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Molecular Analysis of Mahabadi Goat Population based on ND6 gene 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 ND6 gene sequence in Mahabadi native goat in Iran. For this study blood samples were taken randomly from 30 goats. After extracting DNA, ND6 gene 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 goats breeds obtained from GenBank. InThe phylogenic tree, Mahabadi native goat was clustered with Switzerland, Romania, Austria, Cyprus, Jordan, Spain, Saudi Arabia, Albania , Turkey, Egypt, Kurdi Iran, Malaysia, Kyrgyzstan, and Italya native goat breeds. This is possible because of the conserved area is ND6 in goats**.**

Keywords**:** mtDNA, DNA sequencing, ND6 gene , phylogeny, Mahabadi 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; Mannen2004; 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 NADH dehydrogenase (ubiquinone) ND6 gene is a component of respiratory chain complex I (Howell, 1989, ESposti *et al,* 1993). Length of ND6 gene is 639 bp and has some stable sequences which were used for suggestion of universal primers and some variable sequences used for animal identification. The Mahabadi 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 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 and cheeses are appreciated by consumers (Boyazoglu et al. 2005). Furthermore, meat of suck ling 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

protein 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 gene of mitochondrial DNA (mtDNA), the ND6 gene 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 Mahabadi goat population based on ND6 gene of mitochondrial DNA were investigated to develop molecular markers for breed identification.

MATERIALS AND METHODS

We collected blood samples of native goat from Mahabadi goat. Blood samples (5ml in EDTA Containing tubes) randomly collected from 20 animals and stored at -20°C until used at biotechnology laboratory. Amplification and sequencing The complete ND6 gene was amplified by using forward primer ND6-F: 5'-CgATACATACACgCAAACggA-3' and reverse primer ND6-R: 5'AgAAggTTgTTTTCAATggTgC -3'. The forward and reverse primers were designed 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). Therminal cycling was performed on a PTC-200 thermocycler (MJ Research Inc.) under the following conditions; 2 minute denaturation at94°C, followed by 35 cycles of 30 s at 94°C, 30 s at60°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 QIA quick 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 ND6 gene from different breeds were aligned in CLUSTAL W (Thompson etal., 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 ND6 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 divergence distances.

RESULTS AND DISCUSSION

Sequence composition and variation of the ND6 gene The full length coding sequences of the ND6 genes in 30 individuals were determined. All sequences spanned 693 bp , started with an ATG translational start codon and ended with an AGA stop codon. length variation was detected in these sequences (Fig. 2). According to the data in Fig. 3 , to assume sequence index in Mahabadi native goat, we used consensus sequence using BioEdit software in 639 pair bases. As presented in Fig. 3, the Composition procedure of BioEdit software implied that 270 nucleotides was in group (A), 182 nucleotides in group (C), 50 nucleotides in group(G) and 137 nucleotides in group (T), respectively. Additionally, the G + C ratio was 36.31 and A+T was 63.69 percent. Furthermore, the molecular weight of this sequance was 197801 daltons and the Molecular weight of pairs was 386997daltons. These patterns were very similar to those of a previous report which analyzed goat breeds (Amer, 2014). Based on the alignment of the ND6 gene initial fragment, phylogenetic trees were constructed. Fig 5. demonstrates the diagram 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. The ND6 gene sequences were not highly polymorphic. Our 30 sequences gave just 2 different haplotypes with 1 variable sites defined. The largest haplotype group consisted of 18 individuals, , and other haplotypes included 11 individuals (table1). Mahabadi goat divided tow cluster within population (Fig 1). This result indicates that Mahabadi goat The between group distances were computed using the MEGA 5.0 software (Fig. 5).

