IJAS Iranian Journal of Applied Animal Science

Mitochondrial Diversity and Phylogenetic Structure of Marghoz Goat Population

Research Article

H.R. Seyedabadi[1*], K. Pahlevan Afshari[2] and M. Abdolmaleki[2]

[1] Department of Animal Biotechnology, Animal Science Research Institute, Karaj, Iran
[2] Department of Animal Science, Abhar Branch, Islamic Azad University, Abhar, Iran

Received on: 27 Nov 2015
Revised on: 11 Jan 2016
Accepted on: 31 Jan 2016
Online Published on: Sep 2016

*Correspondence E-mail: h_seyedabadi@asri.ir

Online version is available on: www.ijas.ir

ABSTRACT

The genetic diversity and phylogenetic structure was analyzed in Marghoz goat population by mitochondrial DNA sequences. Phylogenetic analysis was carried out using hyper variable region 1 (968 bp) obtained form 40 animals. Marghoz goat proved to be extremely diverse (average haplotype diversity of 0.999) and the nucleotide diversity values 0.022. A total of 40 Marghoz goats were grouped into six haplotypes and the large majority of haplotypes were present in 15 animals. All Marghoz haplotypes were classified into C haplogroup and revealed remarkably high genetic distances within the population when compared with other Asian goat populations, indicating high genetic variation in the Marghoz goat. These results indicate high-divergence status of the Marghoz goat and will influence breeding and conservation strategies adopted for this breed.

KEY WORDS genetic diversity, Marghoz goat, mtDNA, phylogenetic.

INTRODUCTION

The mitochondrial DNA (mtDNA) polymorphism, especially the displacement loop (D-loop) region, has been largely applied to understand phylogenetical relationships in many animal species, including cattle (Ilie *et al*. 2015), pig (Li *et al*. 2014), sheep (Agaviezor *et al*. 2012), chicken (Hoque *et al*. 2013), horse (Zernekova *et al*. 2013) and goat (Hoda *et al*. 2014; Pakpahan *et al*. 2015; Çinar Kul and Ertugrul, 2011). Previous studies on domestic goats identified at least four major mtDNA lineages (Joshi *et al*. 2004; Luikart *et al*. 2001; Sultana *et al*. 2003). Lineage A is the most diverse and widely distributed across all continents. Lineage B is confined to eastern and southern Asia, including Mongolia, Laos, Malaysia, Pakistan and India. Lineage C is present in low frequencies in Mongolia, Switzerland, Slovenia, Pakistan, India and Iran. Finally, lineage D is rare and only observed in Pakistani and Indian local goats (Naderi *et al*. 2007). The time since divergence among these four lineages (more than 200000 years ago) far predated the time of domestication around 10000 years ago (Joshi *et al*. 2004; Luikart *et al*. 2001; Sultana *et al*. 2003). The control region (D-loop) is the most variable and non-coding portion of the mitochondrial genome (Wilson *et al*. 1985). This region controls the mitochondrial DNA (mtDNA) replication by regulating the activities of various enzymes and proteins that are coded by the nuclear genes (Ghivizzani *et al*. 1993; Nass, 1995). The sequences of the control region vary greatly in different mammalian species, however, preservation of several conserved regions indicate fundamental harmony in its function (Saccone *et al*. 1991). It contains the origin of heavy strand and the promoters for the light and heavy strand replication (Anderson *et al*. 1982). Because of their rapid evolution (Brown *et al*. 1979), the control region sequences are valuable for investigating the genetic diversity and evolutionary relationships among

