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Genetic Diversity and Molecular Phylogeny of Iranian Goats Based on Cytochrome Oxidase I (COXI) Gene Sequences

(KERAGAMAN GENETIK DAN FILOGENI MOLEKULER KAMBING-KAMBING IRAN BERDASARKAN SEKUENS GEN CYTOCHROME OXIDASE I (COXI))

Reza Seyed Sharifi[1], Sima Savar Sofla[2], Hamid Reza Seyedabadi[*2]

[1]Department of Animal Science, Faculty of Agricultural and Natural Resources,
University of Mohaghegh Ardabili, Ardabil, Iran
[2] Animal Science Research Institute of Iran (ASRI), Agricultural Research,
Education and Extension Organization (AREEO), Karaj, Iran
*Corresponding Author: Hamid Reza Seyedabadi
Address: Animal Science Research Institute of Iran (ASRI),
Agricultural Research Education and Extension Organization (AREEO),
Karaj, Iran; Tel: +98-26-34430010; Email: hseyedabady@gmail.com

ABSTRACT

Mitochondrial DNA has been one of the most widely used molecular markers for phylogenetic studies in animals because of its simple genomic structure. This study examines the genetic characteristic of domestic goat using sequence analysis of mitochondrial DNA Cytochrome oxidase subunit I (COXI) to identify and differentiate among three common breeds (Adani, Najdi and Markhoz) of Iran. The genomic DNA was isolated by salting out method and amplified cytochrome oxidase I gene using Polymerase Chain Reaction (PCR) method with a pair of primer. Phylogenetic trees and pairwise calculations were obtained by using Mega 6 software. A partial sequence of cytochrome oxidase I gene of Iranian goats is 1286 bp and contained four variable sites and three haplotypes. Phylogenetic analysis of haplotype in the combination with the goat from GenBank showed that Iranian goatclustered in a separate lineage. This study was found informative for establishing relationships between breeds from different parts of the world. This study may facilitate the future researchers and breeders for better understanding the genetic interactions and breed differentiation for devising future breeding and conservation strategies to preserve the rich animal genetic reservoir of the country.

Keywords: mitochondrial cytochrome oxidase I gene; genetic diversity; phylogenetic analysis; Iranian goat.

ABSTRAK

Senyawa DNA mitokondria telah menjadi senyawa yang paling banyak digunakan sebagai penanda molekuler studi filogeni pada hewan karena struktur genomnya sederhana. Penelitian ini mengkaji karakter genetik ternak kambing di Iran menggunakan analisis sekuens Cytochrome oxidase subunit I (COXI) DNA mitokondria guna mengidentifikasi dan membedakan tiga ras kambing (Adani, Najdi, dan Markhoz) yang umum di Iran. Senyawa DNA genome diisolasi dengan metode *salting out* dan *amplified cytochrome oxidase I gene* menggunakan metode Polymerase Chain Reaction (PCR) dengan sepasang primer. Pohon filogeni dan pairwise calculations diperoleh dengan menggunakan piranti lunak Mega 6. Sebagian sekuens gen cytochrome oxidase I kambing-kambing Iran adalah 1286 bp dan memiliki empat variable sites dan tiga haplotypes. Analisis filogenetik terhadap haplotype kambing yang ada di GenBank menunjukkan bahwa kambing Iran memiliki kelompok turunan yang terpisah. Studi ini menemukan informasi yang mengungkap adanya keterkaitan antar ras kambing dari berbagai tempat di dunia. Studi ini di masa yang akan datang mungkin dapat mempermudah para peneliti dan para pembibit kambing untuk memahami lebih baik interaksi genetik dan perbedaan ras guna merancang pembiakan dan strategi konservasi di masa depan guna menjaga kekayaan *genetic reservoir* yang dimiliki Negara Iran yang melimpah.

