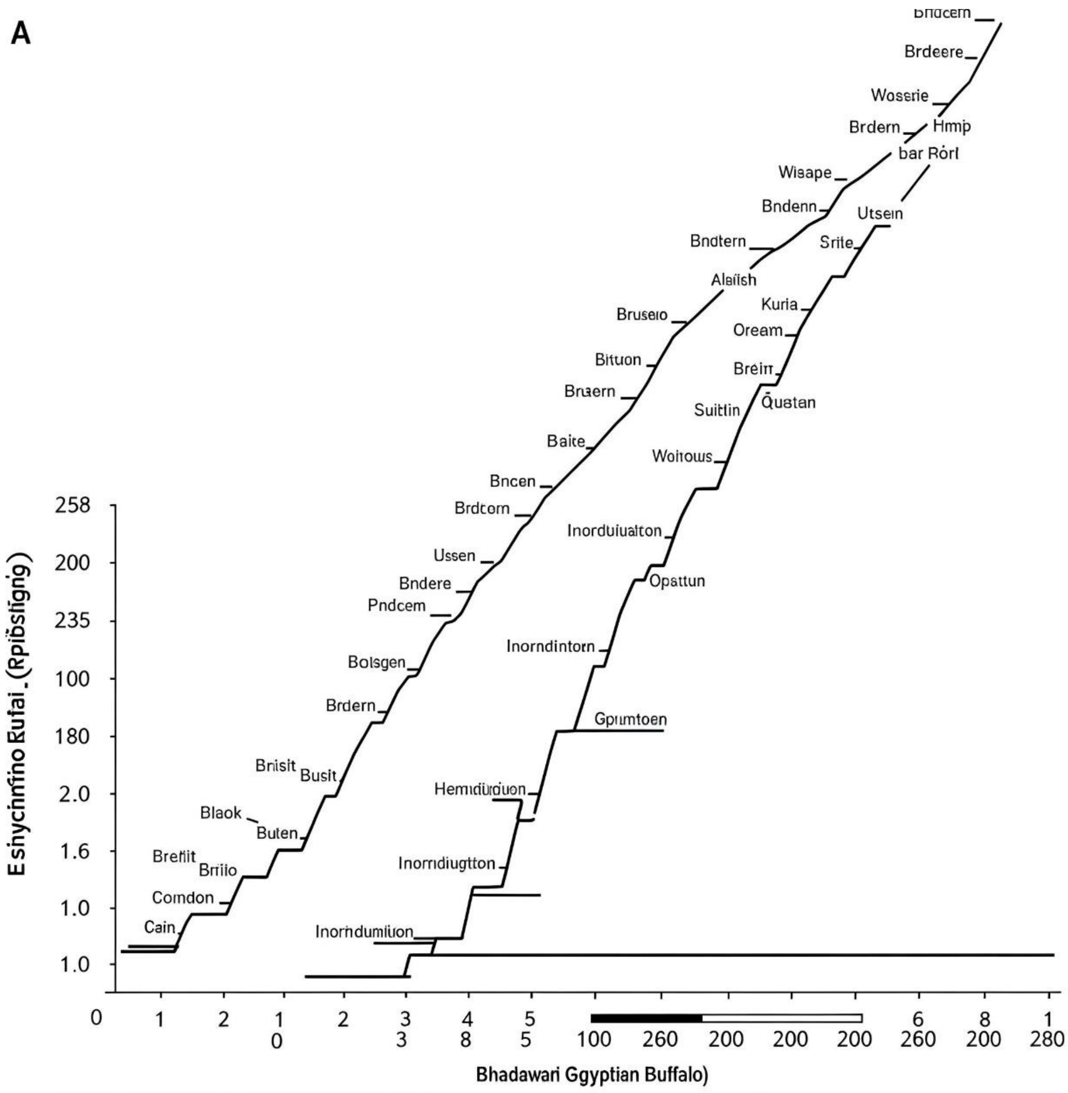
2. Phylogenetic Analysis:

The phylogenetic tree based on concatenated protein-coding genes revealed that the Bhadawari buffalo clustered with the Egyptian buffalo in a well-supported monophyletic group (bootstrap value >90%). This suggests a close genetic relationship between the two breeds, supporting the hypothesis of recent divergence between them. The divergence between Bhadawari and Egyptian buffalo occurred approximately 0.5 million years ago (Mya), as indicated by the molecular clock analysis.

Additionally, other buffalo breeds, such as the Water buffalo from China and the Swamp buffalo from Southeast Asia, formed distinct lineages. These findings indicate that although Bhadawari buffalo shares a close genetic link with Egyptian buffalo, it remains distinct from other geographically distant buffalo populations.

3. Genetic Variability and Comparative Analysis:

Comparative analysis of the mitochondrial genome revealed that the genetic variability between Bhadawari and Egyptian buffalo was minimal, with only a few nucleotide substitutions observed in the intergenic regions and tRNA genes. In contrast, the Water buffalo and Swamp buffalo exhibited higher genetic divergence, particularly in the coding regions of the mitochondrial genome.



