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Mitochondrial diversity and phylogenetic structure of native Iranian goat population compared with 4 important livestock species

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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 important livestock species obtained from Gen Bank. In The phylogenic tree, Mahabadi native goat was clustered with such as sheep, cattle, camel and swamp buffalo breeds. This is possible because of the conserved area is ND6 in goats.

Keywords: Genetic diversity, marghoz goat, mtDNA, phylogenetic and livestock species

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) [8, 9, 10]. Mitochondrial DNA is very useful for its multiple presences in cells. The most of animal mtDNA is coding 37 genes (Avise, 1994) [3]. 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) [6, 5]. 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) [4]. 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: Gen Bank AF533441) was deposited in 2003 (Parma *et al*. 2003) [11]. 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 [2]. 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) [8, 2] 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 (Gen Bank 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 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 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 *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) [7]. The Neighbor-joining (NJ) tree (Saitou and Nei, 1987) [12] 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 Bio Edit software in 639 pair bases. As presented in Fig. 3, the Composition procedure of Bio Edit 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).

Table 1: situation and number of frequent sequence ND6 gene analyzed

Situation		
Haplotyp	**Frequent**	**389**
1	18	A
2	11	G

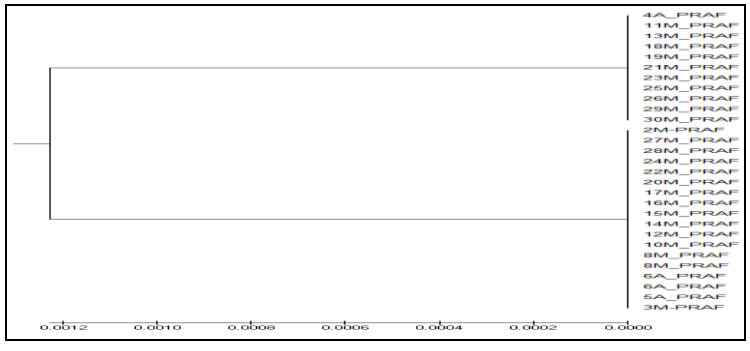

Fig 1: Phylogenetic tree within population Mahabadi breed based on ND6 gene


10 20 30 40 50 60 70 80 90 100 110 120 130 140 150
2M-PRAF CCACAACACCAATTAACAAAGATCAACCAGTAACAATAACTAATCAAGTACCATAACTGTATAAAGCAGCAATCCCTATGGCCTCCTCACTGAAGAACCCAGAATCCCCTGTATCATAAATAACCCAATCCCCCATACCATTAAACTCAAACACA
3M-PRAF
4A PRAF
5A PRAF
6A PRAF
6A PRAF
8M PRAF
8M PRAF
10M PRAF
11M PRAF
12M PRAF
13M PRAF
14M PRAF
15M PRAF
16M PRAF
17M PRAF
18M PRAF
19M PRAF
20M PRAF
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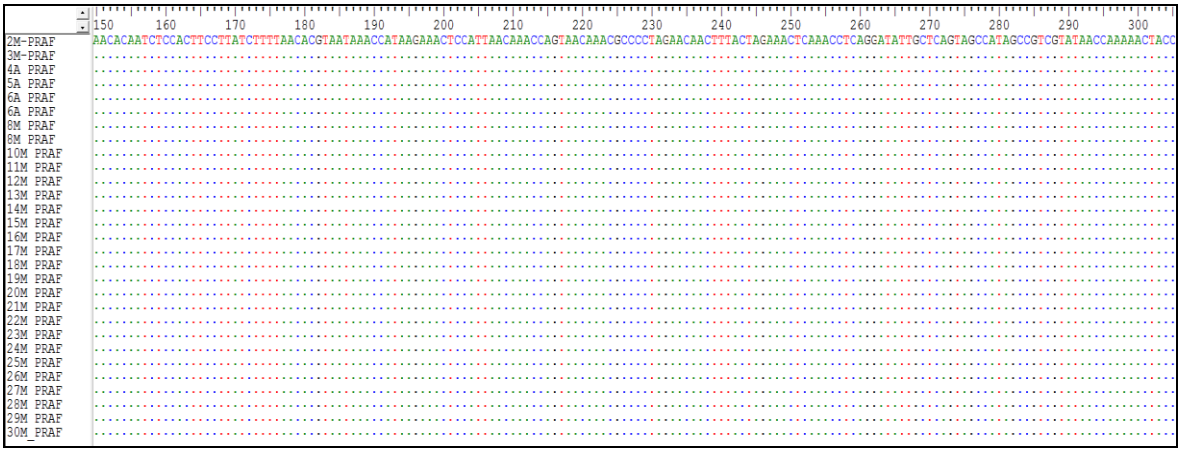
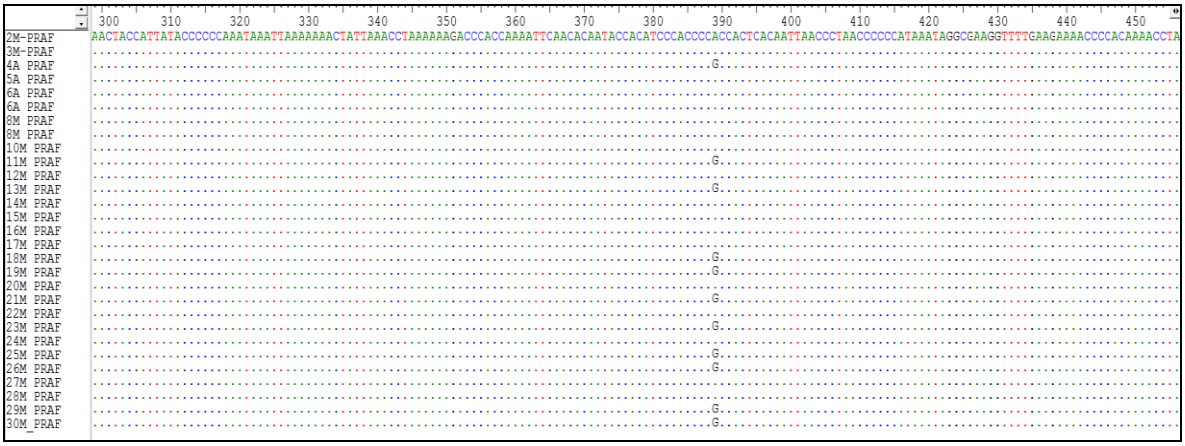
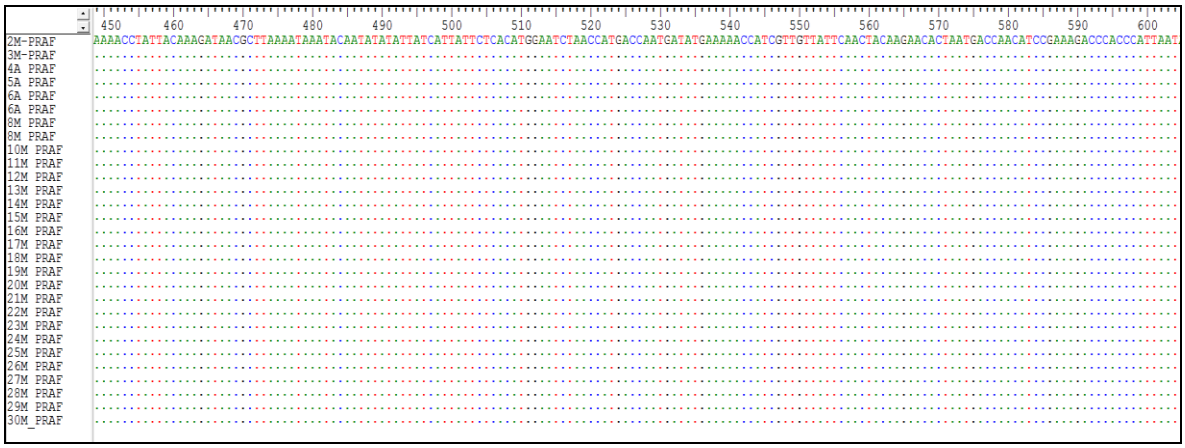
26M PRAF
27M PRAF
28M PRAF
29M PRAF
30M PRAF


150 160 170 180 190 200 210 220 230 240 250 260 270 280 290 300
2M-PRAF AACACAATCTCCACTTCCTTATCTTTTAACACGTAATAAACCATAAGAAACTCCATTAACAAACCAGTAACAAACGCCCCTAGAACAACTTTACTAGAAACTCAAACCTCAGCATATTGCTCAGTAGCCATAGCCGTCGTATAACCAAAAACTACC
3M-PRAF
4A PRAF
5A PRAF
6A PRAF
6A PRAF
8M PRAF
8M PRAF
10M PRAF
11M PRAF
12M PRAF
13M PRAF
14M PRAF
15M PRAF
16M PRAF
17M PRAF
18M PRAF
19M PRAF
20M PRAF
21M PRAF
22M PRAF
23M PRAF
24M PRAF
25M PRAF
26M PRAF
27M PRAF
28M PRAF
29M PRAF
30M_PRAF