Kata-kata kunci: mitochondrial cytochrome oxidase I gene; keragaman genetik; analisis filogenetik; kambing Iran

INTRODUCTION

Goat was the first animal to be domesticated by humankind. Since they have small size, the power of high compatibility, low expectations and high resistance against many diseases and hard environmental conditions has led to the preservation and breeding of these animals. There are numerous local goat breeds in Iran, which have a strong ûtness and foraging capability under a wide range of habitats, from the dry, cold, and harsh land. Due to the large environmental difference across Iran, it is natural that there are phenotypic differences among different native breeds.

According to the latest livestock census, conducted in 2008, the Iranian goat population is around 25,300,000 animals (http:// faostat.fao.org). Iranian goats are mainly reared in traditional systems by small holders. Since nomadic tribes are almost completely economically dependent on animal rearing, these stakeholders play an important role in the conservation of animal genetic resources, especially of small ruminants. More than 20 breed of goats have been recognized in Iran and according to the Animal Breeding Center of Iran report, Morghoz, Adani and Najdi goat breedsare at risk of extinction. Marghoz, the small breed of goat is distributed over the western and North-West of Iran near to the Turkey and Iraqi borders. They produce quite fine Mohair with different colors e.g. white, golden, brown, gray and even black. Adani dairy goat is one of the most important breeds in southern Iran, and despite the high average temperatures, humidity and lack of good pasture, the breed has adapted well to the severe environmental conditions. Najdi is a breed of domestic goat native to the Khozestan providence of the Iran. They have long with drooping ears and short and soft hairs. Ewes and rams are horned or polled. Though its meat may be consumed locally, it is especially valued for its milk.

Molecular systematic uses genetic markers to make inferences about population process and phylogeny and in doing so creates substantial comparative database for specific genes or proteins. Mitochondrial DNA has been one of the most widely used molecular markers for phylogenetic studies in animals because of its simple genomic structure (Akhilesh *et al*., 2015). Cytochrome C Oxidase Subunit 1 (COX1) is one out of the three mitochondrial DNA subunits, the others being MT- CO2, MT- CO3 that are part of respiratory complex IV (Tsukihara *et al*., 1996).

Inrespiratory complex IV, this enzyme completes the electron transport system, and is function of catalyzing the reduction of water into oxygen. Most Eukaryotes and all vertebrates have this protein (Tsukihara *et al*., 1996). DNA sequences of the mitochondrial cytochrome oxidase I (COI) gene can serve as a DNA barcode for identifying all kinds of animals (Hebert *et al*., 2003). Phylogenetic analysis using COI gene sequences were extensively carried out by several workers in different group of organisms like southern house mosquito *Culex quinquefasciatus* (Rukhsana *et al*., 2014), Armigeressubalbatus mosquito (Bindu and Sebastian, 2014), green bottle fly Luciliasericata (PriyaBhaskaran and Sebastian, 2014), Herpetogrammastultalis (Akhilesh and Sebastian, 2014), white backed plant hopperSogatellafurcifera (Sreejith and Sebastian, 2014), Asian honeybee Apiscerana (Rukhsana *et al*., 2014) and lepidopteran species (Pavana and Sebastian, 2014). The purpose of this study was to investigate the genetic diversity and phylogenetic evolution of Iranian goat (Morghoz, Adani and Najdi) based on the analysis of the partial sequence of the cytochrome oxidase I (COI) gene. This investigation will be helpful for the conservation, utilization, and exploitation of the genetic resources of the indigenous Iranian goat.

RESEARCH METHODS

Population Sampling

Blood samples from three Iranian goat breeds (Morghoz, Adani and Najdi) were considered for the study. Samples were collected from goats that were judged to be true to type with the phenotypic characteristics of that breed. The individuals selected had unrelated parents and grandparents based on the information provided by the owners and also cross checked with their neighbors. A total of 60 individuals from different locations were sampled and the blood was stored at 4°C. Genomic DNA was extracted from fresh blood according to standard procedures (Javanrouh *et al*., 2006) and was quantitated by spectrophotometry (Nanodrop ND1000).

