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Genetic Diversity & Sequences Analysis for *Cyprinus carpio L.*, in IRAQ

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Abstract

The common carp, *Cyprinus carpio*, is of very importance to the economy of our country. In this study, the *cyt B* fragment was chosen to measure the genetic diversity of 30 individuals of the common carp. These fish were caught from one of the fish farms in the Nasiriyah city, they were similar in appearance to wild carp. The length of the selected *cyt B* gene fragment was 581 bp. A sequencing analysis was conducted by the Korean company Macrogen, A Tools and software were used to analyze the results of the *cyt B* sequences. The results of the molecular analysis indicated that the number of mutations in the *Cyt B* gene sequences in our current study reached 9 mutations, resulting in the formation of 3 haplotypes. The importance of gene diversity in haplotypes, Hd, was recorded as 0.43, while the value of Nucleotide diversity (per site), Pi: 0.00564, was evident from According to the results, the molecular variation AMOVA for the Cyt B gene of common carp fish within the population groups is greater and this is due to the fact that all individuals of these groups belong to the same mother, which makes the variation between the population groups less than the variation within them.

The neutrality test's findings revealed that the value of Tajima's D for the *Cyt B* gene for the local common carp fish was a positive value and was 0.692112. As for the fish of the United States and China, it recorded a negative result of - 0.93302 and - 0.49083. As for the statistical values of Fu's Fs, it was a positive result for both the local Iraqi fish and the American and Chinese fish, and the result was Fu's Fs. 0.9830, 0.25500 and 0.36300 respectively.The results of the network of haplotypes that were designed using Network V.10.2 software indicated that the sequences of the *cyt B* gene of the local carp fish were distributed into three individual haplotypes that were independent from the main haplotypes belonging to the sequences of Chinese fish. The Chinese sequences of the **cyt B** gene took 7 independent haplotypes and participated in the ninth haplotype is found in Russian fish, Taiwanese fish, and some American fish sequences are independent haplotypes derived from Chinese sequences. These results are similar to the results of the phylogenetic tree when comparing the sequences of the original study with reference copies.

Keywords: ***Cyprinus carpio, Cytochrome b, genetic divergence, haplotype.***

I. Introduction

Carp (Cyprinus carpio L., 1758) is among the first freshwater fish to be domesticated. Its cultivation dates back thousands of years and it is considered one of the most important fish currently farmed in the world. Common carp are distributed in Europe through western Asia to Japan, China and Southeast Asia. (Barus et al. 2001). The common carp (Cyprinus carpio) is an extremely important fish economically since it provides wholesome food for people all over the world. (Kohlmann et al., 2003). Carp farming plays a vital role in strengthening the local economy, as it is considered a major source of animal protein for the population, as well as providing many job opportunities. Carp farming in Iraq remains a promising field, with great potential to expand production and increase efficiency, provided that appropriate support is available and existing challenges are overcome. Population genetic structure can be complexly influenced by both anthropogenic and biological causes, particularly in cases when forms are propagated for commercial purposes. (Zenger et al., 2007). Biological and human factors influence the genetic variations of populations in a complex way, especially when the intent is commercial (Bruford et al., 2003; Zenger et al., 2007). Many

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scientific researches have dealt with the genetic characterization of the mitochondrial genome mtDNA in general or the use of some individual markers or genes within the mitochondrial genome as *Cyt B* Gene Choudhury, & Das, (2018) or control Region in mtDNA Thai, (2006) & Shuli, et al., (2022).

The molecular structure, phylogeny, genetic distribution, and origin of common carp populations have all been studied extensively using mtDNA techniques. This is due to the fact that mtDNA is a helpful molecule that may be used to establish genealogical ties among populations within species due to its quick rate of mutation and stochastic loss of haplotypes. (Avise 2000). These investigations, which used either the RFLP technique or sequencing, have shown cases of introduction and demonstrated that European common carp populations have the usual European haplotype (Lehoczky et al. 2005), proving that they belong to the evolutionary group of common carp populations that includes Europe and Central Asia. (Memis & Kohlmann 2006). (2002) Froufe et al.

