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STUDY OF GENETIC DIVERSITY OF SOME MITOCHONDRIAL GENES OF IRAQI MARSH BUFFALO

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ABSTRACT

This study aimed to analyze the genetic diversity of two mitochondrial genes (16 S ribosomal RNA 16 S rRNA) and cytochrome b for some buffalo populations in the Iraqi marshlands in southern Iraq. In the present study, 20 local buffaloes were used from the Chabayish marshes and Hammar district. Blood samples were collected from the jugular vein of the animals, and DNA extraction was performed using the kit from Geneaid company. Two regions of the mitochondrial genome (mtDNA) were used, the first piece of cytochrome b gene was 674 bp, and the other piece was from 16 S ribosomal RNA (16 S rRNA) gene and its size was 600 bp. After confirming the success of the amplification process by using electrophoresis of the PCR product using 2% agarose gel and Demand dye, the PCR reaction products were sent to the Chinese company (Yang Ling Tiantun Aoka Biotechnology) to obtain the sequence of nitrogenous bases of cytochrome b and 16S rRNA genes Bioinformatics programs were used to analysis of the nitrogenous base sequences of the genes targeted in this study.. The results of the phylogenetic tree of the 16S rRNA gene for the local Iraqi buffalo, compared to the buffalo of some countries, showed the presence of two main branches, the first branch included the Iraqi buffalo, and the other main branch branched into secondary branches that included Chinese, Indian, Iranian, Russian in addition to Iraqi buffaloes as well. As for the results of the phylogenetic tree of the ctochrome b gene two main branches were also formed, the local Iraqi buffalo participated in the first main branch with countries, China, Russia, Pakistan, India, America, Japan and Romania. The second main branch included the Indonesian buffalo. The results of the phylogenetic tree almost coincided with the results of the network of individual patterns of 16 S rRNA and ctochrome b genes, where the buffalo participated with a group of buffaloes of some countries under comparison (China, India, Pakistan, Japan and some other countries, in addition to the Iraqi local buffalo with one haplotype). It is clear from the results of the hplotypes network and the results of the phylogenetic tee for the 16 S rRNA and ctochrome b genes that the local Iraqi buffalo, and Chinese, Indian and Pakistani buffaloes in general, are same origin. The studied segments of the target genes in the current study, S16 rRNA and ctochrome b of Iraqi local buffaloes, were recorded in the NCBI, EMBL and DDBJ GenBank sites with independent accession numbers starting from LC481471 to LC481490.

Keywords: mtDNA; buffalo; genetic diversity.

1. INTRODUCTION

The latest availability of databases in GenBank and the development of bioinformatics programs have revolutionized the analysis of genetic data as a working basis for the development of DNA markers[1]. In addition, the development of molecular genetics techniques in detecting genetic markers has helped well in knowing and evaluating the genetic diversity of different species and breeds of animals and preserving them as an important source of this diversity that can be relied upon in producing wealth in most countries [2]. Since its maternal inheritance, absence of introns, accessibility of single-copy orthologous genes, lack of recombination measures, and quick mutation rate, mitochondrial DNA (mtDNA) has been widely employed in genetic investigations. The displacement loop (D-loop) region and 37 genes make up bovine mtDNA, which is a double-stranded circular molecule [3].Mitochondrial DNA (mtDNA) has been widely used in research and studies to detect traits and use it in scientific applications in humans and animals in the study of evolution and diversity and in the heredity of populations [4]. One of the characteristics of this molecule is that its high copy number in the cell as well as its overlapping genes and their affinity with each other and the absence of introns in it [5,6]. The molecular variation in the mtDNA region was considered one of the most important sources of genetic variation due to the similarity of roles between DNA in the nucleus and mitochondrial DNA in terms of transcribing different types of RNA, rRNA, tRNA and mRNA, and it can also translate mRNA into proteins within the mitochondria 7. The buffalo's cytochrome b gene has a size of 1139 bp, which encodes for 379 amino acids, and is located in the region between the tRNA-Glu and tRNA-Thr genes [8,9]. This gene is considered one of the international standard genes for comparing the rates of evolution, emergence and inheritance of breeds and classification of different species [19]. The cytochrome b gene was used to study variation in many agricultural animals such as goats [11], cows [12], and buffaloes [13]. The S16 rRNA gene is one of the rRNA genes located between the tRNA-Val and tRNA-Leu genes, with a size of 1568 bp. The S16 RNA gene has been shown to be very useful for species identification [14]. The S16 RNA gene was also used to distinguish between animal meat and detect fraud in meat [15,16].