PCR Amplification and Sequencing

The first cytochrome oxidase I (COX I) of the mtDNA was amplified and sequenced. To amplify the COXI region of goat mtDNA, a pair of primers was designed using the known goat mtDNA sequence (GenBank Accession No NC_001941.1). The primers COXI-F 52 -GACATCGGCACCCTCTAC-32 and COXI-R 52 -TCAGAGTATCGTCGTGGT-32 were used to amplify a 1286-bp DNA fragment. PCR amplifications were conducted in a 30 µl volume containing 5 µl of 10x reaction buffer, 1.5 mM $MgCl_2$, 0.2 mMdNTPs, 0.2 uM each primer, 1U Taq DNA polymerase (TaKaRaBiosystems), and approximately150 ng genomic DNA. The PCR mixture underwent 4 min at 95°C, 35 cycles 50s at 94°C, 1 min at 60°C and 1 min at 72°C, and 5 min at 72°C. PCR products were purified by using Watson PCR Purification Kit (Watson BioTechnologies, Shanghai). PCR products were sequenced using ABI PRISM BigDyeTM Terminator Cycle Sequencing Ready Reaction Kit and ABI PRISM 3130 Geneti Analyzer (Applied Biosystems, Foster City, USA).

Phylogenetic Reconstruction

The quality of the 1286bp COXI gene sequence for individuals was ûrstly evaluated on the basis of sequencing peak value and then these sequences were manually edited using program Chromas version 2.23. Then sequences were arranged using the BioEdit program and were aligned using CLUSTALW (http://ebi.ac.uk/clustalw) software. These results were compared with other sequences obtained from GenBank. To investigate genetic relationship between mitochondrial sequences, an unrooted neighbor-joining phylogeny (Saitou and Nei, 1987) was constructed using the Tamura–Nei distance method (Tamura and Nei, 1993). The phylogenetic tree construction is incorporated in the MEGA version.6.1 (Tamura *et al*., 2013). Diversity parameters including haplotype diversity (hd), nucleotide diversity (ð) and the average number of nucleotide differences were estimated using DnaSP (Sequence Polymorphism Software) 4.1 (Rozas *et al*., 2003).

RESULTS AND DISCUSSION

Nucleotide Composition of Iranian Goat COX1

The PCR-amplified COX1 DNA sequences of 60 individuals were 1286 bp in length on average. There were no insertions/deletions in 60 sequences of COX1 region .The BLAST analysis revealed these sequences were closely related to the mitochondrial sequences of goat, showing 100% sequence identity with a COX1 sequence (1286bp; GenBank accession No. KY305183.1). The sequence analysis of COX1 DNA revealed an open reading frame encoding 514 amino acids, with an ATG initiation codon, and no introns. The average nucleotide ratios in the COX1 gene were: 28.97% for A, 29.66% for T, 15.86% for G, and 25.52% for C. Percentage of nucleotide pairs A+T was 58% and the C+G was 42%, suggesting that A+T nucleotides were higher in the COXI gene of mtDNA Iranian goat breeds. Because of the well-known gene structure and lack of recombination, the COXI gene has been generally used alone or in combination with other mtDNA encoding genes and hyper variable regions for phylogenetic studies between species (Chen et al., 2006). Generally speaking, the AT content is always higher than the GC content in COXI gene (Chen et al., 2006). Our study was consistent with that, showing proportions of 58:42.

COX1 Genetic Diversity

Sixty sequences rendered 3 divergent haplotypes with 2 variable sites defined. The largest haplotype group consisted of 44 individuals. The COX1 sequences of 60 individuals showed a haplotype diversity of 0.47 and a nucleotide diversity of 0.0005, comprising three haplotypes (I, II, and III). The statistical parsimony network constructed for COX1 haplotypes showed that 74% of sequences belonged to haplotype I, 18% to haplotype II, and 8% to haplotype III. As an encoding gene of mtDNA, the occurrence of mutation in the COX1 gene is low compared to mutation in the D-loop and other encoding genes (Chen *et al*., 2006). Haplotypes diversity (H_d) and nucleotide diversity (ð) values were low in Iranian goat population (H_d=0.47, ð= 0.0005). Haplotypes diversity (H_d) values were low in three populations. Values ranged from 0.541± 0.023 in Morghoz to 0.44± 0.021 in Adani and Najdi goat populations. If haplotype diversity and nucleotide diversity of mtDNA are greater, polymorphism of the population will be higher (Table1). Nucleotide diversity and haplotype diversity of mtDNACOX1 region are the important indices for assessing population polymorphism and genetic differentiation. It was far lower than that of the D-loop region

