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Molecular Analysis of Khalkhali Goat Population based on cytB region of Mitochondrial DNA

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ABSTRACT: Native goats of importance in the economy of rural households are also important as genetic reserves that account for the reserving genetic diversity in native goat breeds of Iran because of the little population size is necessary for breeding goals and increasing their production. The first step is determination of genetic diversity in existing populations. Among the genetic markers, mtDNA sequencing is one of the most useful and common methods employed for inferring phylogenetic relationship between close related species and population and conservation of species. The object this study was carried out for determination of the mitochondrial cytB sequence in Khalkhali native goat in Iran. For this study blood samples were taken randomly from 20 goats. After extracting DNA, cytB region of mtDNA was amplified with specific primers using PCR and after purification was sequenced. The phylogenic tree was drawn with the consensus sequence of other similar sequences of different chicken breeds obtained from GenBank. In The phylogenic tree, Khalkhali native goat was clustered with China, Japan, France and Italya native goat breeds. This is possible because of the conserved area is cytB in goats.

Key words: mtDNA, DNA sequencing, cytB, phylogeny, Khalkhali native goat.

INTRODUCTION

The goat is the earliest ruminant to have been domesticated (Mason, 1984). The domestic goat Capra hircus is one of the most important livestock species in the world for providing good animal production even under harsh environmental conditions. Recently, molecular studies of goats based on mitochondrial DNA (mtDNA) sequences have been carried out to investigate the origin and phylogeny of goats (Luikart *et al.* 2001; Mannen *et al.* 2001; Mannen 2004; Naderi *et al.* 2007). Mitochondrial DNA is very useful for its multiple presences in cells. The most of animal mtDNA is coding 37 genes (Avise, 1994). One of them is gene for cytochrome b (CytB). Cytochrome b is a component of respiratory chain complex III (Howell, 1989, ESposti *et al*, 1993). Length of CytB gene is 1140 bp and has some stable sequences which were used for suggestion of universal primers and some variable sequences used for animal identification. The Khalkhali is the autochthonous goat from Iran and belongs to the same indigenous population that lives throughout west north Iran. It is a long haired and a small-sized goat and reared in extensive mixed farming systems, together with sheep and cows, or semi-intensive oasis systems. The breed produces mainly meat, but it shows a high genetic potential for milk production. National projects for development of the small ruminant sector and biodiversity conservation strategies are currently developed in Iran for the native goat (FAO 2007). Goat milk can be used as food for people with cow-milk allergy and cheeses are appreciated by consumers (Boyazoglu *et al.* 2005). Furthermore, meat of suckling kids is a delicacy and prices paid to farmers are constantly higher than that of lamb meat. Goat milk-derived products are an important source of pro?t in France and Greece, as these countries have started to exploit the value of their typical products. Indeed, under well-organized management, goat farming is a profitable way of marketing marginal natural resources without endangering the environment. The study of autochthonous breeds can play an important role in the preservation of natural resources and the rural environment and landscape, in particular the protection of biodiversity. To extend the knowledge of goats reared in the Mediterranean area, we studied a particular region of mitochon drial DNA (mtDNA), the cyt-B region.

To date, sequences from many species are known and the complete sequence of goat mitochondrial genome (Accession number: GenBank AF533441) was deposited in 2003 (Parma *et al.* 2003). Many studies used mtDNA as an important means of population studies. Luikart *et al.* made the first important research in 2001; Naderi *et al.,* using a large mtDNA analysis, identified six haplogroups mtDNA in 2007, and Amills *et al.* analyzed the genetic diversity of South and Central American goats in 2009. These studies confirmed a weak phylogeographic structure in goat species, when compared to cattle. This result has been explained by some authors (Luikart *et al.* 2001; Amills *et al.* 2009) because goat, owing to its moderate size and ability to adapt to different environments, well-suited to the intercontinental transportation in ancient times. Based on previous literatures, in this study, molecular analysis of Khalkhali goat population based on cyt-B region of mitochondrial DNA were investigated to develop molecular markers for breed identification.

MATERIALS AND METHODS

A. Animals

We collected blood samples of native goat from Khalkhali goat. Blood samples (5ml in EDTA containing tubes) randomly collected from 20 animals and stored at -20°C until used at biotechnology laboratory.