Table1: situation and number of frequent sequence ND6 gene analyzed

		situation
haplotyp	frequent	389
1	18	A
2	11	G

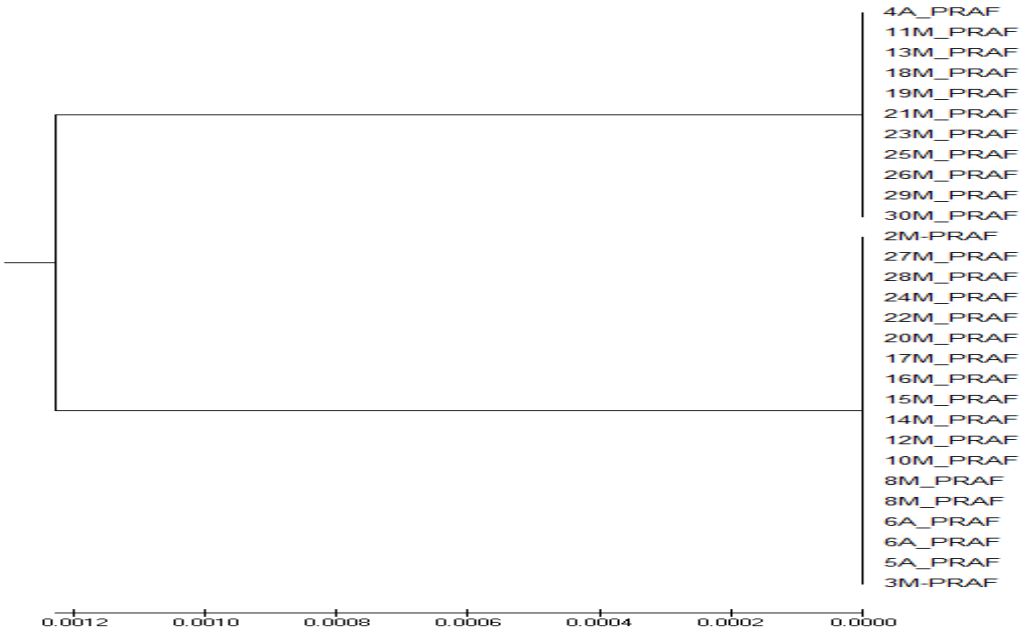

Fig. 1. Phylogenetic tree within population Mahabadi breed based on ND6 gene

5

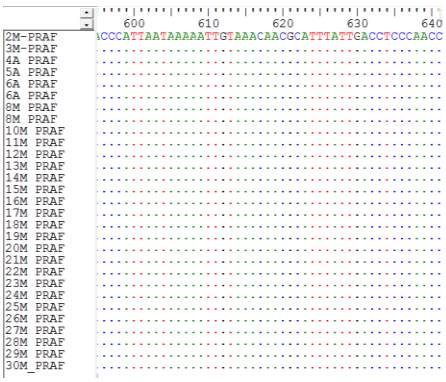

Fig 2. Sequence variation of mtDNA ND6 gene of 30 individuals of the Mahabadi goat.

1 CAA CAC CAA TTA ACA AAG ATC AAC CAG TAA CAA TAA CTA ATC AAG
46 TAC CAT AAC TGT ATA AAG CAG CAA TCC CTA TGG CCT CCT CAC TGA
91 AGA ACC CAG AAT CCC CTG TAT CAT AAA TAA CCC AAT CCC CCA TAC
136 CAT TAA ACT CAA ACA CAA TCT CCA CTT CCT TAT CTT TTA ACA CGT
181 AAT AAA CCA TAA GAA ACT CCA TTA ACA AAC CAG TAA CAA ACG CCC
226 CTA GAA CAA CTT TAC TAG AAA CTC AAA CCT CAG GAT ATT GCT CAG
271 TAG CCA TAG CCG TCG TAT AAC CAA AAA CTA CCA TTA TAC CCC CCA
316 AAT AAA TTA AAA AAA CTA TTA AAC CTA AAA AAG ACC CAC CAA AAT
361 TCA ACA CAA TAC CAC ATC CCA CCC CAC CAC TCA CAA TTA ACC CTA
406 ACC CCC CAT AAA TAG GCG AAG GTT TTG AAG AAA ACC CCA CAA AAC
451 CTA TTA CAA AGA TAA CGC TTA AAA TAA ATA CAA TAT ATA TTA TCA
496 TTA TTC TCA CAT GGA ATC TAA CCA TGA CCA ATG ATA TGA AAA ACC
541 ATC GTT GTT ATT CAA CTA CAA GAA CAC TAA TGA CCA ACA TCC GAA
586 AGA CCC ACC CAT TAA TAA AAA TTG TAA ACA ACG CAT TTA TTG ACC
631 TCC CAA CCC

Fig. 3. Consensus Sequence in Mahabadi goat.

