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Research Article

MITOCHONDRIAL DNA GENOME SEQUENSING OF 12 S rRNA AND tRNA REGIONS IN KHORASAN'S NATIVE CHICKENS

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ABSTRACT
According to the study done by FAO up to 30% of Global mammalian and avian Livestock breeds are faced currently at risk of being lost and could not be replaced (Soysal *et al.,* 2003). In order to perform breeding programs and improve production of the native chickens, the preserving genetic diversity in different areas of Iran is important because of their few populations. Mitochondrial sequencing of 12S rRNA due to highly conserved is considered as most functional approach to determine the phylogeny relation between close populations. Aim of this project was to evaluation the phylogeny and genetic nucleotide sequences of 12 S rRNA and tRNA regions in mitochondrial genome of khorasan native genome chickens. Blood samples were collected from randomly 6 khorasan native chickens and after DNA extraction, the 12 S rRNA and tRNA regions with the length of 852 bp were amplified using specific primers. Sequencing was done according to Sanger method and based on automate system. The phylogeny tree and matrix of the genetic distances between khorasan native chickens and other breeds for 12 S rRNA and tRNA of mitochondrial genome were drawn using the same sequences of mitochondrial genome in other available breeds in NCBI database. Results showed no haplotype difference between the studied samples sequences. The results of phylogeny test revealed that lowest genetic distance was observed between khorasan native chicken and other Asian chickens such as Huang Lang, Lv'erwu for the 12 S rRNA and tRNA genes. So we could conclude that there is close relationship between khorasan native chickens and other Asian chickens.

Keywords: *Khorasan Native Chicken, 12 S rRNA, tRNA, DNA Mitochondrial, Phylogeny Tree*

INTRODUCTION
In recent years the use of genomics for conservation genetic or preserving of the species has attracted an increasing attention. Knowing relationships in the conservation genetic to minimize the rate of inbreeding between individuals and prevent the loss of genetic diversity in breeding programs is very important. It is well acknowledged that the reduction in genetic variation reduces the ability of a population to adapt to environmental changes and therefore decreases its long term survival. The fate of small populations often suffers from genetic changes. In order to perform breeding programs and improve production of the native chickens the preserving genetic diversity in different areas of Iran is important because of their few population. Mating of relatives increase inbreeding, the loss of genetic variation is involved, as well as the survival and reduces reproduction and increases the risk of extinction (Arif and Khan, 2009). There are several advantages in using the indigenous poultry as a genetic resource in breeding programs and it appears necessary to preserve the genetic resources in order to have more accurate knowledge and learn more about them. One way is to identify these breeds using molecular techniques, the sequencing of the mitochondrial genome (mtDNA). It is a good method to determine the mitochondrial phylogenetic relationships between populations, because of the relatively high rate of evolution of mitochondrial genes compared to nuclear genome (Kyung *et al.,* 1998)
The mitochondrial genome in the identification of species and phylogenetic relationships benefits include a large number of copies per cell (transcripts) the smaller size of the genomic DNA, maternal heritability, being haploid, the absence of recombination in the presence of protected and in unprotected regions (D-Loop) for evolutionary studies related species (Bellagamba *et al.,* 2001). The mitochondrial genome nucleotide displacement rate is 5-10 times higher than in nuclear DNA possibly by reducing replication

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and high levels of oxygen radicals in electron transfer processes. The mitochondria evolve in the cytoplasm of most cells of the body and from the evolutionary viewpoint, mtDNAs are "small genomes" that co evolve at their own rate with the organism in which they are lodged. Thus, mtDNA sequences are widely used to construct phylogenetic trees (Christiane, 1998). These organelles exist in most animals by 15 to 17 kb in length and able to produce energy for the cell. A specific circular DNA is independent of nuclear DNA and the gene encoding the 37 animal species, which contains 13 genes encoding respiratory chain, 22 genes coding for tRNA and two proprietary ribosomal genes encoding 12 S rRNA and 16 S rRNA. Expression of these genes in vertebrates is essential for energy production, metabolism, cellular homeostasis and cell death (Dimauro, 2004). The 12 s rRNA gene is less variable than D-loop, because evolutionary rates in ribosomal RNA genes is low from variable D-loop region (Arif and Khan, 2009). The 12 S rRNA gene from mitochondrial genome used in the detection of genetic variations due to greatly protected. Lei *et al.,* (2003) studied mitochondrial ribosomal genes of mountain goats to investigate the extent of genetic diversity.