No existing mtDNA research has been conducted on common carp populations in Iraq, so the first aim of our study was the molecular characterization of common carp from Iraq south. As we included in our molecular analysis individuals in our study, The second goal was to use reference copies from Genebank to investigate the evolutionary relationships of our common carp.

II. MATERIALS AND METHODS

A total of 30 individuals of common carp were collected and analyzed. These fish were caught from a fish farm in the city of Nasiriyah and were similar in appearance to wild carp, We used the Genaid Taiwan kit to extract DNA from the blood of fish using the protocol followed by the company, while changing the amount of blood taken from each fish to about 5 µl of blood instead of 100 µl due to the red blood cells (RBC) in the fish containing DNA, and after completing the extraction process, it was measured. Concentration and purity of DNA using a UV spectrophotometer nanodrop device at wavelengths 260/208. The concentration of DNA among the studied samples ranged between 55-120 ng/ µl.

In a PCR, the Cyt B gene was amplified in a 25 µl reaction volume with 1X PCR buffer (Green master mix 2x (preomega), 10 pmol of each primer and 50 ng genomic DNA using a thermal cycler (multigene, Germany). The primer pairs used for PCR were F: (5' CGCATTCCACTTCCTACTACC 3') and R: (5' CTAACCATCCTGCTAGTCGC 3') (Thai, 2006). One cycle of initial denaturation at 95 °C for 4 min was followed by 34 cycles (denaturation at 95 °C for 30 s, annealing at 55 °C for 1 min, and extension at 72 °C for 2 min) and a final extension at 72 °C for 7 min. This was the PCR temperature profile that was employed. Following 1% agarose gel testing of the PCR results, the most intense and specific products were chosen for sequencing. Sequencing of the studied region of Cyt B gene for the PCR product for all studied samples of common carp fish was done in Korean company Macrogen, after receiving the sequence results, a filtering process was carried out for the Cyt B gene sequences, and alignment of the sequences were performed using the Bio Edit program (Hall, 1999). The sequences were aligned using the Bio Edit application after the sequencing findings for every sample were compared to the standard sequences made available online by the National Center for Biotechnology Information (NCBI). (Hall, 1999).

Analysis of molecular variance (AMOVA), neutrality test including Tajima (D) and Mismatch distribution performed using by Arlequin software ver. 3.5.1.2 (Excover and Lesher, 2010). The phylogenetic tree was drawn by the neighbor-joining tree (NJT) method using the program (MEGA 7.0) (Kumar et al., 2016). Neighborhood trees (NJT) are used to clarify the relationships between the Cyt B Gene sequences of the fish samples in the current study among themselves or with the sequences that we obtained from the GenBank database. Haplotype diversity (HD) and nucleotide diversity (π) and other molecular parameters were calculated using DnaSP v5.10 (Librado and Rozas, 2009).

III. Results and Discussion

The results of agarose gel electrophoresis showed the successful amplification of the *Cyt B* gene, where the size of the PCR product was 581 bp (fig.1).