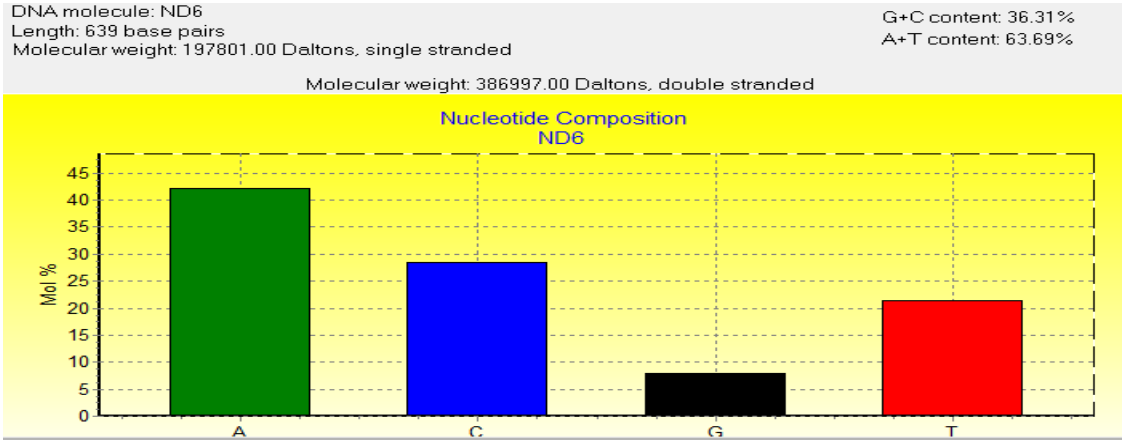

Fig. 4. Nucleotide Composition Percentage of Consensus Sequence in Mahabadi goat.

Distribution of the samples between the groups was made in accordance with the clusterization obtained. Apparently, the longest distance separated theKR059159 (Egypt) and KR059220 (Malaysia) from the others. The shortest distances were among KR059205 (Italy) , KR 059147 (Switzerland), KR059198 (Romania),KR059163(Cyprus), KR059169 (Jordan), KR059156 (Spain), KR059146(Albania) andKR059200(Turkey). This is possible because of the conserved area is ND6 in goats.

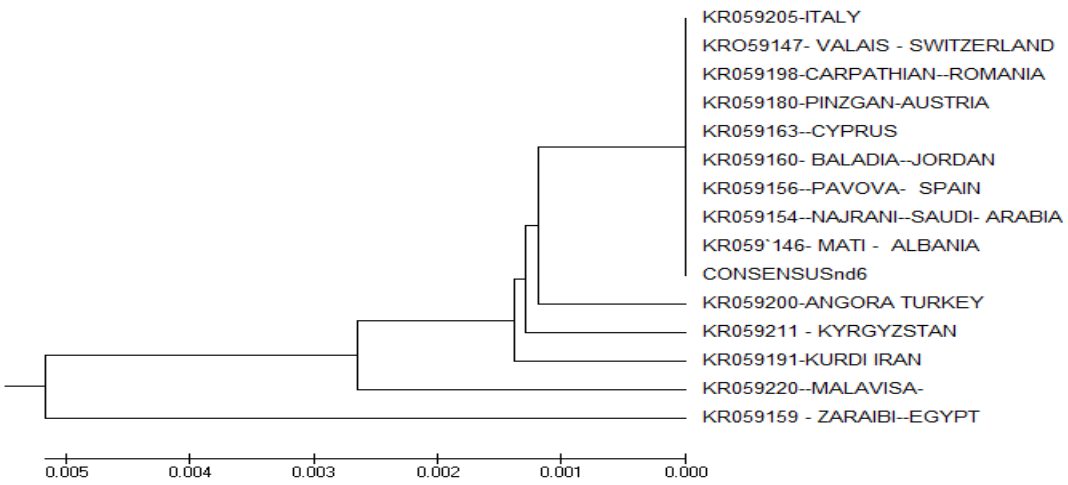

Fig. 5. Phylogenetic relationship among 12 GenBank accession number of ND6 gene from goat breeds.

