



Detection of genetic diversity of local Iraqi black goats by using Cytochrome b gene

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Summary

This study was conducted at the College of Agriculture / University of Basrah. The study aimed to characterize the genetics of local Iraqi black goats using mitochondrial DNA (Cytochrome b). A total of 160 blood samples were collected from local Iraqi black goats aged (1-2) years in the governorates of Al-Muthanna, Dhi Qar, Basrah and Maysan (40 samples each). A segment of 1140 bp of Cytochrome b gene were amplified. The number of haplotypes of the Cytochrome b gene was 20 haplotypes. The total number of transition mutations was 18, the highest number was in Al-Muthanna (8), the lowest was in Thi-Qar and Maysan, (only 2 mutations). The total number of transversion mutations was 9 (7 in Al-Muthanna and 2 in Basrah). The overall haplotype diversity (HD) was (0.864), the highest was in Basrah (0.838). The overall nucleotide diversity was 0.00294, with the highest value in Al-Muthanna (0.00548). The presence of three haplotype pattern that were unique to the Iraqi black goat breed. The phylogenetic tree showed two main branches, the 1st included the animals of Al-Muthanna and Maysan, and the 2nd included Thi-Qar and Basrah. The phylogenetic tree for black Iraqi local goats exhibited a unique branch, goat breeds in the world showed another branch. The highest genetic distance between the animals of Basrah and Al-Muthanna (0.833), the lowest between animals of Maysan and Thi-Qar (0.505). The results of the mismatch distribution showed a mono peak distribution in the animals of Thi-Qar and a multi-peaks distribution in the animals of Al-Muthanna and Basrah.

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Key words: Iraqi goats, cytochrome b, genetic diversity, haplotypes, nucleotide diversity

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Introduction

Goats are characterized by the ability to survive and thrive under very harsh climatic conditions, such as high ambient temperatures, water shortages and scarcity of pastures. Goats have been widely used as a good source of meat, milk and hair, especially in areas with harsh climatic conditions that negatively affect the performance and production of other animals. Despite the large contribution of goats to food security in arid and semi-arid areas compared to other farm animals, studies on goat production systems and phenotypic and genetic

characterization are few (Al-Araimi et. al., 2017).

Phenotypic and genetic characterization to assess biological diversity and differences between the current Iraqi goat breeds is a prerequisite and an important condition to facilitate their conservation and use in breeding and improvement programs, as Iraqi goats are not classified and the information available about them is very limited. Therefore, the phenotypic characterization of animal genetic resources is the process of identifying the different groups and breeds and describing



their external and productive characteristics in a particular environment and under a certain administration, and the information provided by phenotypic studies is necessary in planning and managing animal genetic resources at the local, national, regional and global levels (Othman and Mahfouz, 2016).

Cytochrome b gene is one of the genes encoded by mtDNA, which is widely used in the study of evolution for its ease of access and has a high value in evolution and follows a pattern of inheritance consistent with phylogenetic reconstruction (Jain et al, 2007).

The Cytochrome b gene in goats has a length of 1140 base pairs of 337 amino acids, preceded by an ATG-encoded start codon and terminated by an AGA stop codon (Chen et al, 2016; Pietro et al, 2003). The Cytochrome b gene is involved in the transfer of electrons in the respiratory chain and can be used for the purpose of phylogenetic analysis and species identification. In addition, it is particularly useful for comparing species within the same genus or family and can also be used to study genetic diversity through mtDNA sequences (Castersana, 2001). The Cytochrome b gene is unique in that it is one of the protein-encoding genes that has an eternal role in determining the level of species so that it can be used to classify a particular breed or to determine the genetic relationship between species.

The present study was part of a project to recognize the genetic structure of Iraqi local camel (Ayied et al., 2018) and cattle (Faraj et al, 2020 and 2021).

Aim of the study were to count the number of haplotypes within and between lineages, as well as calculating the haplogroup. As well as compare the Iraqi goat breed with the goat breeds in the world, calculate Neutrality, genetic stability of breeds and expansion time in the evolutionary history of Iraqi Goats.