species (Wilson *et al*. 1985). The extent and pattern of genetic variability in livestock species will contribute to the conservation of livestock genetic resources. On the basis of the previously mentioned archaeological studies and biogeography, it is likely that the molecular studies of diversity in Iranian goats will yield new understanding of the origin and process of goat domestication, and will contribute to the resolution of goat phylogeny. More than 20 breed of goats have been recognized in Iran but only breeds namely Marghoz and Raeni produces attractive and expensive Mohair and Kashmir fiber. These small breeds of goat are distributed over the western and north-west of Iran near to the Turkey and Iraqi borders. Marghaz goats are fertile animals with twin kidding over 30% (Moradi *et al*. 2014). It is believed the Angora goats are originated from this breed (Moradi *et al*. 2014). Angora goats appear in one color which is white to silver, but Marghoz goats produce mohair in different natural colors which are a unique character of this breed. Keeping above in view in present study, the current phylogenetic status and genetic diversities of Marghoz goat has been investigated in order to understand the genetic basis of this breed.

MATERIALS AND METHODS

Animals

Blood samples from 40 Marghoz goats were collected. All the goats were selected according to the herd book and the phenotypic standard for this breed. They came from several farms located in different areas of Kurdistan province of Iran. Genomic DNA was extracted from fresh blood according to a standard phenol and chloroform method.

PCR amplification and sequencing

The first hyper variable segment (HVRI) of the mtDNA control region sequences was amplified and sequenced. To amplify the HVRI region of goat mtDNA, a pair of primers was designed using the known goat mtDNA sequence (GenBank Accession No. NC005044). The primers CAP-F 5′-ACTCCACAAGCCTACAGA-3′ and CAP-R 5′-GGAAAGGTGGAGCGGATG-3′ were used to amplify a 968 bp DNA fragment. PCR amplifications were conducted in a 30 uL volume containing 5 uL of 10x reaction buffer, 1.5 m*M* $MgCl_2$, 0.2 mM dNTPs, 0.2 uM each primer, 1.5 U Taq DNA polymerase (TaKaRa Biosystems) and approximately 150 ng genomic DNA. The PCR mixture underwent 3 min at 95 ˚C, 35 cycles 50s at 94 ˚C, 1min at 58 ˚C and 1 min at 72 ˚C and 5 min at 72 ˚C. PCR products were purified by using Watson PCR Purification Kit (Watson Bio-Technologies, 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 sequences obtained were compared by alignment to the international Gene Bank data-base in 25 Chinese (DQ089106–DQ089131), 9 Japan (AB110552-AB110555, AB162196-AB162201) and 14 Pakistan (AB110558–AB110572) using the BioEdit software program version 7.2 (Hall, 1999). 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 distance computation and phylogenetic tree construction are incorporated in the MEGA package version. 5.1 (Kumar *et al*. 2004). Haplotype diversity (h_d), nucleotide diversity (p) and average number of nucleotide were calculated using DnaSP software version 4.2 (Rozas *et al*. 2003).

RESULTS AND DISCUSSION

mtDNA variation in Marghoz goat

In the present study, we analyzed the mtDNA control region sequences of 40 Marghoz goats to further elucidate its diversity. There were no insertions/deletions in 40 sequences of HVRI of the control regions. The HVRI sequences were highly polymorphic. Our 40 sequences gave 6 different haplotypes with 20 variable sites defined. The largest haplotype group consisted of 15 individuals, two haplotype groups included more than 5 individuals, and three haplotypes included 2 individuals (Table 1).

The average base composition of the control region sequences was as follows; A: 29.6%, C: 25%, G: 16.2% and T: 29.1% (Figure 1). This indicates that the caprine mtDNA control region has high A/T contents as well as in all artiodactyls (cattle, sheep, goat and pig) and other mammalian species such as mouse (Ameur *et al*. 2011), donkey (Xu *et al*. 1996) and whale (Arnason *et al*. 1991). However, in the case of primates (Foran *et al*. 1988), seal (Arnason *et al*. 1993) and rabbit (Mignotte *et al*. 1990), the control region is rich in A/C contents. This is attributed to the species-specific variations that exist in the control regions among different mammalian species.