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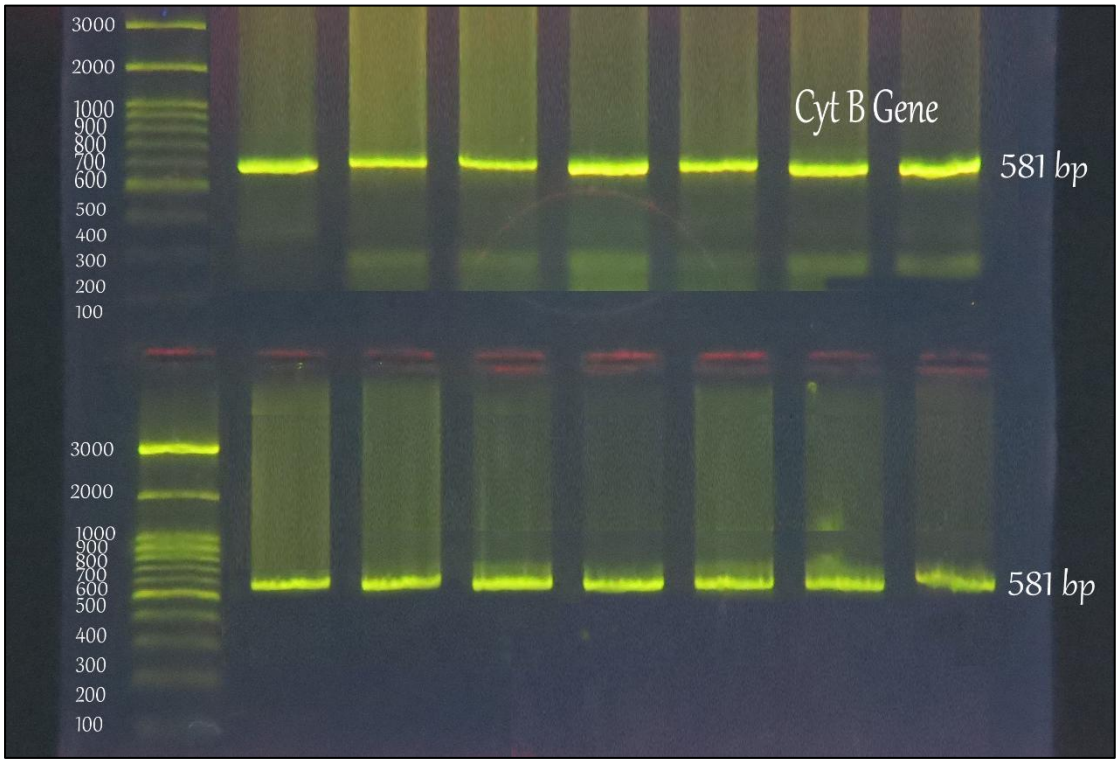

Figure. 1. *Cyt B* electrophoresis on agarose gel concentration 1.5%.

Genetic diversity

The results of the genetic diversity Tabel (1) of the *Cyt B* gene showed that the number of its total sequences is 113 sequences in all regions studied from the gene. The sequences were distributed to 30 fish in our current study and 83 reference copies were taken from the NCBI gene bank data, including 59 sequences from China, 12 sequences from Canada, 6 from the United States of America, 4 from Greece, in addition to one sequence for each of Russia and Taiwan.

The number of mutations in the sequences of the *Cyt B* gene in our current study reached 9 mutations, resulting in the formation of 3 haplotypes. The value of haplotype (gene) diversity (Hd) was recorded as 0.43, while the value of Nucleotide diversity (per site), Pi: 0.00564.

The results of the genetic diversity of the *Cyt B* gene were recorded. The number of reference copies was 83 sequences distributed among the countries of China, the United States of America, Canada, Greece, Taiwan and Russia. The number of variable sites was 18, resulting in 13 haplotypes. The percentage of GC bases in the Cyt gene sequence was recorded. B is 43.5%, and the values of Haplotype diversity (Hd) and Nucleotide diversity

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(Pi) for the comparison fish were 0.676 and 0.00530, which are close to the values we obtained for our local fish in the current study.

parameter	Current study	Reference Copies (NCBI)	All
Number of sequences	30	83	113
Number of variable sites	18	18	20
G+C content (%)	43.4	43.5	43.5
Number of Haplotypes	3	13	16
Haplotype diversity(Hd)	0.432	0.676	0.787
Nucleotide diversity(Pi)	0.00564	0.00530	0.00636
Average number of nucleotide differences(k)	2.77701	2.61005	3.13148

Table (1): It shows the results of the genetic diversity of the Cyt B gene for local fish and Reference Copies (NCBI)

AMOVA analysis

AMOVA analysis Tab.2 is an extension of analysis of variance (ANOVA), which divides the total genetic variance into genetic variance between populations and genetic variance within each population. AMOVA compares the ratio of Sum Square of Differences (SSD) to Mean Square Deviations (MSD) between group hierarchies (Excoffier and Lischer, 2010).