The current study aimed to study the genetic diversity of two distinct regions of the mitochondrial genome, cytochrome b gene and S16 RNA genes in buffaloes, analyze some molecular parameters of the gene, and record the sequence of nitrogenous bases for both species in the NCBI GenBank.

2. MATERIALS AND METHODS

Twenty local buffaloes were used from the Chabayish marsh area. Blood samples were collected from the jugular vein of the animals. The DNA extraction process was performed using the attached kit from Geneaid company. Electrophoresis was used to ensure the success of the DNA extraction process with agarose gel. The purity and concentration of the DNA were also confirmed using the Nanodrop 2000 device. The concentration of the DNA was between 25.4-63.6 nanograms per microliter. The purity for wavelengths (260/280) ranged between 1.67-1.93. These values are within the recommended ranges to ensure the success of the process amplification. In this study, two regions of the mtDNA were used. The first from the cytochrome b gene, the size of the selected segment was 674 bp, and the other segment was from the 16 S ribosomal RNA (16 S rRNA) gene and its size was 600 bp. Mixture of PCR was 25 µl, containing 12 µl Master Mix, 1µl for each primer forward & reverse, 3 µl DNA template Concentration 30-65 ng and 8µl dd water. The PCR program for cytochrome b gene was 95°C for 3 min for initial denaturation, followed by 35 cycles of 95°C for 1 min, 57 °C for 30 Sec., 72°C for1 Min.. and a final extension at 72°C for 10 min. While the program of PCR for 16 S rRNA gene was 95°C for 3 min for initial denaturation, followed by 35 cycles of 95°C for 45 Sec., 60 °C for 30 Sec., 72°C for 45 Sec.. and a final extension at 72°C for 10 min.

Table (1) shows the sequence of primers used in the study and product size for stuidies genes.

Gene	Primers	Product size (bp)
Cytochrom e b	F5'GCCTGTTTATCAAAAACAT-3 R5'CTCCGGTTTGAACTCAGATC-3	647
16 S rRNA	F5' CTCACCGGCCTATTCCTAG -3 R5' AACTACACCCCAGCAAACCC -3	600

After completing the amplification process and validating its success, the result was electrophoresed on a 2% agarose gel. The sequence of the nitrogenous bases of cytochrome b and 16 S rRNA genes was obtained by sending the PCR reaction results to a Chinese company (Yang Ling Tiantun Aoka Biotechnology).

3. RESULTS AND DISCUSSION

The electrophoresis of the 16S rRNA and Cytochrome b gene products revealed that the amplification process was successful , As the results revealed the expected sizes from the amplification process using a standard DNA marker of size 100 bp, with the size of the 16S rRNA gene product being 600 bp and the size of the cytochrome b gene product being 647 bp.

Figure (1) shows a map of the mitochondrial genome of buffaloes (water buffalo), showing the locations of the genes targeted in this study. The 16S rRNA gene starts from the end of the 12S rRNA gene at site 1094 until the start of the tRNA-Leu gene at site 2662. Therefore, the size of the 16 S rRNA gene is S rRNA is (1568 bp). As for the cytochrome b gene, its size is (1139 bp) and its location in relation to the mitochondrial genome of buffaloes starts from the end of the tRNA-Glu gene. At locus 14154 up to the start of the tRNA-Thr gene at locus 15293 [17].