This study analyzed the genetic and phylogenetic regions 12 S rRNA and tRNA from the mitochondrial DNA of khorasan native chickens and the obtained sequence compared with other breeds from local population recorded in gene Banks.

MATERIALS AND METHODS

In this study, 6 blood samples of native birds were collected from Breeding Center of Khorasan Razavi Agricultural Production and ensure that there is no family relationship between samples. Blood samples were collected in tubes containing EDTA and were stored in a freezer at -20 ° C. Quantity of the extracted DNA was measured according to spectrometry method using nano drop –nd 2000 spectrophotometer of USA Thermo Company and the quality was calculated on Agarose gel 1%. Specific primers for amplifying 12SrRNA and tRNA fragments designed by using Primer premier 5 Software as mentioned in below:

Forward (12SrRNA) :(5`- AAGGAGCAGGTATCAGGCACACT -3`)

Reverse (12 S rRNA) :(5`- ACACTTACCTTGTTACGACTTGCCT-3`)

Polymerase chain reaction for amplifying 818 bp of D-loop and tRNA fragments were carried out using T-personal model Biometra thermo cycler according to standard method. The components in a final volume of 25 microliter PCR reactions contained 100 ng of target DNA, Taq polymerase, a unit of dNTPs 10 mM, 5.0 microliter, 1 microliter of $MgCl_2$ 50 mM, 1 micro liter of 5 pico-molar of primer mix, 2.5 microliter of 10 X buffer and 18 microliter of double-distilled water were used.

The application of heat and time of the polymerase chain reaction for 12 S rRNA and tRNA genes included the denaturation at 94 °C for 30 s, 54 °C annealing temperature for 35 seconds, amplification at 72 °C for 30 s, an initial denaturation step temperature of 94 °C for 10 min and a final amplification step at 72 °C for 10 min followed by 35 cycles of amplification. The PCR Products were electrophoresed on 1% Agarose gel and staining of gel was performed using etidium bromide. ml of PCR products was purified and sent with 50 ml of each used primers forward and reverse (10 pmol) to MacroGen company(south Korea)for sequencing. These sample are sequenced using the ABI3130 machine according to Sanger automate approach. The obtained sequences homology level was measured using accurate BLAST tool and blastn method in NCBI database. To investigate the phylogenetic relationship of breeds studied, the tree was drawn using UPGMA procedure equivalent sequence, the software MEGA 5 (Tamura *et al.,* 2011).

RESULTS AND DISCUSSION

DNA extraction from all samples was performed successfully. The spectrometry results showed that the extracted DNA had good quality. The amplified products were electrophoresed on 1% agarose showed that the designed primers worked well and 12 S rRNA and tRNA genes in specific parts of length 852 bp, respectively (Figure 1).

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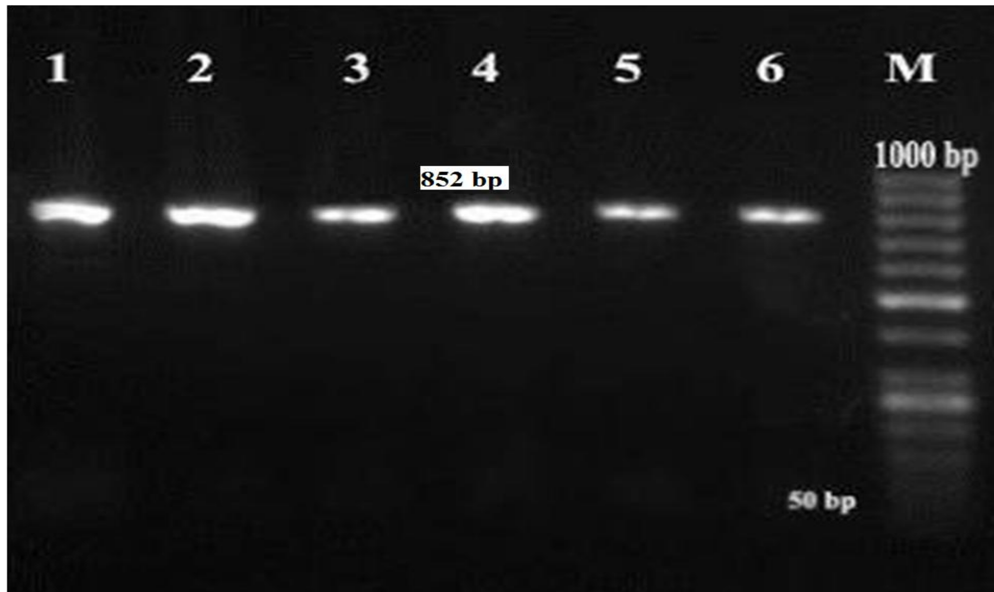