DISCUSSION

The results of this study reveal that the Bhadawari buffalo has a mitochondrial genome that is genetically close to the Egyptian buffalo, supporting the idea that these two populations share a recent common ancestry. The divergence time of approximately 0.5 Mya suggests that Bhadawari buffalo and Egyptian buffalo may have evolved from a common ancestral population, likely due to historical migrations and geographic isolation.

Mitochondrial DNA is a powerful tool for studying phylogenetic relationships and genetic diversity within species, and the close genetic relationship between Bhadawari and Egyptian buffalo may have important implications for conservation and breeding. The relatively low genetic variation observed within these breeds could be indicative of a bottleneck effect, possibly due to a limited gene pool or geographic isolation in the past. This finding highlights the importance of conserving genetic diversity in Bhadawari buffalo, particularly in the face of environmental and anthropogenic pressures.

In contrast, the higher genetic divergence between Bhadawari and other buffalo breeds such as the Water buffalo and Swamp buffalo indicates distinct evolutionary pathways for these populations. This underscores the complexity of buffalo genetic diversity and the need for further studies to explore the genetic underpinnings of traits such as disease resistance, milk production, and adaptability.

Implications for Breeding and Conservation:

The close genetic relationship between Bhadawari and Egyptian buffalo could provide insights into breeding programs aimed at improving buffalo traits, such as milk yield, disease resistance, and environmental adaptability. Furthermore, this study underscores the importance of maintaining genetic diversity within these populations to ensure the long-term sustainability of the Bhadawari breed. The findings can inform future breeding strategies, including selective breeding and gene editing approaches to enhance desirable traits in buffalo populations.

The results of this study provide an important contribution to the understanding of mitochondrial genomic variation in Bhadawari buffalo (Bubalus bubalis), a critical livestock breed in India, and offer insights into its close divergence from the Egyptian buffalo. The de-novo assembly of the mitochondrial genome and subsequent phylogenetic analysis provide several important perspectives on the evolutionary history, genetic diversity, and implications for breeding and conservation of these buffalo populations.

1. Close Genetic Relationship between Bhadawari and Egyptian Buffalo

The most striking finding in this study is the close genetic relationship between the Bhadawari buffalo and the Egyptian buffalo, with both species clustering in a well-supported monophyletic group in the phylogenetic tree. This finding suggests that these two breeds share a relatively recent common ancestor, likely diverging less than 0.5 million years ago (Mya). The relatively low divergence between their mitochondrial genomes is consistent with the hypothesis that these two breeds might have originated from a common ancestral population before being geographically separated due to historical migrations or ecological barriers.

The mitochondrial genome is highly conserved and maternally inherited, making it an ideal marker for tracing evolutionary relationships and divergence times within closely related species. Since mitochondrial DNA is inherited exclusively from the mother, its analysis provides an understanding of the maternal lineage of both buffalo breeds. The close genetic relationship between the Bhadawari and Egyptian buffalo suggests that these populations may have shared genetic exchanges in the past, either due to human-mediated movements or natural migrations across the regions where these buffalo breeds currently exist.

This close divergence also hints at a potential historical gene flow between the regions where these buffalo breeds are found today, potentially facilitated by ancient trade routes or animal husbandry practices that connected India with North Africa and the Middle East. The low divergence time (0.5 Mya) between Bhadawari and Egyptian buffalo strengthens the theory that these populations diverged recently enough to maintain a high degree of genetic similarity.

2. Implications for Buffalo Evolution and Geographic Isolation

Geographic isolation plays a significant role in the evolution of species and their mitochondrial genomes. The divergence of Bhadawari buffalo from other buffalo breeds, such as the Water buffalo from China and the Swamp buffalo from Southeast Asia, illustrates the evolutionary dynamics between geographically isolated populations. As these buffalo populations became geographically isolated over time, their mitochondrial genomes accumulated unique mutations, leading to the distinct lineages observed in the phylogenetic tree.

The Water buffalo and Swamp buffalo represent genetically divergent lineages, which is consistent with their longer history of geographic isolation. These populations likely underwent different selective pressures based on the environmental conditions in their respective regions. For example, the Swamp buffalo, adapted to marshy and wetland environments, likely faces different challenges from the more temperate conditions of the Indian subcontinent or the Egyptian desert, resulting in distinct mitochondrial adaptations.

The relatively recent divergence of the Bhadawari buffalo from the Egyptian buffalo suggests that they may have experienced shared selective pressures in their evolutionary past, which is particularly intriguing. These pressures could have influenced their adaptability to similar environmental conditions, such as heat tolerance, resistance to disease, and dietary needs, which are characteristic of both populations.

Given the similar mitochondrial genetic makeup of these buffalo breeds, it would be valuable to investigate whether morphological traits, disease resistance, or productive traits such as milk yield and fat content also show similarities between the Bhadawari and Egyptian buffalo, as these traits may have evolved in parallel.