Material and methods

Animals

160 samples were collected from the local Iraqi goat breed, 40 samples from each of the governorates of Muthanna, Dhi Qar, Basrah and Maysan, and the animals were not related to each other.

DNA extraction kit

The gSYNC™ DNA Extraction Kit was used for DNA extraction, manufactured by the Taiwanese Geneaid Company, and according to the instructions in the leaflet attached by the company. Primer was 'F-5'-GGAATCTAACCATGACCAAT-3, 'R-5'-GCTTCTTCCTTGAGTCTTAG-3

Polymerase Chain Reaction

The special materials were prepared using PCR technique, then they were placed in a container containing pieces of ice to protect them from heat. The work was done in a sterile and clean place in a special cabinet (PCR Cabinet), which contains ultraviolet (UV) rays, to sterilize the micropipettes, tubes and tubes. The PCR reaction mixture was prepared in a 100 µlEppendorf tube and the final volume of components was (50 µl) and then the tubes were placed in a microcentrifuge for 30 seconds (Table 1).

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Table (1) Quantities (µl) of materials used in PCR-mtDNA technique

Master mix	Template DNA	Primers		D. W.	Total volume
		Forward	Reverse		
25	6	2	2	15	50

statistical analysis

Forward bar sequences and Reverse bar sequences were collected, and sequences were aligned and aligned using Bio Edit software (Hall, 1999). Number of polymorphic (NH), number of transitions and transversions, and nucleotide composition were calculated using the Arlequinver program. 3.5.1.2 (Excoffier and

Lischer,2010). Haplotype diversity (HD) and nucleotide diversity (π) were calculated using DnaSP v5.10 (Librado and Rozas, 2009).The network of haplotypes of the D-loop region and the Cytb gene was drawn based on the Median Joining (MJ) algorithm using Network 5.0.0.0 (Bandelt et al., 1999). While the phylogenetic tree was drawn by the Neighbor-joining tree



(NJT) using MEGA 7.0 program (Kumar et al., 2016). Genetic distance was calculated using Arlequinver software. 3.5.1.2 (Excoffier and Lischer, 2010). Analysis of molecular variance (AMOVA) using Arlequinver software. 3.5.1.2 (Excoffier and Lischer, 2010). Analyze the neutrality test using Arlequin ver. 3.5.1.2 (Excoffier and Lischer, 2010). The mismatch distribution was analyzed using DnaSP v5.10 (Librado and Rozas, 2009).

Spssanalysis

The data were statistically analyzed using a fully randomized design for one factor with four parameters, and significant differences between the means were tested using the Revised Least Significant Differences (RLSD) using the ready-made statistical program SPSS (V.26) (2020).

Result and Discussion

Molecular diversity

Total number of sequences was 160 in all studied regions (40 for each governate). The total number of genetic formations (NH) reached 31 formations distributed among the

governorates of the studied breed, where 17 formations appeared in Al-Muthanna, two formations in Dhi Qar, 10 formations in Basrah and two formations in Maysan. The total number of transitions was 18, distributed (8, 2, 6 and 2) between the governorates of Al-Muthanna, Dhi Qar, Basrah and Maysan respectively. While the total number of mutations showed 9 mutations distributed (7 and 2) in each of Al-Muthanna and Basrah, respectively, while there was no transformation within the breed in each of Dhi Qar and Maysan. As for the content of nitrogenous bases, it was rich in nitrogen bases content of adenine A, cytosine C and thymine T (Table 2).

Genetic diversity

The results of the genetic diversity of the Cytochrome b gene (table, 3) showed that the total haplotype diversity (HD) values were (0.864), and the highest haplotype diversity was for the animals of Basrah (0.838) followed by Al-Muthanna and Maysan (0.828 and 0.517, respectively).The lowest haplotype diversity in Dhi Qar (0.492).