Figure 1: Electrophoresis of 852 bp PCR Products on 1 % Agarose gel

The regions of the 12 S rRNA and tRNA mitochondrial genome for 6 samples were sequenced, but the results of one of the samples were excluded due to poor quality and it was not used in further studies. After sequencing the samples, the comparison was conducted between the five sequences using Glusta multiple alignment and Bio Eddit software (Hal, 1999). After evaluating the quality of sequencing, nucleotides that were of poor quality in the ends of the sequences were removed and the common areas between the five sequences were isolated and thereby leading to a five-piece was used with a length of 685 nucleotides of 12 S rRNA and tRNA genes, including in all the samples. As a means of comparison between the sequences, showed that there is no difference between the sequences studied (P = 0) and the reason may be due to low sample size and the homogeneity of the studied population several successive generations of selection with business goals (Figure 2).

breed Lv'erwu	caaacgaaaa	aggacgtgaa	acccgccctt	agaaggagga	tttagcagta
breed Huang Lang	caaacgaaaa	aggacgtgaa	acccgccctt	agaaggagga	tttagcagta
silky chicken	caaacgaaaa	aggatgtgaa	acccgccctt	agaaggagga	tttagcagta
Khorasanian native chicken	CAAACGAAAA	AGGACGTGAA	ACCCGCCCTT	AGAAGGAGGA	TTTAGCAGTA
White Leghorn	caaacgaaaa	aggacgtgaa	acccgccctt	agaaggagga	tttagcagta
lafayettei	cagacgaaaa	aggacgtgaa	acccgccctt	ggaaggagga	tttagcagta
Bambusicola thoracica	caaacgaaaa	aggatgtgaa	acccaccctt	ggaaggagga	tttagcagta
Bambusicola fytchii	caaacgaaaa	agggtgtgaa	acccaccctt	ggaaggagga	tttagcagta
Francolinus pintadeanus	tagacgaaaa	aggatgtgaa	acccatcctt	agaaggagga	tttagcagta
Tragopan caboti	caaacgaaaa	aggacgtgaa	acccgtcctt	ggaaggagga	tttagcagta
Tetraophasis obscurus	caaacgaaaa	aggacgtgaa	actcgccctt	agaaggagga	tttagcagta

Figure 2: The comparison nucleotide sequences of 12 S rRNA and tRNA regions for khorasanian native chicken samples and other breeds with length of 852 bp.

The nucleotide diversity[1] showed that a point mutation is seen in the position (nucleotide number 852) of pyrimidine nucleotide substitution mutation resulting from transversion of a pyrimidine for a purine (T to A). The phylogenetic tree analysis showed that 12 S rRNA and tRNA sequences from the mitochondrial genome of the native chicken and the sequence of Asian chicken breeds, Huang Lang and Lo'erwu with greater relativity and placed in a group and the chicken breed of teraophasis obscures the farthest place (Figure 3).