300 310 320 330 340 350 360 370 380 390 400 410 420 430 440 450
2M-PRAF AACTACCATTATACCCCCCAAATAAATTAAAAAAACTATTAAACCTAAAAAAGACCCACCAAAATTCAACACAATACCACATCCCACCCCACCACTCACAATTAACCCTAACCCCCCCATAAATAGGCGAAGGTTTTGAAGAAAACCCCACAAAACCTA
3M-PRAF .....G.....
4A PRAF .....G.....
5A PRAF .....G.....
6A PRAF .....G.....
6A PRAF .....G.....
8M PRAF .....G.....
8M PRAF .....G.....
10M PRAF .....G.....
11M PRAF .....G.....
12M PRAF .....G.....
13M PRAF .....G.....
14M PRAF .....G.....
15M PRAF .....G.....
16M PRAF .....G.....
17M PRAF .....G.....
18M PRAF .....G.....
19M PRAF .....G.....
20M PRAF .....G.....
21M PRAF .....G.....
22M PRAF .....G.....
23M PRAF .....G.....
24M PRAF .....G.....
25M PRAF .....G.....
26M PRAF .....G.....
27M PRAF .....G.....
28M PRAF .....G.....
29M PRAF .....G.....
30M_PRAF .....G.....


450 460 470 480 490 500 510 520 530 540 550 560 570 580 590 600
2M-PRAF AAAACCTATTACAAAGATAACGCTTAAAATAAATACAATATATATTATCATTATTCTCACATGGAATCTAACCATGACCAATGATATGAAAAACCATCGTTGTTATTCAACTACAAGAACACTAATCGACCAACATCCGAAAGACCCACCCATTAAT
3M-PRAF
4A PRAF
5A PRAF
6A PRAF
6A PRAF
8M PRAF
8M PRAF
10M PRAF
11M PRAF
12M PRAF
13M PRAF
14M PRAF
15M PRAF
16M PRAF
17M PRAF
18M PRAF
19M PRAF
20M PRAF
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27M PRAF
28M PRAF
29M PRAF
30M_PRAF