Genetic distances by comparing with other Asian goat populations

Using previously published Asian goat mtDNA sequence information, mean sequence divergence values between four Asian goat populations (Iran, Pakistan, China and Japan) and within each population were calculated (Table 2). The highest sequence divergence value, 0.014, was observed between China and Pakistani goat populations. When compared mean sequence divergence values within population in Iranian goats with those of china, Japanese and Pakistani domestic goats, the level of diversity within Iran (nucleotide diversity, 0.022) is higher than that other

populations with the haplotype diversity values 0.999 in the Marghoz goat.

The result highly supports the evidence of the high variability in the breed.

Table 1 Mitochondrial hyper variable region 1 (HVR1) sequence variations among Marghoz goat

Haplotypes	Base position[1]																			
	85	108	114	120	249	265	304	330	348	379	384	408	417	434	439	453	472	473	479	584
Type 1 (15)[2]	T	G	C	T	A	T	C	C	A	A	C	T	T	T	A	T	C	G	T	C
Type 2 (10)	C	-	-	C	G	-	A	-	T	G	T	C	C	C	G	-	T	-	C	T
Type 3 (9)	C	-	-	C	-	C	-	T	-	-	T	C	-	-	G	C	T	A	-	T
Type 4 (2)	C	-	T	C	G	-	-	T	-	-	T	C	-	C	G	-	T	A	C	-
Type 5(2)	C	-	-	C	-	C	-	T	-	-	T	-	-	-	G	C	T	A	-	T
Type 6 (2)	C	A	-	C	-	-	-	T	-	-	T	C	-	-	G	-	T	A	-	T

[1] Numbers indicate nucleotide base position in caprine mtDNA HVR1 region and hyphen represents the identical nucleotide with the type 1 sequence.
[2] Numbers in parentheses indicate number of animal observed in Marghoz goat.