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among populations	6	44.961	0.51657	29.63
Within populations	107	131.258	1.22664	70.37
Total	113	176.211	1.74320	

Table (2): It shows the results of AMOVA analysis for Cyt B gene among & within populations

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The results of molecular variation between *Cyt B* gene sequences of local carp and comparative sequences (Reference copies) from the NCBI Gene Bank showed that the genetic variation within populations outweighed the molecular variation between populations, with values of 70.37 and 29.63, respectively. The results gave sum of squares values of 131.258 and 44.961 for variation within populations and outside populations, respectively. It is clear from the results that the AMOVA molecular variation of the Cyt B gene for common carp fish within populations is greater. This is due to the fact that all members of these populations are due to the same mother, and this is what makes the variation between populations there is less variation than within them.

Neutrality Test

The Neutrality Test (Tajima's D test) is important in analyzing haploid data, such as mtDNA sequence data. The Tajima's D test is used to compare pairwise sequences (Tajima, 1989). The value of Tajima's D for the *Cyt B* gene for the local common carp fish recorded a positive value and was 0.692112, the United States and China recorded a negative result of - 0.93302 and - 0.49083, respectively. As for Canadian and Greek fish, the value of Tajima's D was 0.00000 because there were no genetic differences between the fish. The result of Tajima's D, calculated on the basis of all the totals studied (current and comparison from the Genebank) for all countries, showed a negative result. It recorded -0.48525 without any significant effect.

The reason for the positive Tajima's D value for local fish may be due to their obtaining a greater number of mutations with high and medium frequency, and this indicates genetic drift. In general, the negative value of the Tajima's D test for fish from the comparison countries is due to the stability of the populations and they were not under any pressure. Evolutionary, (Aris-Brosou and Excoffier, 1996).

On the other hand, the statistical value of Fu's Fs recorded a positive result for both local Iraqi fish and American and Chinese fish. The result of Fu's Fs was 0.9830, 0.25500 and 0.36300, respectively. Canadian and Japanese fish did not record any significant result due to the lack of genetic differences between the fish studied for these two groups, with

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no significant statistical significance. The medium but not high positive Fs values are due to the presence of fewer haplotypes than expected in the population, The total Fu's Fs value for all fish studied and compared from the gene bank for all countries was negative and was recorded as - 2.162, this is due to the heterogeneity of haplotypes between countries and due to the presence of more haplotypes than expected if we consider all the fish studied and compared as one population group. (Fu, 1997)

Country	Sample size	Tajima's D Statistic	Fu's Fs Statistic
Iraq	30	-0.69112	0.98300
USA	6	- 0.93302	0.25500
Canada	12	0.00000	*
China	60	- 0.49083	0.36300
Greece	4	0.00000	*
All	113	-0.48525 N. S	- 2.162 N.S

Table. 3: It shows the results of Neutrality Test for Cyt B gene

Phylogenetic tree & Haploypes Network

The phylogenetic tree for the *Cyt B* gene was designed using the Neighbor-joining tree (NJT) model. The results of the fig.2 indicated that the sequences of the Cyt B gene for local fish were distributed into three regions within the tree in a manner similar to what is found in the Haplotype Network, where the majority of individuals for fish were found in two positions. Within the current study, there is a secondary branch within the main branch, which was mostly Chinese, Canadian, American, and Greek fish, according to the order of their numbers, respectively, and in another secondary branch, we also find that the rest of the local sequences were present within another main branch consisting only of Chinese sequences.