Table (2) Molecular diversity of Cytochrome b gene in Iraqi local black goat breed

Parameters	Governates				Overall
	Al-Muthanna	Thi Qar	Basrah	Maysan	
No. of sequences	40	40	40	40	160
No. of polymorphic (NH)	17	2	10	2	31
No. of transitions	8	2	6	2	18
No. of transversion	7	0	2	0	9
Nucleotide composition	C: 29.26% T: 26.38% A: 31.16% G: 13.20%	C: 29.31% T: 26.42% A: 31.21% G: 13.06%	C: 29.24% T: 26.46% A: 31.21% G: 13.10%	C: 29.30% T: 26.43% A: 31.16% G: 13.11%	C: 29.28% T: 26.42% A: 31.19% G: 13.12%

Nucleotide diversity (π) of the studied breed was 0.00294. The highest nucleotide diversity was for a group of animals in Al-Muthanna (0.00548), followed by animals in the provinces

of Basrah and Dhi Qar (0.00250 and 0.00103 respectively). The lowest value (0.00059) was of animals in Maysan (Table 3).

Table (3) Genetic diversity of the Cytochrome b gene in the Iraqi local goat breed

Governorate	Haplotype diversity (HD)	Nucleotide diversity (π)
Al-Muthanna	0.828	0.00548



DhiQar	0.492	0.00103
Basrah	0.838	0.00250
Maysan	0.517	0.00059
Overall	0.864	0.00294

Haplotypes network

Fig. (1) showed that the total number of haplotypes for Cytochrome b was 20, distributed as 6 haplotypes in Al-Muthanna, 5 in Dhi Qar, 7 in Basrah and 5 in Maysan. The haplotype H6 was shared between Dhi Qar and Al-Muthanna, while haplotype H10 is common

between the provinces of Dhi Qar and Basrah, finally the haplotype H16 is common between Basrah and Maysan. The haplotypes (1-5) were specific to Al-Muthanna, (7-9) were specific to Dhi Qar governorate, (11-15) were specific to Basrah governorate, and (17-20) were specific to Maysan governorate (Fig. 1)

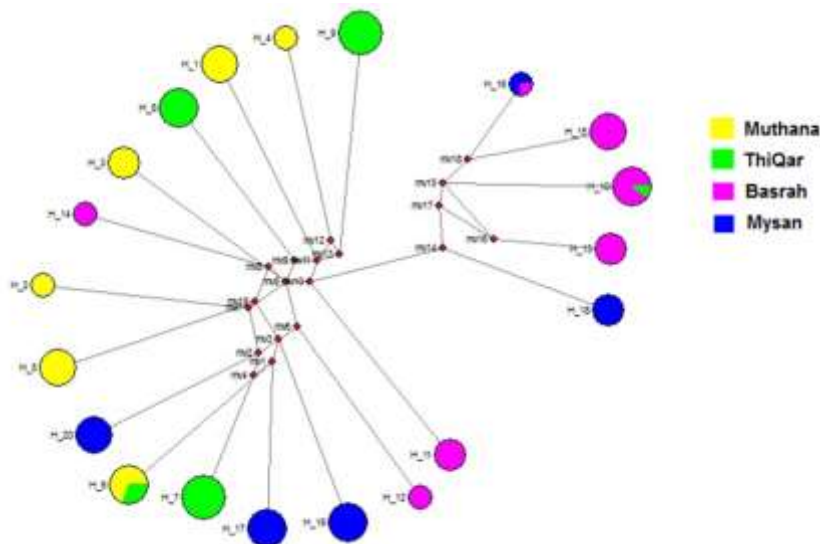


Fig. (1) The network of haplotypes of the Cytochrome b gene of the Iraqi local goat breed among the governorates

As for comparison with goat breeds in some countries of the world, the total number of haplotypes of the Cytochrome b gene was 3 haplotypes, two of them in Iraq and one in a group of countries participated. The H3

haplotype was common among the goat breeds in Bangladesh, China, India, Ireland, Italy, Russia, Turkey and the United Kingdom, while the H1 and H2 haplotypes were specific to the goat breeds in Iraq (Fig. 2).