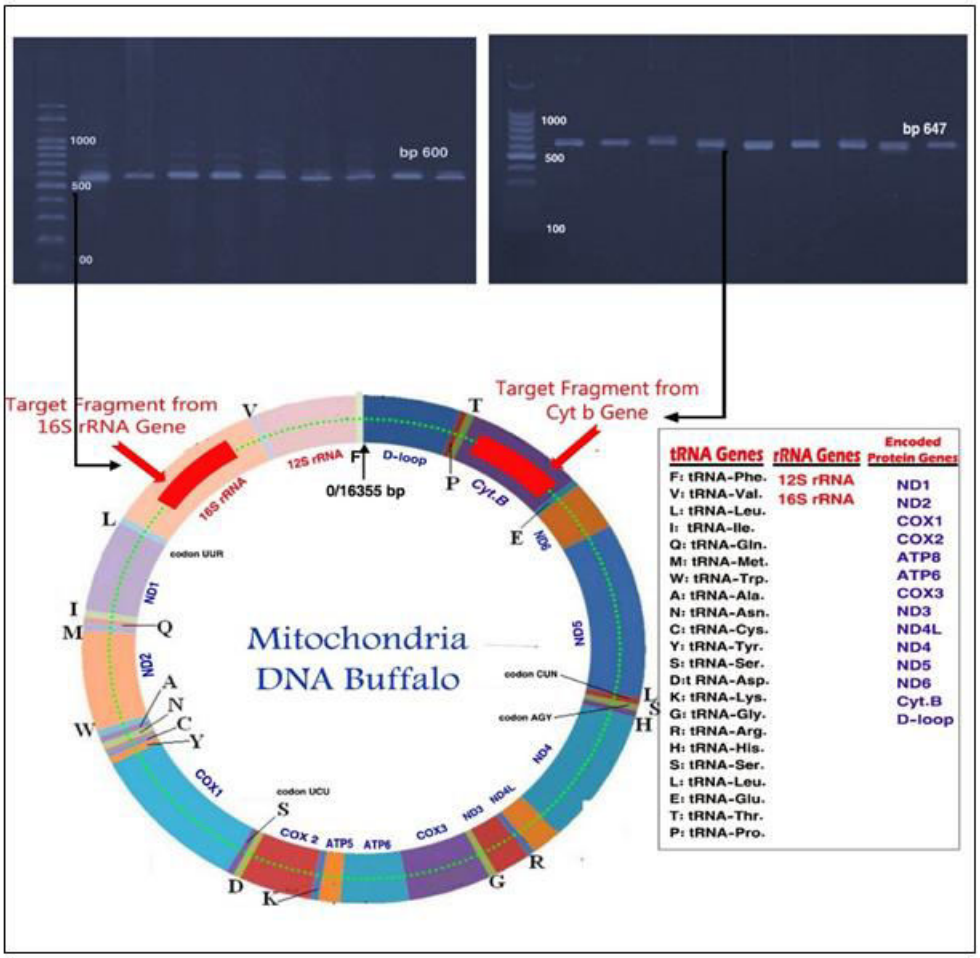

Figure 1, Buffalo mitochondrial genome map showing target segments of 16S rRNA and cytochrome b genes and electrophoresis images.

In the Iraqi indigenous buffalo under research, there are two haplotypes of the 16 SrRNA gene. For the Iraqi buffalo, one is a common H1 and the other is an isolated haplotype H7. When compared to buffalo from a set of nations around the world using reference copies from the GenBank (Table, 2). The total number of haplotypes was discovered to be 9, and the H1 was found to be common to a group of countries that included (Iraq, China, Iran, and India, and America) , Hplotype of Iraqi, Chinese, and Indian branched off from the H1 and formed a haplotype for them (figure, 1).

The results of Analysis of molecular variation (AMOVA) for 16 S rRNA gene table (2) in buffaloes showed that the proportion of molecular variation between countries was 41.08% and the proportion of molecular variation within countries was 52.92%.

Table 2, The molecular variation of the 16 S rRNA gene between the Iraqi local buffalo and other countries.