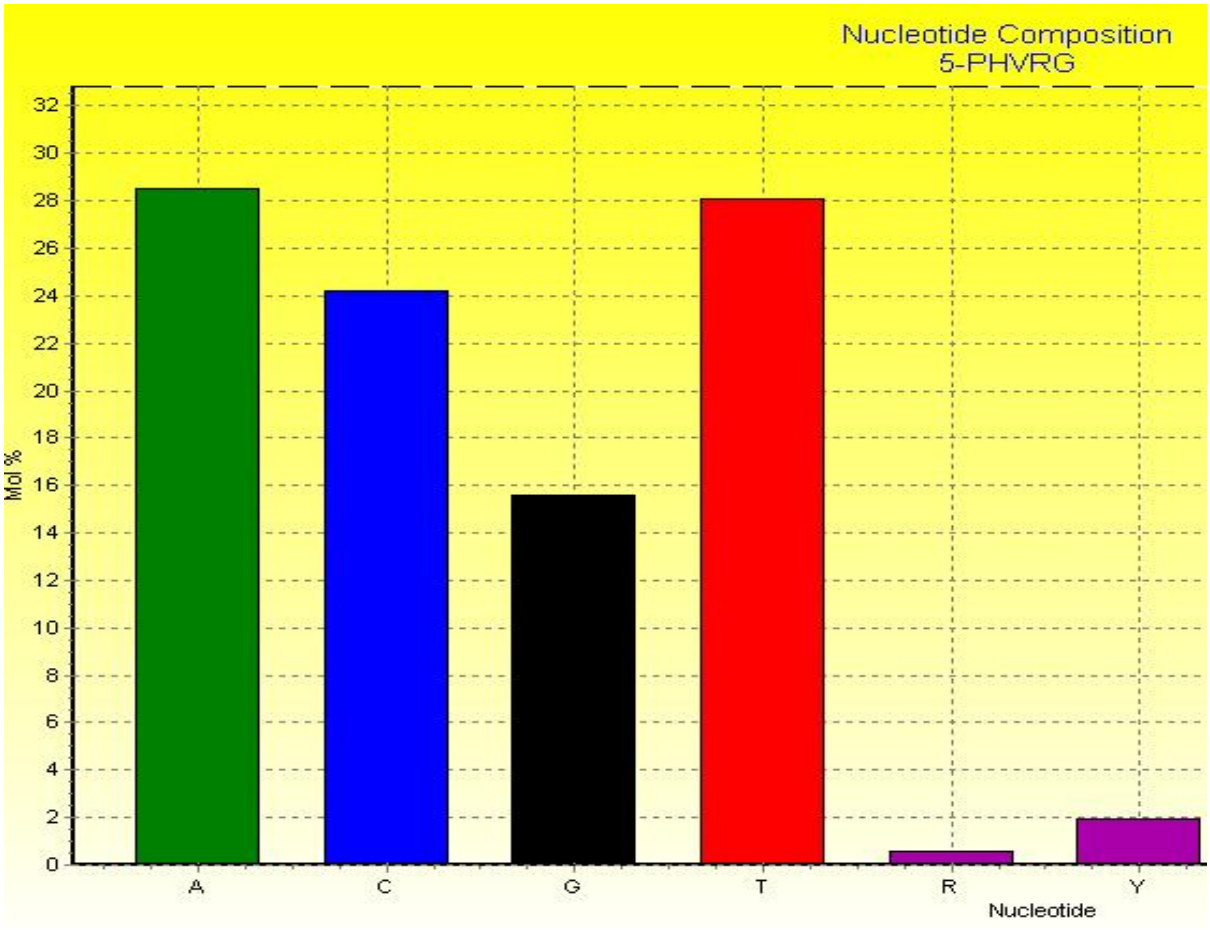

Figure 1 Average base composition of the HVR1 region sequences in Marghoz goat

Table 2 Sequence divergence of Asian goat populations[1]

Countries	Iran	Pakistan	Japan	China
Iran	0.022	0.013	0.001	0.013
Pakistan	*0.206*	0.016	0.008	0.014
Japan	*0.203*	*0.013*	0.009	0.008
China	*0.99*	*0.96*	*0.95*	0.014

[1] Above the diagonal and on the diagonal are the average sequence divergences between populations and within populations, respectively. Below the diagonal (italics) are genetic distances between populations calculated by Tamura-Nei model in MEGA software.

When comparing with Iranian population, high divergence value of 0.013 was observed with China and Pakistan populations. This supported that Iranian population was related more closely to Japanese goats than China and Pakistan. The estimated genetic distances between populations also indicated that the Iranian and Chinese goat populations are far away (0.99) and Iranian goats are closely related with Japanese (0.203) and Pakistani (0.206) goats.

Phylogenetic analysis for the Asian goats

Phylogenetic tree construction of the Marghoz goat was performed with hyper variable region 1 (HVR1) in the caprine mtDNA of present study and published sequences from Pakistani, Chinese and Japanese goat populations (Figure 2). The unrooted neighbor-joining tree indicates three major goat lineages (A to C). Phylogenetic tree also indicates that the largest lineage A (22 haplotypes) includes Chinese population and lineage B (22 haplotypes) includes Pakistani and Japanese populations. Lineage C (6 haplotype) includes only Iranian goats.

The phylogenetic tree indicated that the Pakistani and Chinese goats were wide-spread all over the mt lineage B and A, respectively. However, Iranian goats located in a separate lineage, which indicates the high genetic variation of the Iranian native goats. High genetic variability of the Iranian goats was observed, compared with other Asian goat populations. Luikart *et al*. (2001) examined 406 individuals from 88 breeds partially sequenced the mtDNA hyper variable region (HVR) in distributed across the world and defined lineages A, B and C of which lineages B detected in India, Malaysia, Mongolia and Pakistan. Liu *et al*. (2006) examined 50 mtDNA HVR (481 bp) sequences of lineage B from six Asian goats, which were classified into two subclasses and considered that lineage B. Naderi *et al*. (2007) investigated 2430 goats from different countries and shows six very different groups according to mitochondrial haplogroup are referred to as A, B, C, D, F and G. The results of the research show that the haplogroup A is at most at 53%. A haplogroup spread almost all over the world and is the first haplogroup. Haplogroup B is found throughout most of Asia and a small portion coming from Sub-Saharan Africa and goat Europe from Greece. Goats of haplogroup C is present in central Asian, Middle east, Iran and haplogroup D is present throughout Asia and northern Europe. Group F goat came from Sicily and the group G is present in the Middle east and north Africa. The results were in general agreement with the pattern described in previous study (Naderi *et al*. 2007). However more detailed molecular studies are required in near future. It is urgent to take measures that promote a sustainable management of these genetic resources (Taberlet *et al*. 2008). We also estimated haplotype diversity for the three haplogroups (Table 3).