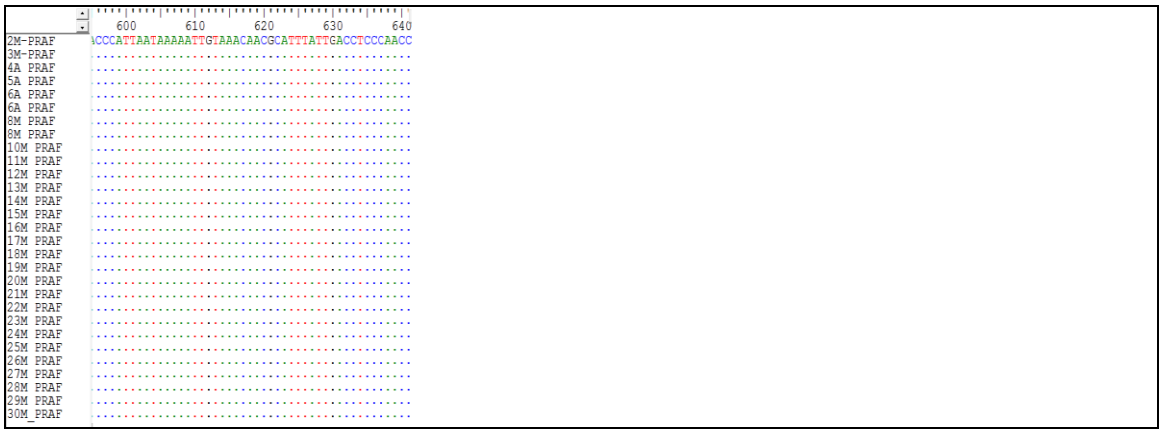

Fig 2. Sequence variation of mtDNA ND6 gene of 30 individuals of the Mahabadi goat.

```
  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

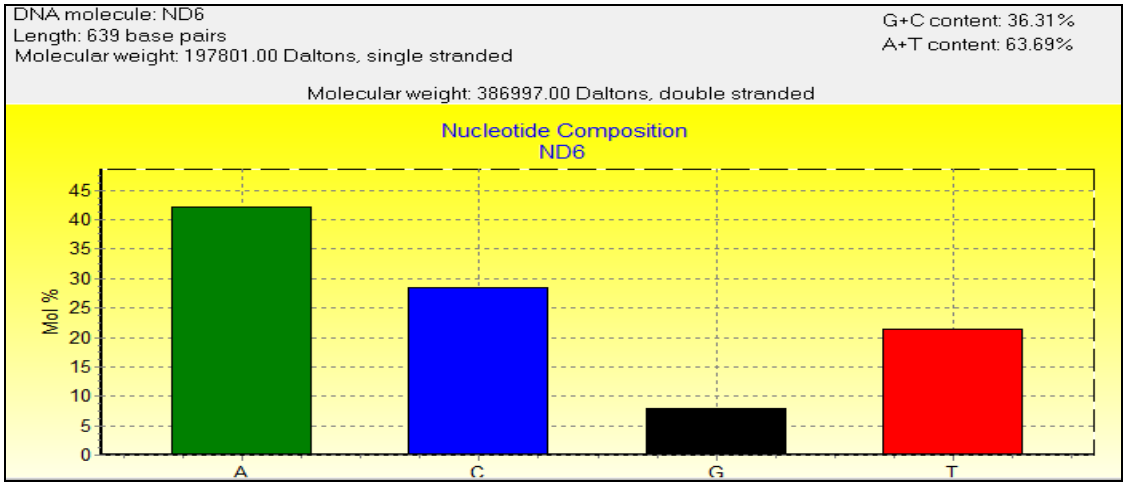

Fig 4: Nucleotide Composition Percentage of Consensus Sequence in Mahabadi goat

The complete water buffalo (Bubalus bubalis) mtDNA in is 16.355 bp in length with Gen Bank: Gen Bank: AY702618.1 and the full length coding sequences of the ND6 genes based on NCBI site was determined and Sequences spanned 525 bp which is located from 13551 to 14078 in length whole mtDNA genome. The complete sheep (Ovis aries) mtDNA is 16166 bp in length with Gen Bank: AF010406.1 and the full length coding sequences of the ND6 genes based on NCBI site was determined and Sequences spanned 527 bp which is located from 13558 to 14085 in length whole mtDNA genome. The complete camle (Camelidae; Camelus) mtDNA is 16643bp in length with Gen Bank: EU159113.1 and the full length coding sequences of the ND6 genes based on NCBI site was determined and Sequences spanned 577 bp which is located from 13504 to 14081 in length whole mtDNA genome. The complete cattale (Bos taurus) mtDNA is 16338 bp in length with Gen Bank: AY526085.1 and the full length coding sequences of the ND6 genes based on NCBI site was determined and Sequences spanned 527 bp which is located from 13913 to 14440 in length whole mtDNA genome. The complete horse (Equus caballus) mtDNA is 16660 bp in length with Gen Bank: X79547.1 and the full length coding sequences of the ND6 genes based on NCBI site was determined and Sequences spanned 566 bp which is located