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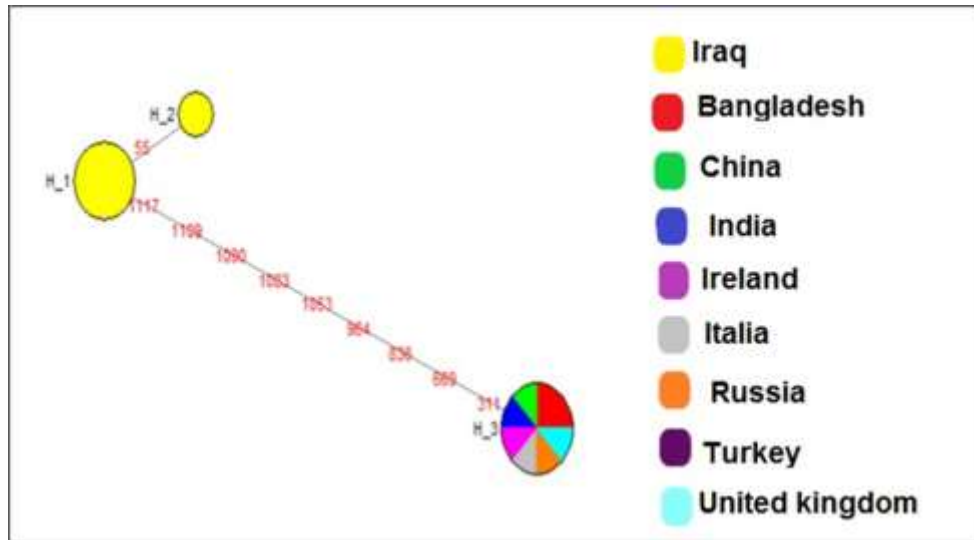


Figure (2) The network of haplotypes of the Cytochrome b between the local Iraqi goat breed and the goat breeds in some countries

Phylogenetic tree

The results of the phylogenetic tree for the Cytochrome b showed the presence of two main branches, the first branch included the

animals of Dhi Qar and Basrah, while the second branch included all the animals of Al-Muthanna and Maysan (Fig. 3).

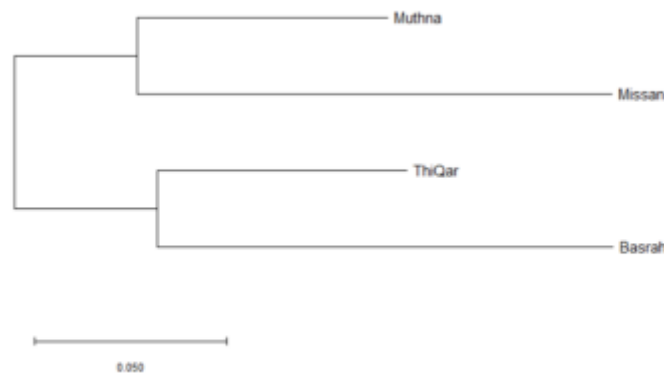


Fig. (3) Phylogenetic tree of the Cytochrome b gene of the Iraqi local goat breed

In comparison with some countries, the results for Cytochrome b showed the presence of two main branches, the first branch included the Iraqi local goat breed, and the second branch included the breeds of Russia, Turkey, Italy,

Ireland, India, China, Bangladesh and the United Kingdom (Fig. 4). This corresponded to a network of haplotypes that included four haplogroups.