Among them, haplogroup C (Iranian goats) had the highest haplotype diversity and haplogroup B (Pakistani and Japanese goat breeds) had the lowest value.

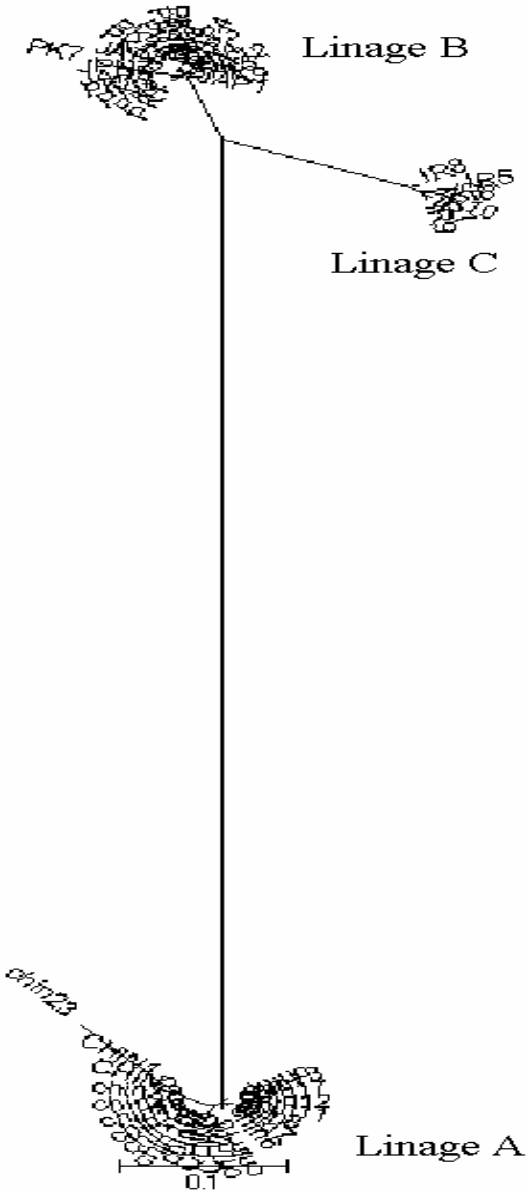

Figure 2 Unrooted neighbor-joining tree constructed from Pakistan, China, Japanese and Iranian goat populations

In Marghoz goat the haplotype diversity was 0.999. This haplotype diversity is in accordance with the previously described values in Albanian and Turkish goat breeds (Hoda *et al*. 2014; Çinar Kul and Ertugrul, 2011). The level of haplotype diversity of 18 goat breeds (0.7121-0.9804) by Chen *et al*. (2005) is lower than that of Marghoz goat (0.999) in our study. Haplotype diversity of mtDNA is important indices for assessing population polymorphism and genetic differentiation. High values of haplotype indicate high polymorphism of the population (Liu *et al*. 2006).

Table 3 Genetic diversity of goat mtDNA haplogroups

Haplogroup	Number	Number of haplotypes	Haplotype diversity (±SD)
Haplogroup A	96	22	0.949±0.028
Haplogroup B	85	22	0.879±0.012
Haplogroup C	40	6	0.999±0.001

The high mtDNA diversity was probably because of the high variability level of the mitochondrial genome (Cozzi *et al*. 2004).