from 13578 to 14144 in length whole mtDNA genome. The complete pig (Sus scrofa) mtDNA is 16613 bp in length with Gen Bank : KP294522.1 and the full length coding sequences of the ND6 genes based on NCBI site was determined and Sequences spanned 527 bp which is located from 14739 to 15266 in length whole mtDNA genome.

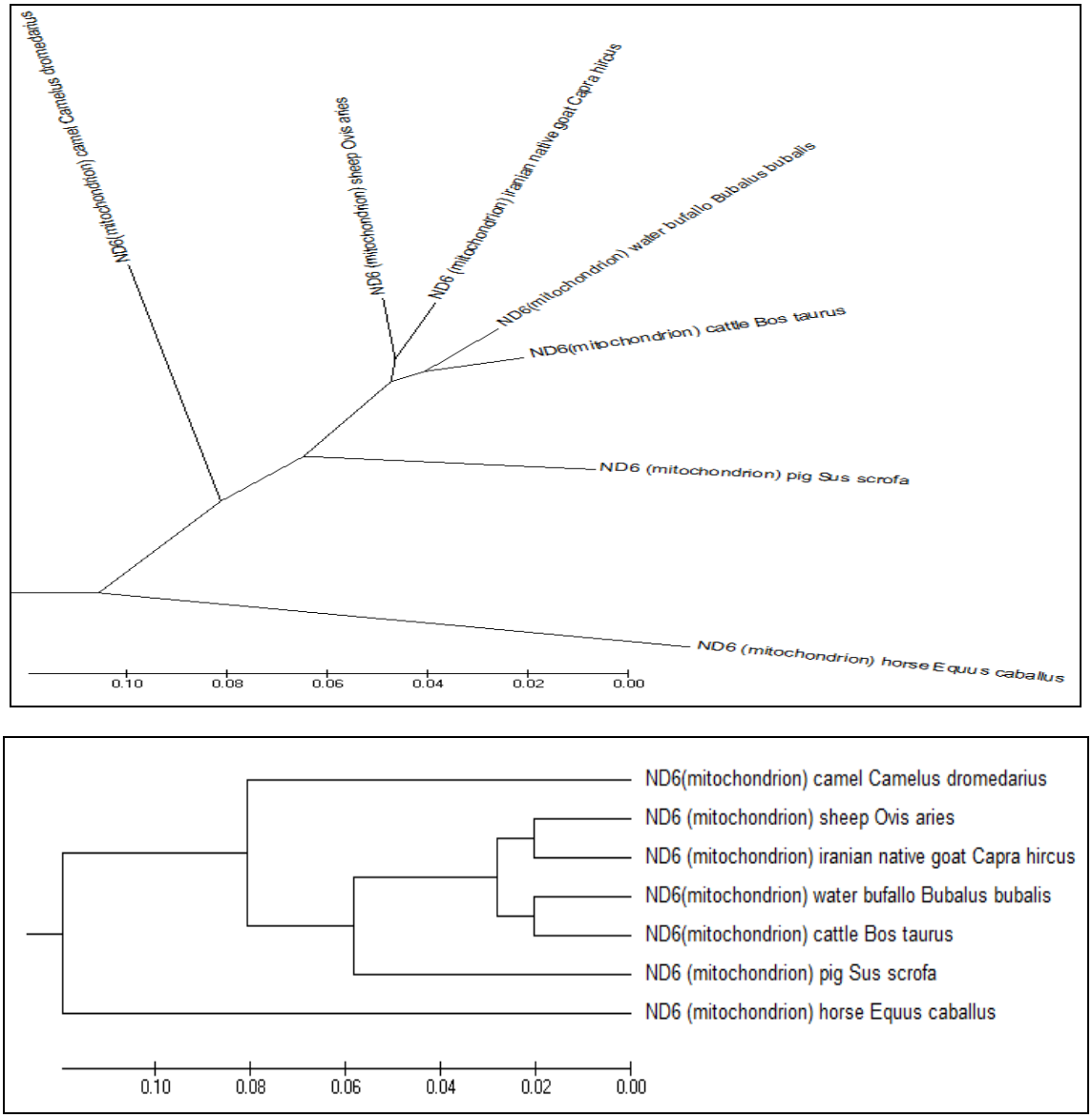

Fig 5: Phylogenetic relationship among Iranian goat and Gen Bank accession number of ND6 gene from livestock breeds.

Iranian native Mahabadi goats are compered of 7 different livestock by drawing phylogenetic that is showed in bellow (Table 4).

Table 4: Phylogenetic relationship among 8 Gen Bank accession number of ND6 gene from livestock breeds.

	1	2	3	4	5	6	7
1. ND6(mitochondrion) cattle Bos taurus							
2. ND6 (mitochondrion) horse Equus caballus	0.2303						
3. ND6(mitochondrion) water bufallo Bubalus bubalis	0.0408	0.2375					
4. ND6(mitochondrion) camel Camelus dromedarius	0.1676	0.2669	0.1608				
5. ND6 (mitochondrion) iranian native goat Capra hircus	0.0528	0.2375	0.0468	0.1744			
6. ND6 (mitochondrion) sheep Ovis aries	0.0649	0.2160	0.0588	0.1409	0.0408		
7. ND6 (mitochondrion) pig Sus scrofa	0.1214	0.2448	0.1149	0.1608	0.1149	0.1149	

Distribution of the samples between the groups was made in accordance with the cluster obtained. Apparently, the longest distance separated the horse (Equus caballus) from the others and iranain native Mahabadi goat has shortest genetic distance with sheep (Ovis Aries) This is possible because of mtDNA of specific livestock, geographical distance and distributed.

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