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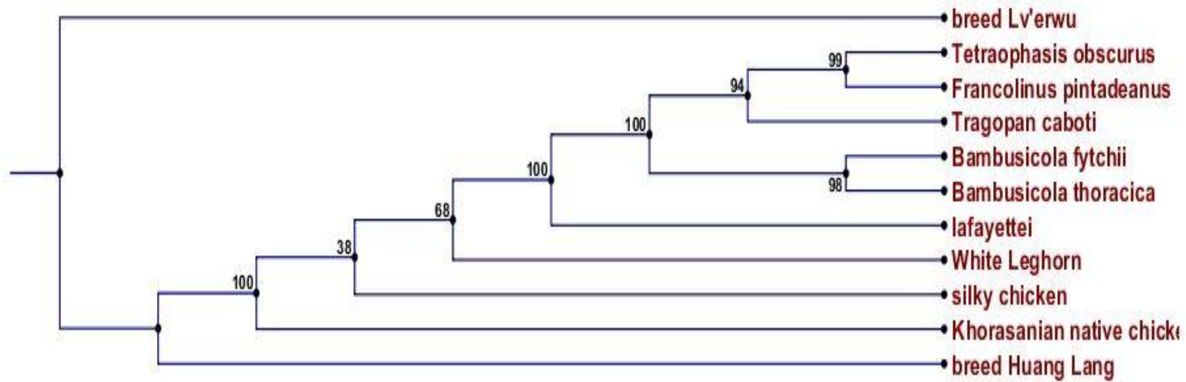

Figure 3: Phylogenetic tree based on consensus sequences of Khorasan's native chicken and other chicken breeds are taken from GenBank

Genetic distances Matrix is obtained by pair wise comparison of each sequences between species using Maximum Composite Likelihood model (Tamura *et al.,* 2004). Since the resulting numbers represent the nucleotide substitution rates between breed sequences, the genetic distance between the two breeds with the phylogenetic relationship are studied between them. The Iranian native poultry breed overlap[2] the China Huang Lang and Lv'erwu with the 99/76%, which is equivalent to replacing two nucleotides and the maximum genetic distance between native chicken breed overlap with hens Teraophasis obscures 84/78%, these results confirm the validity of drawing a phylogenetic tree (table 1).

		1	2	3	4	5	6	7	8	9	10	11
breed Lv'erwu	1		100.00	99.88	99.76	99.88	95.74	92.83	90.52	87.45	89.12	84.78
breed Huang Lang	2	0		99.88	99.76	99.88	95.74	92.83	90.52	87.45	89.12	84.78
silky chicken	3	1	1		99.63	99.76	95.62	92.95	90.64	87.58	89.00	84.66
Khorasanian native chicken	4	2	2	3		99.63	95.49	92.59	90.52	87.45	88.88	84.78
White Leghorn	5	1	1	2	3		95.86	92.95	90.64	87.58	89.24	84.90
lafayettei	6	35	35	36	37	34		91.99	89.32	87.20	89.12	84.06
Bambusicola thoracica	7	59	59	58	61	58	66		94.27	86.82	89.71	85.49
Bambusicola fytchii	8	78	78	77	78	77	88	47		86.55	88.01	85.21
Francolinus pintadeanus	9	104	104	103	104	103	106	109	110		85.04	87.88
Tragopan caboti	10	90	90	91	92	89	90	85	99	124		85.68
Tetraophasis obscurus	11	126	126	127	126	125	132	120	121	96	118	

Percent Identity

Divergence

Table 1: Percent identity and divergence matrix of 12 S rRNA and tRNA genes in khorasan native chickens in relation to other breeds

According to the results of the phylogenetic tree and checking matrix of genetic distances for the two genes studied, it can be said that, use of these two markers showing genetic similarity with native chicken breeds studied that are important and the mitochondrial genome sequences to determine genetic diversity in Asian Chicken Has been were used successfully (Sulandari *et al.,* 2008). The results of this study agreed with assay reported by Ahmed *et al.,* (2010). The results showed moderation to low genetic diversity in this population. Low genetic diversity in these birds requires special attention to protect genetic resources (Hillel *et al.,* 2003). The similarity indicates the number of polymorphic loci close

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genetic of this population breed of poultry and other countries. Log in foreign chicken breeds pursuant to the neglect capacity of native hens. Since the conservation of genetic resources activity alone is costly and indigenous chicken breeds adapted to suit their local conditions and their relative resistance to diseases, assisting breeding methods, they are able to increase production capacity. Regions of 12 S rRNA and tRNA mitochondrial genome sequencing by considering the short length and easy method of data analysis and the comparison with some breeds can get full commercial production these breeds having some similarities with highly productive breeds represents the ability to produce these breeds. Also, using these sequences and registered with the World Bank in the name of their genes can be identified as an appropriate indicator of native breeds of chicken.

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