B. Amplification and sequencing

The complete cyt B gene was amplified by using forward primer cytB-F: 5'-CgATACATACACgCAAACggA-3'and reverse primer cytB-R: 5'- AgAAggTTgTTTTCAATggTgC -3'. The forward and reverse primers were designed from tRNA-Glu and tRNA-Thr sequences of the mtDNA genome (GenBank accession no. V00654). Polymerase chain reaction (PCR) was carried out in a total volume of 25 ul, containing 10 ng of genomic DNA, 2.5 ul of 10ul buffer, 0.2 mM of dNTP, 10 pM of each primer and 1.5 units of Taq polymerase (TaKaRa, Japan). Thermal cycling was performed on a PTC-200 thermocycler (MJ Research Inc.) under the following conditions; 2 min denaturation at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 60°C, 60 s at 72°C, and a final 5 min at 72°C before cooling to 4°C for 10 min. The amplified products were separated by electrophoresis on 1% agarose gels, and were visualized under UV illumination after staining with ethidium bromide. The PCR products were purified using a QIAquick PCR purification Kit (Qiagen, USA), and were directly sequenced on an ABI 3130xl Genetic Analyzer (PE Applied Biosystems, USA).

C. Statistical and phylogenetic analyses

The sequences of the cyt b gene from different breeds were aligned in CLUSTAL W (Thompson *et al.,* 1994). Numbers of nucleotide polymorphic sites (S) and haplotype (h), nucleotide diversity (Pi), haplotype diversity (Hd) and nucleotide divergence (Dxy) were performed in DNA sequence polymorphism Version 5.1 (Librado and Rozas, 2009). The Neighbor-joining (NJ) tree (Saitou and Nei, 1987) among haplotypes based on the cyt b gene sequences was reconstructed in MEGA 5.05 package (Tamura *et al.*, 2011), with the reliability of the tree topology assessed by 1,000 bootstrap replications (Felsenstein, 1985). The NJ tree among breeds was constructed in MEGA 5.05 package on the basis of Dxy distances.

RESULTS AND DISCUSSION

A. Sequence composition and variation of the cyt b gene

The full-length coding sequences of the cyt b genes in 20 individuals were determined. All these sequences spanned 1,140 bp, started with an ATG translational start codon and ended with an AGA stop codon. No insertion/deletion or length variation was detected in these sequences (Fig. 1). According to the data in Fig. 2, to assume sequence index in Khalkhali native goat, we used consensus sequence using BioEdit software in ~ 830 pair bases. As presented in Fig. 3, the Composition procedure of BioEdit software implied that 255 nucleotides was in group (A), 240 nucleotides in group (C), 113 nucleotides in group (G) and 224 nucleotides in group (T), respectively. Additionally, the G + C ratio was 42.43 and A+T was 57.57 percent. Furthermore, the molecular weight of this sequance was 255296 daltons and the the molecular weight of pairs was 504737 daltons. These patterns were very similar to those of a previous report which analyzed goat breeds (Amer, 2014). Based on the alignment of the cytochrome b gene initial fragment, phylogenetic trees were constructed. Fig. 4 demonstrates the cladogram obtained by use of the method of minimal evolution. Clusterization of the samples in tree corresponded to their species affiliation. Currently, four tree branches can be distinguished. This result indicates that Khalkhali goat The between-group distances were computed using the MEGA 5.0 software (Fig. 4).