REFERENCES

Amer, S.A.M. (2014). Mitochondrial DNA Variability among Some. Saudi Arabian Goat Breeds. *British Biotechnology Journal.* 4(8): 877-882.

Amills M., Ramirez O., Tomas A., Badaoui B., MarmiJ.,Acosta J., Sanchez A., Capote J. (2009). Mitochondrial DNA diversity and origins ofSouth and Central American goats. *Anim.Genet.,* 40, 315-322.

Avise, J. C. (1994). Molecular markers, natural historyand evolution. Chapman & Hall, New York,N.Y.

Boyazoglu J., Hatziminaoglou I., Morand-Fehr P.(2005). The role of the goat in society: past,present and per spectives for the future. *Small Rumin. Res.* 60, 13-23.

Esposti M.D., Crimi M., Ghelli A., Patarnello T.,Meyer A., De Vries S. (1993). Mitochondrial cytochrome b: evolution and structure of theprotein. In: *Biochim. Biophys. Acta* 1143(3).243-271.

Howell N. (1989). Evolutionary conservation ofprotein regions in the protonmotivecytochrome b and their possible roles inredox catalysis. In: *J. Mol. Evol.* 29(2): 157-169.

Librado, P. and J. Rozas (2009). DnaSP v5: Asoftware for comprehensive analysis ofDNA polymorphism data. *Bioinformatics,* 25:1451-1452.

Luikart G., Gielly L., Excoffer L., Vigne J.D., BouvetJ., Taberlet P. (2001). Multiple maternal originsand weak phylogeographic structure in domesticgoats. *Proceedings of the National Academy ofSciences* USA 98, 5927-32.

Mannen H., Nagata Y., Tsuji S. (2001).Mitochondrial DNA reveal that domestic goat(*Capra hircus*) are genetically affected by twosubspecies of bezoar (*Capra aegagrus*).*Biochemical Genetics,* 39, 145-54.

Mason I.L. (1984). Evolution of DomesticatedAnimals. Longman, London.Naderi S., Rezaei H.R., Taberlet P., Zundel S., RafatS.A., Naghash H.R., el-Barody M.A., ErtugrulO., Pompanon F., Econogene C. (2007). Largescalemitochondrial DNA analysis of thedomestic goat reveals six haplogroups with highdiversity. PLoS ONE 2, e1012.

Parma P., Feligini M., Greppi G., Enne G. (2003). Thecomplete nucleotide sequence of goat (*Capra hircus*) mitochondrial genome. *DNA Seq.,* 14,199-203.

Saitou N and Nei M. (1987). The neighbor-joiningmethod: A new method for reconstructingphylogenetic trees. *Molecular Biology andEvolution,* 4: 406-425.

Tamura K, J. Dudley, M. Nei and Kumar S.(2007). MEGA4: Molecular EvolutionaryGenetics Analysis (MEGA) softwareversion 4.0. *Molecular Biology and Evolution,*24: 1596-1599.

Thompson J.D., Gibson T.J., Plewniak F., JeanmouginF., Higgins D.G. (1997). The ClustalX windows interface:flexible strategies for multiplesequence alignment aided by quality analysistools. *Nucleic Acids Res.,* 24, 4876-4882.

Hoque M.R., Choi N.R., Sultana H., Kang B.S., Heo K.N., Hong S.K., Jo C. and Lee J.H. (2013). Phylogenetic analysis of a privately-owned Korean Native chicken population using mtDNA D-loop variations. *Asian-Australas J. Anim. Sci*. 26, 157-162.

Ilie D.E., Cean A., Cziszter L.T., Gavojdian D., Ivan A. and Kusza S. (2015). Microsatellite and mitochondrial DNA study of native eastern European cattle populations: the case of the Romanian Grey. *PLoS One*. 10, 1-18.

Joshi M.B., Rout P.K., Mandal A.K., Tyler-Smith C., Singh L. and Thangaraj K. (2004). Phylogeography and origin of Indian domestic goats. *Mol. Biol. Evol*. 21, 454-462