CONCLUSION

We analyzed hyper variable segment (HVRI) of the mtDNA control region to test Iranian goat phylogeny as well as to discern the genetic diversity of Marghoz goat breed. All the Marghoz goats, representing 6 haplotypes, were classified into lineage C. Our results were in general agreement with the pattern described in previous study (Naderi *et al*. 2007). However, additional molecular studies are required in the near future.

ACKNOWLEDGEMENT

The authors acknowledged the three reviewers for constructive comments on the manuscript. We gratefully acknowledge all farmers who took part in the present study, giving access to the animals.

REFERENCES

Agaviezor B.O., Adefenwa M.A., Peters S.O., Yakubu A., Adebambo O.A., Ozoje M.O., Ikeobi C.O.N., Ilori B.M., Wheto M., Ajayi O.O., Amusan S.A., Okpeku M., De Donato M. and Imumorin I.G. (2012). Genetic diversity analysis of the mitochondrial D-loop of Nigerian indigenous sheep. *Anim. Gen. Resour*. **50,** 13-20.

Arnason U., Gullberg A., Johnsson E. and Ledje C. (1993). The nucleotide sequence of the mitochondrial DNA molecule of the grey seal, Halichoerus grypus and a comparison with the mitochondrial sequences of other true seals. *J. Mol. Evol*. **37,** 323-330.

Arnason U., Gullberg A. and Widegren B. (1991). The complete nucleotide sequence of the mitochondrial DNA of the ¢n whale Balaenoptera physalus. *J. Mol. Evol*. **33,** 556-568.

Ameur A., Stewart J.B., Freyer C., Hagstro E., Ingman M., Ran N., Larsson G. and Gyllensten U. (2011). Ultra-deep sequencing of mouse mitochondrial DNA: mutational patterns and their origins. *PLoS Gent*. **7,** 1-15.

Anderson S., DeBruijin M.H.L., Colson A.R., Eperon I.C., Sanger F. and Young I.G. (1982). Complete sequence of bovine mitochondrial DNA conserved features of the mammalian mitochondrial genome. *J. Mol. Biol*. **156,** 683-692.

Brown W.M., George M.J. and Wilson AC. (1979). Rapid evolution of animal mitochondrial DNA. *Proc. Nat. Acad. Sci.* USA. **76,** 1967-1971.

Chen S.Y., Su Y.H., Wu S.F., Sha T. and Zhang Y.P. (2005). Mitochondrial diversity and phylogeographic structure of Chinese domestic goats. *Mol. Phylogenet. Evol*. **73,** 804-814.

Çinar Kul B. and Ertugrul O. (2011). mtDNA diversity and phylogeography of some Turkish native goat breeds. *Ankara Üniv. Vet. Fak. Derg*. **58,** 129-134.

Cozzi M.C., Strillacci M.G., Valianti P., Bighignoli B., Cancedda M. and Zanotti M. (2004). Mitochondrial D-loop sequence variation among Italian horse breeds. *Genet. Sel. Evol*. **36,** 663-672.

Ghivizzani S.C., Madsen C.S. and Hauswirth W.M. (1993). In organello footprinting: analysis of protein binding at regulatory regions in bovine mitochondrial DNA. *J. Biol. Chem*. **268,** 8675-8682.

Hall T.A. (1999). Bio edit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids. Symp. Ser*. **41,** 95-98.

Hoda A., Bicoku Y. and Dobi P. (2014). Genetic diversity of Albanian goat breeds revealed by mtDNA sequence variation. *Biotechnol. Biotec. Equip*. **28,** 77-81.

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.

Kumar S., Tamura K., Jakobsen I.B. and Nei M. (2004). MEGA3.1: Molecular Evolutionary Genetics Analysis Software. Arizona State University Press, Tempe, USA.