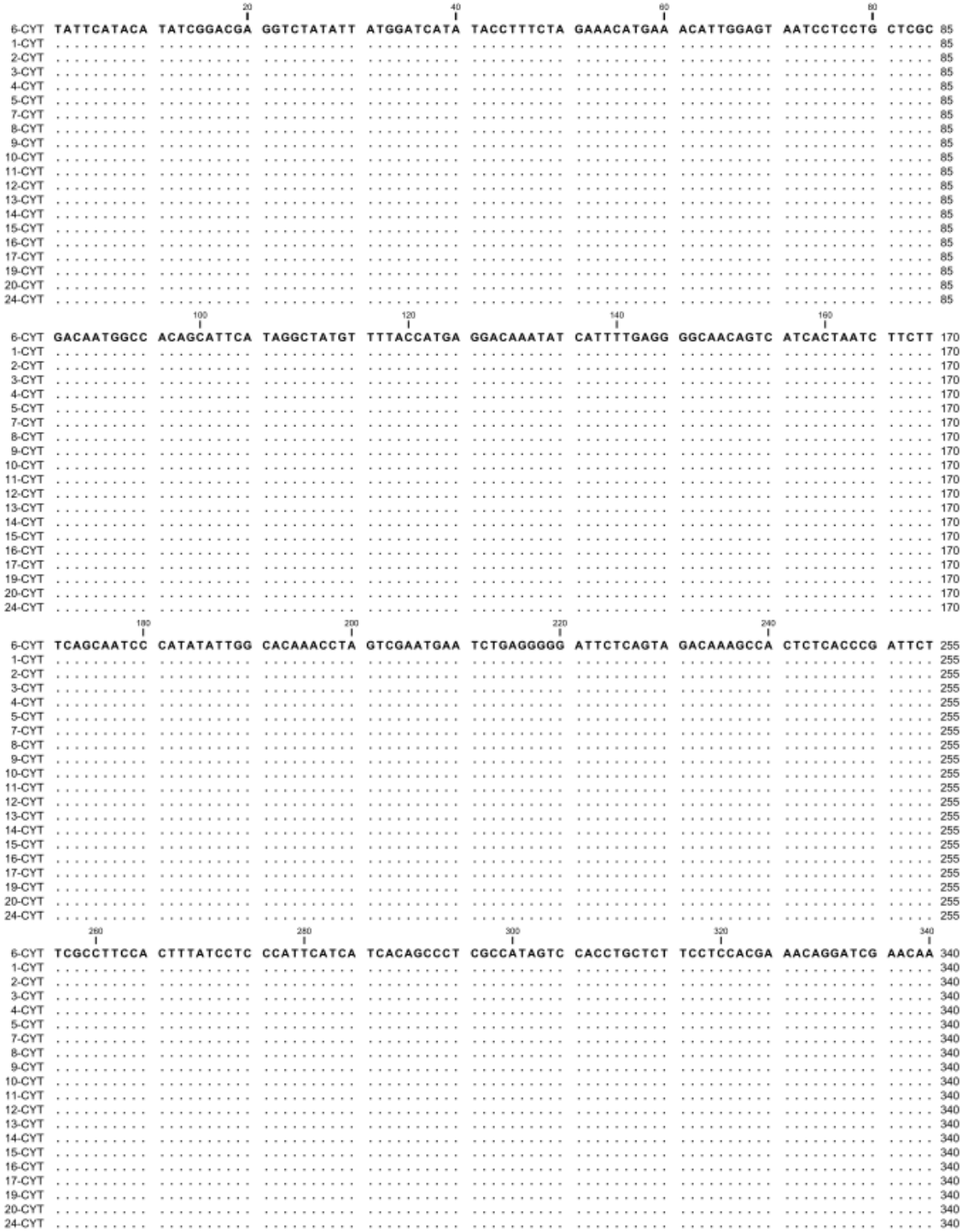

Fig. 1. Sequence variation of mtDNA cyt b gene of 20 individuals of the Khalkhali goat.

Fig. 2. Consensus Sequence in Khalkhali goat.

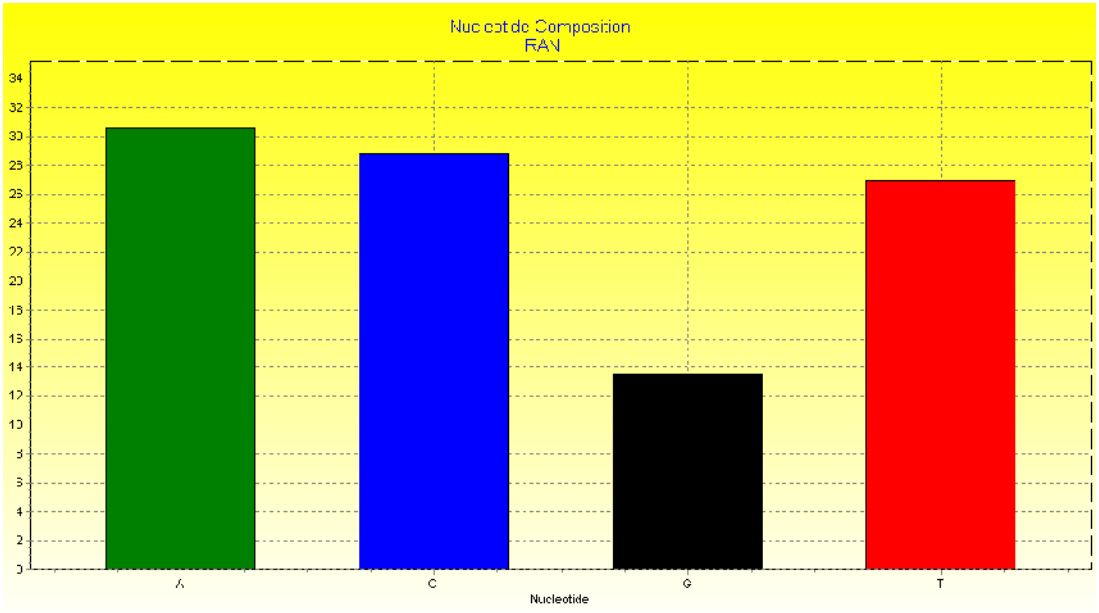

Fig. 3. Nucleotide Composition Percentage of Consensus Sequence in Khalkhali goat.

