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Mitochondrial genome annotation and comparative characterization of the Andamani goat

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Abstract

The Andamani goat is an indigenous goat breed of the Andaman and Nicobar Islands. These goats are well adapted to the hot and humid conditions of the island ecosystem and represent an important livestock germplasm for conservation and genomic characterization. Mitochondrial genome annotation provides useful baseline information for breed documentation, molecular characterization and future conservation genetic studies. In the present study, the mitochondrial genome of Andamani goat was retrieved, annotated and compared with published complete *Capra hircus* mitogenomes. The annotated mitogenome was 16,643 bp in length and showed the typical caprine mitochondrial organization, consisting of 13 protein-coding genes, 22 transfer RNA genes, 2 ribosomal RNA genes and a non-coding control region. The nucleotide composition was AT-rich, with 60.87% AT and 39.13% GC content. Comparative analysis showed that the Andamani goat mitogenome was within the reported range of published complete goat mitogenomes for genome length and base composition. The present study provides a preliminary mitochondrial genome annotation resource for Andamani goat and supports future molecular characterization and conservation-oriented studies of this indigenous island goat population.

Keywords: Andamani goat, mitogenome, annotation, Andaman and Nicobar Islands

Introduction

Goats are among the earliest domesticated livestock species and are believed to be domesticated around 10,000 years ago in the Fertile Crescent (Zeder and Hesse, 2000) [12]. They play an important role in rural livelihood, meat production and smallholder farming systems, especially in developing countries. Indigenous goat populations are valuable animal genetic resources because they are often adapted to local climatic conditions, management systems and ecological pressures.

India possesses diverse goat genetic resources distributed across different agro-climatic zones. Among these, island goat populations are of special interest because their genetic background may be influenced by geographical isolation, local management, founder effects and adaptation to coastal environments. The Andaman and Nicobar Islands represent a unique island ecosystem in the Bay of Bengal. The region has a hot and humid climate, high rainfall and coastal environmental conditions. The Andamani goat is an indigenous goat population of this region and is reared mainly under local island production systems. Previous descriptions of goat populations from the Andaman and Nicobar Islands have highlighted their local importance, farming practices and adaptation to the island environment (Sunder *et al.*, 2019) [10].

Mitochondrial DNA is widely used in livestock genetic studies because of its high copy number, compact genome structure and maternal inheritance. It has been used extensively for studies on genetic diversity, domestication and evolutionary history in domestic goats (Luikart *et al.*, 2001) [1]. Annotation of the mitochondrial genome provides basic information on genome organization and serves as a useful reference for future molecular and conservation genetic studies.

Mitochondrial genome retrieval and annotation provide a simple and useful approach for developing breed-specific mitogenome resources. GetOrganelle has been widely used for de novo assembly and retrieval of organelle genomes from genome sequencing data (Jin *et al.*, 2020) [5]. MITOS2 provides automated annotation of metazoan mitochondrial genomes, including protein-coding genes, rRNA genes and tRNA genes (Donath *et al.*, 2019) [3], while

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GenomeVx enables visual representation of circular organellar genomes (Conant and Wolfe, 2008)^[1].

The present study was undertaken to retrieve, annotate and comparatively characterize the mitochondrial genome of Andamani goat. This work provides a preliminary mitochondrial genome annotation resource for this indigenous island goat population.

Materials and Methods

Mitochondrial genome retrieval

The mitochondrial genome sequence of the goat RefSeq ARS1.2 assembly (GCF_001704415.2) was downloaded from NCBI and used as the reference sequence for mitochondrial genome recovery and comparison. Available sequencing reads of Andamani goat were used for mitochondrial genome retrieval. The mitochondrial genome sequence was recovered using GetOrganelle (Jin *et al.*, 2020)^[5], and the recovered sequence was used for downstream annotation, visualization and comparative analysis.

Mitochondrial genome annotation and visualization

The retrieved mitochondrial genome sequence was annotated using MITOS2 (Donath *et al.*, 2019)^[3] in Galaxy server (usegalaxy.org). Annotation included identification of protein-coding genes, transfer RNA genes, ribosomal RNA genes and the control region. The annotated mitochondrial genome was then visualized as a circular genome map using GenomeVx (Conant and Wolfe, 2008)^[1].

Comparative mitochondrial genome analysis

For comparative assessment, complete *Capra hircus* mitochondrial genome sequences were downloaded from the NCBI Nucleotide database in FASTA format. The downloaded sequences were used for comparison of general mitogenome features. The Andamani goat mitogenome was compared with the downloaded complete goat mitochondrial genomes for genome length and base composition. Sequence length was calculated from the FASTA files, and GC content was estimated as the percentage of guanine and cytosine bases in the complete mitochondrial sequence. AT content was calculated as 100-GC%.

Reference-based mitochondrial sequence variation

The assembled Andamani goat mitochondrial genome was aligned against the goat mitochondrial reference sequence to identify sequence-level differences. Variants were called using bcftools (Danecek *et al.*, 2021)^[2].