Li K.Y., Li K.T., Cheng C.C., Chen C.H., Hung C.Y. and Ju Y.T. (2014). A genetic analysis of Taoyuan pig and its phylogenetic relationship to Eurasian pig breeds. *Asian-Australas J. Anim. Sci*. **28,** 457-466.

Liu Z.G., Lei C.Z., Luo J., Ding C., Chen G.H., Chang H., Wang K.H., Liu X.X., Zhang X.Y., Xiao X.J. and Wu S.L. (2006). Genetic variability of mtDNA sequences in Chinese native chicken breeds. *Asian-Australas J. Anim. Sci*. **17,** 903-909.

Luikart G., Gielly L., Excoffier L., Vigne J.D., Bouvet J. and Taberlet P. (2001). Multiple maternal origins and weak phylogeographic structure in domestic goats. *Proc. Nat. Acad. Sci*. USA. **98,** 5927-5932.

Mignotte F., Gueride M., Champagne A.M. and Mounolou J.C. (1990). Direct repeats in the non-coding region of mitochondrial DNA. Involvement in the generation of intra and inter-individual heterogeneity. *European J. Biochem*. **194,** 561-571.

Moradi M.H., Rostamzadeh J., Rashidi A., Vahabi K. and Farahmand H. (2014). Analysis of genetic diversity in Iranian moh-

air goat and its color types using Inter Simple Sequence Repeat (ISSR) markers. *Agric. Commod*. **2,** 55-62.

Naderi S., Rezaei H.R., Taberlet P., Zundel S., Rafat S.A., Naghash H.R., Elbarody M.A.A., Ertugrul O. and Pompanon F. (2007). Large-scale mitochondrial DNA analysis of the domestic goat reveals six haplogroups with high diversity. *PloS One*. **2,** 1-10.

Nass M.M. (1995). Precise sequence assignment of replication origin in the control region of chick mitochondrial DNA relative to 5ó and 3ó ends, secondary structure, DNA synthesis and protein binding. *Curr. Genet*. **28,** 401-409.

Pakpahan S., Tunas Artama W., Widayanti R. and Suparta G. (2015). Genetic variations and the origin of native Indonesian goat breeds based on mtDNA D-Loop sequences. *Asia J. Anim. Sci*. **9,** 341-350.

Rozas J., Sachez-Delbarrio J.C., Messeguer X. and Rozas R. (2003). DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics*. **19,** 2496-2497.

Saccone C., Pesole G. and Sbisa E. (1991). The main regulatory region of mammalian mitochondrial DNA: structure function model and evolutionary pattern. *J. Mol. Evol*. **33,** 83-91.

Saitou N. and Nei M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol*. **4,** 406-425.

Sultana S., Mannen H. and Tsuji S. (2003). Mitochondrial DNA diversity of Pakistani goats. *Anim. Genet*. **34,** 417-421.

Taberlet P., Valentini A., Rezaei H.R., Naderi S., Pompanon F., Negrini R. and Ajmone-Marsan P. (2008). Are cattle, sheep, and goats endangered species. *Mol. Ecol*. **17,** 275-284.

Tamura K. and Nei M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol. Biol. Evol*. **10,** 512-526.

Wilson A.C., Cann R.L., Carr S.M., George M., Gyllensten U.B., Helm-Bychowski K.M., Higuchi R.J., Palumbi S.R., PragerE.M., Sage R.D. and Stoneking M. (1985). Mitochondrial DNA and two perspectives on evolutionary genetics. *Biol. J. Linn. Soc*. **26,** 375-400.

Xu X., Gullberg A. and Arnason U. (1996). The complete mitochondrial DNA (mtDNA) of the donkey and mtDNA comparisons among four closely related mammalian species pairs. *J. Mol. Evol*. **43,** 438-446.

Zernekova C., Kott T. and Majzlik I. (2013). Mitochondrial D-loop sequence variation among Hucul horse. *Czech J. Anim. Sci*. **58,** 437-442.