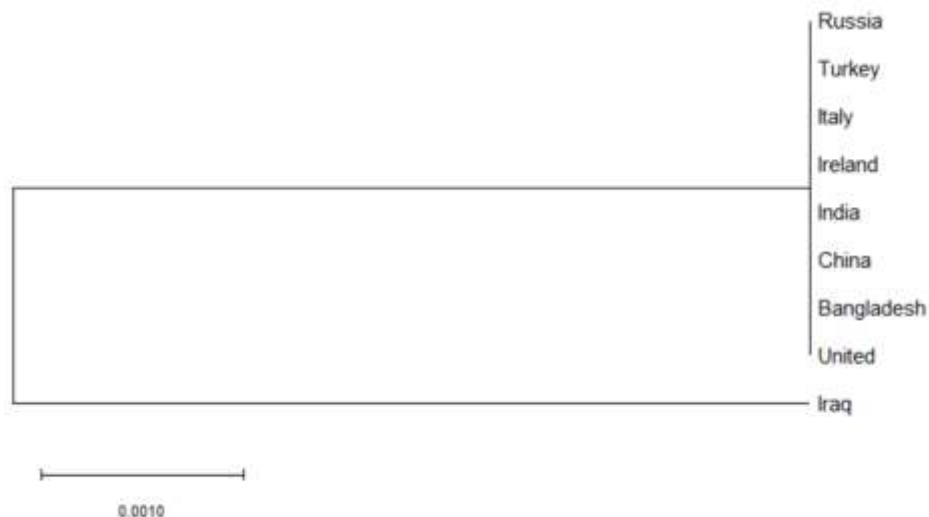


Fig. (4) The phylogenetic tree of the Cytochrome b between the Iraqi local goat breed and the goats of some countries of the world

Genetic distance

The fixation index F_{st} was used to express the genetic distance of the Iraqi local goat breed in the studied areas of Al-Muthanna, Dhi Qar, Basrah and Maysan. Between the provinces of

Dhi Qar and Maysan. Because of the high values in the genetic distance, they indicate the presence of very high genetic differences in the different provinces (Wright 1978; Harti and Clark, 1997) Table (4).

Table (4) Genetic distance of Cytochrome b gene of Iraqi local goat breed

Governorate	Al-Muthanna	DhiQar	Basrah
DhiQar	0.660	-	
Basrah	0.665	0.833	-
Maysan	0.673	0.505	0.678

Mismatch distribution

The results of the mismatch distribution for the Cytochrome b (table, 5) showed the presence of a unimodal distribution in the studied animals of the governorates of Dhi Qar and Maysan with differences peaks (0.985 and 0.569), respectively, and values of the raggedness index

(R) for the animals of the same two governorates (0.742 and 0.175), respectively. As for the animals of Al-Muthanna and Basrah, a multiple distribution appeared with peak differences (5.249 and 2.392), respectively, while the values of the index (R) were 0.0812 and 0.158 respectively.

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Table (5) Mismatch distribution of Cytochrome b region of the local Iraqi breed

Governorate	Mean Pairwise Difference (SD)	Raggedness Index (R)
Al-Muthanna	5.249	0.0182
DhiQar	0.985	0.742
Basrah	2.392	0.158
Maysan	0.569	0.175

The unimodal (Fig. 5) indicates the subordination of the breed to the demographic expansion, and this is what happened in the animals of the provinces of Maysan and Dhi Qar, while the binary form indicates a state of demographic equilibrium, which was seen in the governorates of Al-Muthanna and Basrah for



the Cytochrome b gene (Hudson and Slatkin, 1991; Rogers and Harpending, 1992 and Jobling et al., 2004).