Results and Discussion

Retrieval of the Andamani goat mitochondrial genome

GetOrganelle successfully recovered a circular mitochondrial genome sequence of Andamani goat. The final mitochondrial genome was recovered from the K105 assembly graph and was reported as a circular animal mitochondrial genome. The average mitochondrial k-mer coverage and base coverage were 94.0× and 322.6×, respectively, indicating reliable mitochondrial genome recovery.

Table 1: GetOrganelle assembly summary for retrieval of the Andamani goat mitochondrial genome.

Parameter	Results
Read quality encoding	Sanger/Phred + 33
Mean read length	146.8 bp
Estimated mitochondrial-hitting base coverage	339.05×
Assembly k-mers	21, 45, 65, 85, 105
Final graph	K105
Average mitochondrial k-mer coverage	94.0×
Average mitochondrial base coverage	322.6×

Mitochondrial genome annotation

The recovered Andamani goat mitochondrial genome was annotated using MITOS2. The GFF annotation identified the mitogenome as a circular mitochondrial DNA molecule of 16,643 bp. A total of 37 mitochondrial genomic features were annotated, including 13 protein-coding genes, 22 transfer RNA genes and 2 ribosomal RNA genes. The annotated protein-coding genes included seven NADH dehydrogenase genes (nad1, nad2, nad3, nad4, nad4l, nad5 and nad6), three cytochrome c oxidase genes (cox1, cox2 and cox3), two ATP synthase genes (atp6 and atp8) and one cytochrome b gene (cob).

The annotation also identified 22 tRNA genes distributed across the mitochondrial genome. The two ribosomal RNA genes, rrnS and rrnL, were located toward the terminal region of the circularized annotation. Most of the annotated features were located on the positive strand, while nad6 and several tRNA genes were annotated on the negative strand, which is consistent with the typical strand distribution of mammalian mitochondrial genomes.

A large non-coding interval of approximately 1.2 kb was observed between trnP and trnF in the circularized mitochondrial genome annotation. This region likely

corresponds to the mitochondrial control-region interval in the present genome orientation. Overall, the MITOS2 annotation confirmed that the Andamani goat mitogenome contains the standard caprine mitochondrial gene complement and showed typical mammalian mitochondrial genome organization.

Table 2: MITOS2-based annotation summary of the goat mitochondrial genome.

Feature category	Annotated number
Protein-coding genes	13
tRNA genes	22
rRNA genes	2
Total annotated features	37
Mitogenome length	16,643 bp

Visualization of the annotated mitochondrial genome

The MITOS2 annotation was visualized using GenomeVx, which generated a circular map of the Andamani goat mitogenome. The map represented a mitochondrial genome length of 16,643 bp and displayed the annotated protein-coding genes, tRNA genes, rRNA genes and control-region interval in their respective genomic positions.

mammalian mitochondrial genomes and is consistent with the compact and conserved organization of vertebrate mitochondrial DNA. Therefore, the genome length, nucleotide composition, circular genome structure and conserved gene complement observed in the present study support the reliability of the mitogenome retrieval and annotation workflow used for Andamani goat.

Importance of mitochondrial genome annotation in indigenous goats

Mitochondrial genome annotation is an important component of genomic documentation in indigenous livestock because the mitochondrial genome is compact, maternally inherited and widely used in studies of diversity, domestication and conservation genetics. In goats, mitochondrial DNA has been extensively used to study maternal diversity and domestication history (Luikart *et al.*, 2001) ^[1]. Therefore, annotation of the Andamani goat mitogenome provides a useful baseline resource for future molecular characterization of this indigenous island goat population. Since island livestock populations may be influenced by geographic isolation, founder effects and local adaptation, generation of such genomic resources is important for breed documentation and conservation planning.

Utility of mitochondrial genome retrieval and annotation

The workflow used in the present study demonstrates that mitochondrial genome retrieval, annotation and visualization can be efficiently performed using standard bioinformatic tools. GetOrganelle enabled recovery of a circular Andamani goat mitogenome, while MITOS2 provided automated annotation of mitochondrial genes and GenomeVx supported circular genome visualization (Jin *et al.*, 2020; Donath *et al.*, 2019; Conant and Wolfe, 2008) ^[5, 3, 1]. This approach can be applied to other indigenous livestock populations for developing baseline mitochondrial genome resources useful for molecular characterization and conservation-oriented studies.

Conclusion

The present study reports mitochondrial genome annotation and comparative characterization of the Andamani goat. The annotated mitogenome was 16,643 bp in length and showed the typical caprine mitochondrial genome structure, consisting of 13 protein-coding genes, 22 transfer RNA genes, 2 ribosomal RNA genes and a control region. The genome showed AT-rich nucleotide composition and was comparable to published complete *Capra hircus* mitogenomes in terms of genome length and base composition. This work provides a preliminary mitochondrial genome resource for Andamani goat and may support future molecular characterization and conservation genetic studies of this indigenous island goat population.

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