S. O. V	Df	SS	Variance Components	% Variation
Between Countries	8	22.751	0.2259	21.80
Within Countries	63	51.054	0.81038	78.20
Total	71	73.805	1.03628	

The results of Analysis of molecular variation (AMOVA) for cytochrome b gene Table (3) in buffaloes were identical to that of 16 S rRNA gene. The percentage of molecular variation between countries was 21.80% compared to 78.20 % for molecular variation within countries. It is clear from this result that the molecular variance between the breeds was less than the variance within the breeds, This may be due to the fact that the Iraqi buffalo and the Asian buffalo (China, India and Pakistan and other countries) are due to the same mother.

Table 3, The molecular variation of the cytochrome b gene between the Iraqi local buffalo and other countries.

S. O. V	Df	SS	Variance Components	% Variation
Between Countries	5	67.049	1.09214	41.08
Within Countries	112	175.443	1.56645	52.92
Total	117	242.492	2.65860	

The results of the network of haplotypes for the cytochrome b gene in buffaloes (Fig. 2) showed the presence of 4 haplotypes of the Iraqi local buffalo, including the H1 haplotype shared with other group of buffalo of different countries, and the three haplotypes H2, H3, and H4 independent. In comparison with the buffalo breeds of some countries of the world, the number of haplotypes formed was 20, including the H1 haplotype common between countries (Iraq, China, India, Pakistan, Romania and America). The results also showed that the haplotype H5 that branched from the first haplotype H1 was shared between the Chinese and the Indian buffalo , The haplotype H14 also shared between the Pakistani and the Netherlands buffalo ,

The results also showed that the haplotypes H19 and H20 followed the Indonesian buffalo independently, and the remaining haplotypes were distributed independently as follows , The haplotypes H6, H7, H8, H9 and H10 of the Chinese buffalo, the haplotype H11 belong the Roman buffalo , the haplotypes H12 and H13 for the Pakistani buffalo , and the individual patterns H16, H17 and H18 for the Japanese buffalo.