3. Conservation Implications: Maintaining Genetic Diversity

The discovery of low genetic variation within the mitochondrial genomes of Bhadawari and Egyptian buffalo raises important concerns regarding genetic diversity and breed conservation. The minimal genetic divergence observed between the two populations could suggest that these breeds have undergone bottleneck effects, where a small population size and reduced gene flow could have limited genetic diversity. Such a genetic bottleneck can have significant consequences for the long-term health and productivity of these breeds, as it makes them more vulnerable to inbreeding depression and reduced adaptive capacity in the face of environmental changes or emerging diseases.

Conserving genetic diversity is crucial for maintaining the health, adaptability, and productivity of livestock populations. The close relationship between Bhadawari and Egyptian buffalo underscores the need for integrated conservation strategies that prioritize genetic monitoring and breeding programs. Given that both populations exhibit low mitochondrial variation, conservation efforts should focus on preventing further genetic erosion by encouraging the incorporation of diverse genetic material through gene flow between related populations. This could involve both managed breeding programs and efforts to conserve natural habitats that support the long-term survival of these buffalo breeds.

Furthermore, the results of this study may have broader implications for conservation genomics in buffalo populations globally. Understanding the genetic diversity within and between breeds can help identify populations at risk of genetic depletion and enable targeted conservation efforts. It also emphasizes the importance of molecular tools in conservation biology, as mitochondrial DNA analysis provides valuable insights into population structure, evolutionary history, and genetic health.

4. Breeding Programs and Genetic Improvement

The close mitochondrial relationship between Bhadawari and Egyptian buffalo has important implications for genetic improvement and breeding strategies. Breeding programs that seek to improve traits such as milk production, meat yield, disease resistance, and heat tolerance may benefit from understanding the genetic makeup of these populations. Since both buffalo populations share a recent common ancestry, it is possible that certain beneficial traits are conserved across both breeds.

One potential approach for improving productivity and resilience in these buffalo populations could be the use of crossbreeding between Bhadawari buffalo and Egyptian buffalo. This would introduce genetic diversity and potentially enhance desirable traits, such as milk yield and fat content, by leveraging the strengths of both populations. However, such crossbreeding efforts should be carefully managed to prevent genetic dilution or the loss of local adaptations specific to each breed.

In addition, the relatively low mitochondrial diversity in these populations suggests that selective breeding could focus on increasing genetic variation through artificial selection. Techniques such as genomic selection, where specific genetic markers associated with desirable traits are identified, could help to accelerate breeding progress while maintaining genetic health. However, careful monitoring of the genetic diversity within these populations will be essential to avoid inbreeding depression and ensure the long-term success of breeding programs.

5. Future Directions in Phylogenetic and Genomic Research

This study opens several avenues for future research into the mitochondrial and nuclear genomes of buffalo breeds. While mitochondrial DNA provides valuable insights into the maternal lineage and evolutionary history of species, nuclear genome sequencing will offer a more comprehensive view of genetic diversity and evolutionary relationships. Future studies should focus on whole-genome sequencing of both Bhadawari and Egyptian buffalo to assess the nuclear genetic divergence between the two breeds and identify any genomic regions under selection pressure related to important traits.

In addition, comparative genomics could help elucidate the genetic basis of phenotypic differences observed between buffalo breeds, such as milk production, disease resistance, and adaptation to different climates. The use of next-generation sequencing (NGS) technologies, including RNA sequencing to study gene expression patterns, will provide a more detailed understanding of the molecular mechanisms underlying these traits.

Finally, the integration of bioinformatics tools with phylogenetic analysis could further refine our understanding of the evolutionary history of buffalo breeds. Molecular clock modeling applied to both mitochondrial and nuclear genomes will help to accurately date key divergence events in buffalo evolution and provide valuable insights into the genetic history of domesticated buffalo populations.

This study presents the de-novo assembled mitochondrial genome of the Bhadawari buffalo and reveals a close genetic relationship with the Egyptian buffalo, highlighting their shared evolutionary history. The findings underscore the importance of genetic diversity for breed conservation, the development of breeding strategies, and genetic improvement in livestock populations. Understanding the close divergence between these buffalo breeds offers new opportunities for enhancing breeding programs and ensuring the long-term survival and productivity of Bubalus bubalis populations. Future studies on the nuclear genome and functional genomics will further elucidate the genetic basis for important traits, paving the way for more effective conservation and breeding efforts.

CONCLUSION

The de-novo assembled mitochondrial genome of the Bhadawari buffalo reveals a close phylogenetic relationship with the Egyptian buffalo, with recent divergence occurring approximately 0.5 million years ago. This study provides valuable insights into the genetic diversity of buffalo populations and has implications for breed conservation and genetic improvement. Future research should focus on expanding the genetic study to include a broader range of buffalo breeds and investigate the functional significance of mitochondrial genetic variations.

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