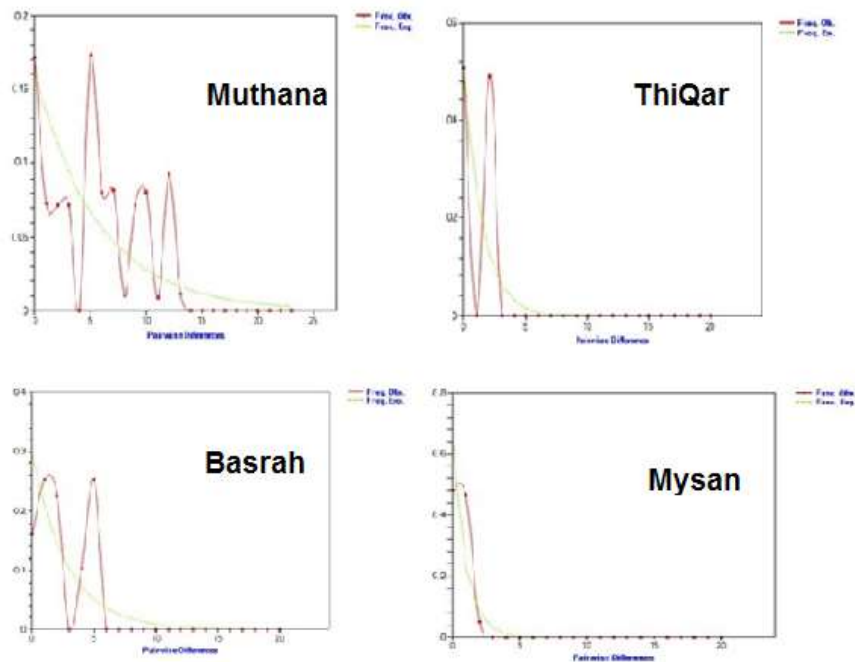


Fig.(5). Mismatch analysis of the Cytochrome b region of the local Iraqi goat breed

Neutrality Test

The results showed that there was no negative value of the neutrality test for Cytochrome b gene for the local Iraqi goat breed studied in all governorates (table, 6). The highest value for Tajima's D were in Dhi Qar (2.06578), followed by Al-Muthanna and Basrah(1.55675 and 0.77572 respectively). The lowest value was in Maysan (0.39770).For Fu's Fs, its value was significant (P<0.05) in all governorates, where the animals of Al-Muthanna governorate recorded the highest value (6.27893), followed by Dhi Qar and Basrah(3.61172 and 2.62857respectively), and the lowest value recorded for the animals of Maysan (0.51400), they were all significant (P<0.05).The results indicated that the breed in the four governorates lose a number of their members

which may lead to an increase in domestic breeding, or it may be about the small size of the herd for the breeders, or the occurrence of genetic drift and the stabilization of some genetic structures and genes, in addition to the occurrence of a loss in many rare alleles that have an effect on many traits that reach the clan to the so-called neck. This means that there is a significant deterioration in genetic diversity, and the reason for the positive values of the Tajima test for the study gene may be due to the lack of organized scientific cross-breeding programs or that cross-breeding between animals is random, which may cause the loss of some genetic structures (Tajima, 1989; Fu , 1997; Aris-Brosou and Excoffier, 1996) (Table 6).

Table (6) Neutrality test for Cytochrome b gene for Iraqi local goat breed



Governorate	Fu's Fs Statistic	Tajima's D Statistic
Al-Muthanna	6.27893	1.55675
DhiQar	3.61172	2.06578
Basrah	2.62847	0.77572
Maysan	0.51400	0.39770
Mean	*3.25828	1.19899

- **There is a significant difference $p < 0.05$**
Analysis of molecular variation AMOVA of gene Cytochrome b

The results of the AMOVA analysis of the Cytochrome b gene between and within the

governorates of the Iraqi local goat breed showed that the percentage of genetic variance between the governorates was 24.58% and the percentage of variance within the governorate was 75.42% (Table 7).

Table (7) Molecular Variation Analysis of Cytochrome b of Iraqi local goat breed

Source of Variation	DF	SS	Variance component	Variance component %
Between Provinces	3	287.131	2.22226	24.58
Within Provinces	156	1063.900	6.81987	75.42
Total	159	1351.031	9.04214	100