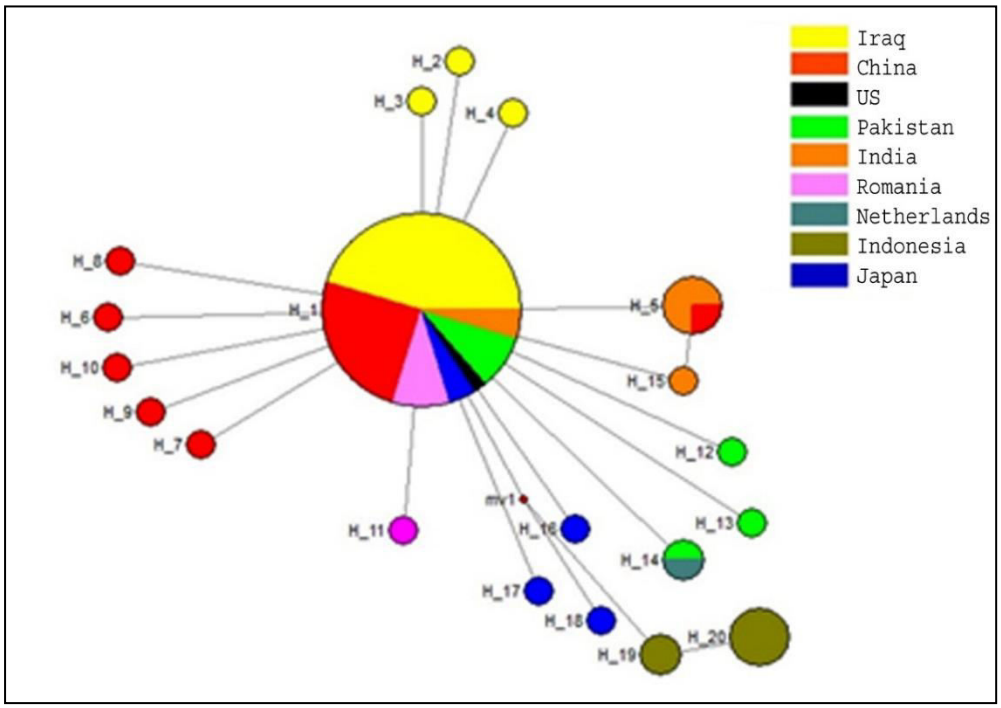

Figure 2, Haplotype network of Iraqi local buffalo and some other countries

The results of the phylogenetic tree of the 16 S rRNA gene, figure (3), for the Iraqi local buffalo compared to the buffalo of some countries, showed the presence of two main branches, the first branch included the Iraqi buffalo. The other main branch branched into secondary branches, including Chinese, Indian, Iranian, Russian, and Iraqi buffaloes.

As for the results of the phylogenetic tree of the Cytochrome b gene for Iraqi buffaloes and buffaloes in some countries, it also branched into two main branches. The local Iraqi buffalo participated in the first main branch with each of China, Russia, Pakistan, India, America, Japan and Romania. The second main branch was independent of the Indonesian buffalo.

The results of the phylogenetic tree almost coincided with the results of the network of individual patterns of 16 S rRNA and cytochrome b genes, where the buffalo lineages of a group of countries (China, India, Pakistan,

Japan and some other countries, in addition to the Iraqi local buffaloes) shared a single haplotype. It is clear from the results of the haplotypes network and the results of the phylogenetic tree for the 16 S rRNA and cytochrome b genes that the local Iraqi buffalo, and Chinese, Indian and Pakistani buffaloes in general are same origin.

According to [18] the mtDNA 16S rRNA gene sequences in 12 bovine breeds revealed a low GC content, did not differ considerably, and a median-joining network based on the mtDNA 16s rRNA gene for cattle phylogenetic analysis would be a major resource analysis marker. Variation in GC content among organisms was discovered to have a significant impact on evolutionary history reconstructions by geneticists [19].The most efficient molecular markers for evaluating genetic diversity, population differentiation, breed relationships, and determining paternity in animal populations are single-nucleotide polymorphisms [20]

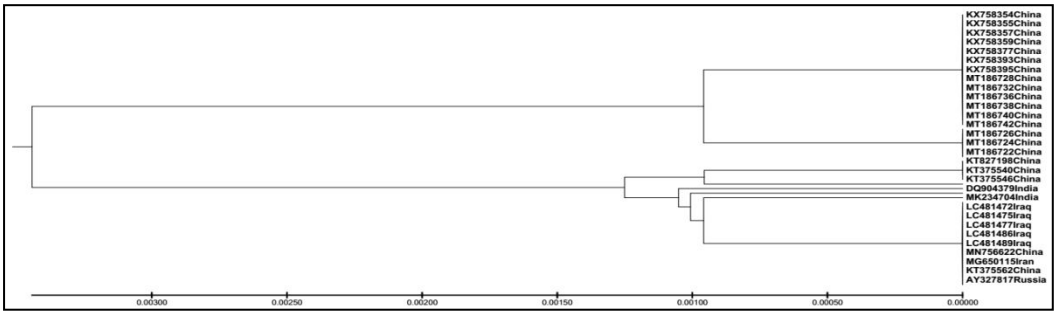

Figure3, Phylogenetic tree of S16 rRNA gene Iraqi local buffalo and some other countries

Figure 4, Phylogenetic tree of cytochrome b gene Iraqi local buffalo and some other countries

The studied segments of the target genes in the current study, S16 rRNA and cytochrome b of Iraqi local buffaloes, were recorded in the NCBI, EMBL and DDBJ Genbank sites with independent accession numbers starting from LC481471 to LC481490.*3.1. Subsection.*

4. CONCLUSIONS

It is clear from the results of the haplotypes network and the results of the phylogenetic tree for the 16 S rRNA and cytochrome b genes that the local Iraqi buffalo , and Chinese, Indian and Pakistani buffaloes in general are same origin.

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Conflict of Interest
There are no potential conflicts of interest for the writers to disclose.

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