Distribution of the samples between the groups was made in accordance with the clusterization obtained. Apparently, the longest distance separated the JX286586 (Pakistan) from the others. The shortest distances were among GU229278 (China), JX286542(Pakistan), EU130779(China), Khalkhali and AB004074(Japan). This is possible because of the conserved area is cytB in goats.

		1	2	3	4	5	6	7	8	9	10	11	12
IRAN	1		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
KJ192229(ITALYA)	2	100.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
GU229278.1(CHINA)	3	100.00	100.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
AB004074((JAPAN)	4	100.00	100.00	100.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
DQ089477_(CHINA)	5	100.00	100.00	100.00	100.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00
DQ514544.1(FRANCE)	6	100.00	100.00	100.00	100.00	100.00		0.00	0.00	0.00	0.00	0.00	0.00
EU130779.1((CHINA)	7	100.00	100.00	100.00	100.00	100.00	100.00		0.00	0.00	0.00	0.00	0.00
JX010746(KOREA)	8	100.00	100.00	100.00	100.00	100.00	100.00	100.00		0.00	0.00	0.00	0.00
JX286542_(PAKISTAN)	9	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00		0.00	0.00	0.00
AB735775(JAPAN)	10	99.88	99.88	99.88	99.88	99.88	99.88	99.88	99.88	99.88		0.00	0.00
JX286586(PAKISTAN)	11	99.88	99.88	99.88	99.88	99.88	99.88	99.88	99.88	99.88	99.76		0.00
KJ192227(ITALYA)	12	99.88	99.88	99.88	99.88	99.88	99.88	99.88	99.88	99.88	99.76	99.76	

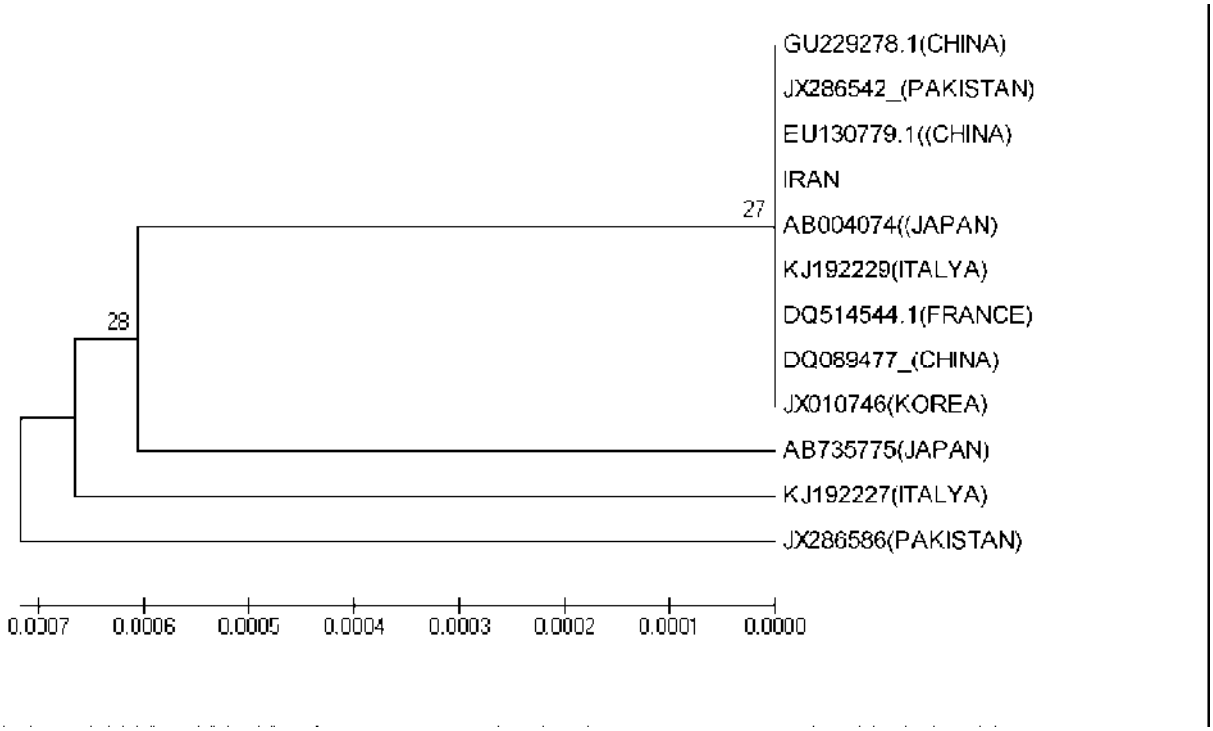

Fig. 4. Phylogenetic relationship among 12 GenBank accession number of cyt b gene from goat breeds.

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