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References

- **Al-Araimi, A. N., Al-Atiyat, R. M. et al. (2017).** Maternal genetic diversity and phylogeography of native Arabian goats. *Livestock Science*, 206:88-94.
- **Aquadro, Charles F and Greenberg, Barry D. (1983).** HUMAN MITOCHONDRIAL DNA VARIATION AND EVOLUTION: ANALYSIS OF NUCLEOTIDE SEQUENCES FROM SEVEN INDIVIDUALS *Genetics*, Volume 103, Issue 2, 1 February 1983, Pages 287-312, <https://doi.org/10.1093/genetics/103.2.287>
- **Aris-Brosou, S. and Excoffier, L. (1996).** The Impact of Population Expansion and Mutation Rate Heterogeneity on DNA Sequence Polymorphism. *Molecular Biology and Evolution*. 13(3): 494-504.
- **Ayied, A.Y., Al-Badran, A.I., Al-Zaalan, A.R. (2018).** Assessment of genetic diversity in Iraqi camel breeds using cytochrome b. *Advances in Animal and Veterinary Sciences*, 6(7), 273-277
- **Castresana, J. (2001).** Cytochrome b Phylogeny and the Taxonomy of Great Apes and Mammals. *Molecular Biology and Evolution*. 18 (4): 465-471.
- **Chen, S., Ma, H.M., Chen, G.S. and Wang, L.Y. (2016)** . Complete mitochondrial genome of the Liuyang black goat and its phylogenetic relationship with other Caprinae. *Genet. Mol. Res.*, 15(2): 1-11.
- **Cozzi M.C., Strillacci M.G., Valiati P., Bighignoli B., Cancedda M. & Zanotti M. (2004).** Mitochondrial D-loop sequence variation among Italian horse breeds. *Genetics Selection Evolution* 36, 663-72.
- **Deniskova, Tatiana; Bakoev, Nekruz; Dotsev, Arsen; Selionova, Marina; Zinovieva, Natalia (2020).** Maternal Origins and Haplotype Diversity of Seven Russian Goat Populations Based on the D-loop Sequence Variability. *Animals*, 10(9), 1603-1603. doi:10.3390/ani10091603.
- **Faraj, S.H., Ayied, A.Y., Al-Rishdy, K.A.H. (2020).** Single nucleotide polymorphisms in the promoter of cyp19 gene in cattle bred in Iraq. *Basrah Journal of Agricultural Sciences*, 33(1), pp. 89-97.



- **Faraj, S.H., Mahde, N.F., Ayied, A.Y. (2021).** Single nucleotide polymorphisms in exon 11 of LHCGR gene in Iraqi Jenoubi cattle Indian Journal of Ecology, 2021, 48, pp. 229–232.
- **Fu, YX.; Li, WH. (Mar 1993).** "Statistical tests of neutrality of mutations". Genetics. 133 (3): 693–09.
- Harti, D.L and Clark, A.G. (1997). Principles of population genetics (3rd edition). Sunderland, MA: Sinauer Associates, Inc.
- Hudson, R. R. and Slatkin, M. (1991). Pairwise Comparisons of Mitochondrial DNA Sequences in Stable and Exponentially Growing Populations. Genetics. 129 (2): 555-562.
- **Jain, S., Brahmabhai, M.N., Rank, D.N., Joshi, C.G. and Solank, J.V. (2007).** Use of Cytochrome b gene variability in detecting meat species by multiplex PCR assay. Indian J. Agric. Sci., 77(9): 880-881.
- **Jobling, M. A.; Hurles, M. E. and Tyler-Smith, C. (2004).** Human Evolutionary Genetics. Garland Science, New York/Abingdon, UK.
- **Mirol P.M., Garcia P.P., Dulout F.N. (2002):** Mitochondrial variability in the D-loop of four equine breeds shown by PCRSSCP analysis. Genetics and Molecular Biology, 25, 25–28.
- **Othman, O.E., Mahfouz, E.R., (2016).** Genetic biodiversity, affinities and phylogeny of four goat breeds in Egypt. J. Biol. Sci. 16, 86.
- **Pietro, P., Maria, F., Gianfranco, G. and Giuseppe, E. (2003).** The complete nucleotide sequence of goat (*Capra hircus*) mitochondrial genome. DNA Seq., 14(3): 199-203. 31.
- **Priskin K., Szabo K., Tomory G., Bogacsi-Szabo E., Csanyi B., Eordogh R., Downes C.S., Rasko I. (2010):** Mitochondrial sequence variation in ancient horses from the Carpathian basin and possible modern relatives. Genetica, 138, 211–218.
- **Rogers, A. R. and Harpending, H. C. (1992).** Population Growth Makes Waves in the Distribution of Pairwise Genetic Differences. Molecular Biology and Evolution. 9: 552-569.
- **Tajima, F. (1989).** Statistical Method for Testing the Neutral Mutation Hypothesis DNA Polymorphism. Genetics. 123: 585-595.
- **Wright, S. (1978).** Evolution and the Genetics of populations. Variability within and among Natural Populations. Volume 14. University of Chicago Press, Chicago.