(Seyedabadi et al., 2016) indicating that the COX1 gene is relatively conserved. Most base substitutionsdid not change the coding of the amino acid. In addition, Javanrouh *et al.* (2009), studied six Iranian indigenous goat populations by investigating their nuclear DNA using Rapid markers, and the result showed that the mean polymorphism information content (PIC) of the six breeds were low. The COX1 gene has beenused to study other aspects, such as intra or interspecific relationships and gene flow (Alves *et al*., 2003).

Table 1. Haplotypes, Values of haplotypes diversity (hd), for each breed

Breed D population	n	Haplotypes	hd ± SD
Morghoz	20	2	0.541± 0.023
Adani	20	1	0.446± 0.021
Najdi	20	1	0.441± 0.021

Phylogenetic Analysis

The phylogenetic tree of Iranian indigenous goat sequences were constructed using unrooted neighbor-joining tree with reported goat sequences from Italy (KJ192235, KR349363), China (KP677511, KY523508, KY523509, KM233163), Saudi Arabia (KT750041), and Ireland (KY564254,KY564266, KY564248, KY564252) (Fig. 1). Phylogeny tree of COX1 gene nucleotide showed that Iranian goat clustered in a separate lineage. This result is supported bythe bootstrap value of 100%. Bootstrap value is a benchmark to determine the level of accuracy ofphylogeny tree. The bootstrap analysis is a method to test how well the set of model data and bootstrap was supported by the software testing, the branches could be trusted (Dharmayanti, 2011). Mitochondrial DNA (mt DNA) has become a very powerful tool in species identification and forensic sciences because of the high number of copies in each cell and the lack of recombination with paternal mtDNA. The high copy number results in increased sensitivity of species identification, a number of studies have adopted the universal primers introduced by Kocher et al. (1989) targeting the cyt b locus (Bellis et al., 2003). However, it is evident that regions residing in the COX1 locus in the mitochondrial genome among mammals are more strictly conserved than the region in the cyt b locus. This study was found informative for establishing relationships between breeds from different parts of the world. In conclusion, this study may facilitate the future researchers and breeders for better understanding the genetic interactions and breed differentiation for devising future breeding and conservation strategies to preserve the rich animal genetic reservoir of the country.

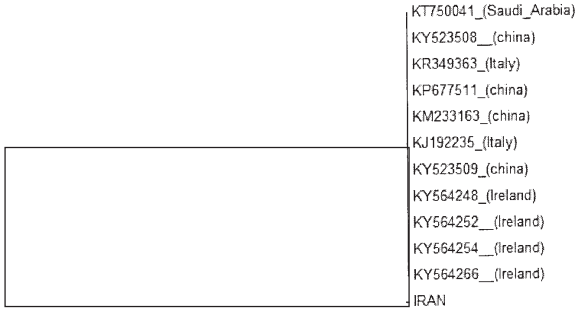

Figure 1. Unrooted neighbor-joining tree constructed from Italy, China, Saudi-Arabia, Ireland and Iranian goat populations

CONCLUSION

The present study is the first example of a COX1 phylogenetic analysis of Iranian goat breeds evaluated at the maximum level of resolution. The COX1 structure has been conserved and positively selected during the evolution of goat breeds. NJ tree constructed based on nucleotide sequence of COX1 gene confirmed that Iranian goat clustered in a separate lineage. The evolutionary divergence into distinct entities of Iranian goat breeds based on mtDNA COX1sequence appear to closely follow their geographical distribution in Iran, and this could have implications for management, improvement and conservation strategies in Iranian goat. This study suggests that in order to better understand the genetic interactions and breed differentiation of Iranian goats, the whole mitochondria genome should be studied.

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