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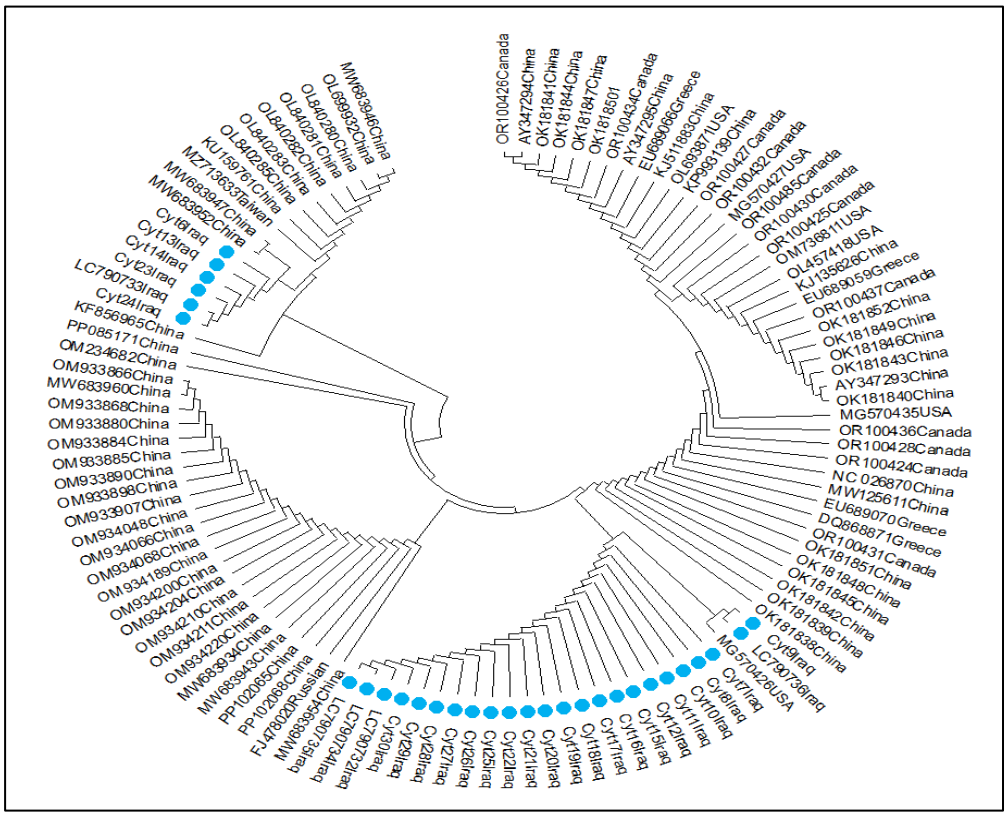

Figure. 2. Phylogenetic tree for Cyt B Gene for local fish and Reference Copies (NCBI) designed by Mega V.11 (Neighbor-joining tree "NJT" model)

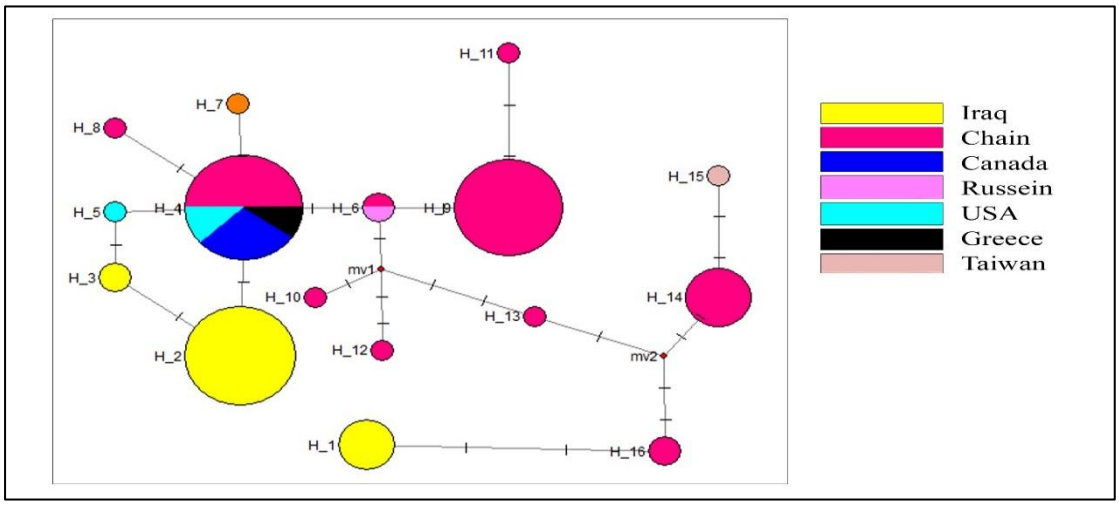

Figure. 3. haplotypes Network for Cyt B Gene for local fish and Reference Copies (NCBI) designed by using Network V.10.2 Software

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Haplotype Network helps us determine the evolutionary relationships between different haplotypes, providing an understanding of how populations evolve from each other over time, usually sequences of individuals from different geographical regions around the world to know the genetic variation between populations and its evolution and the impact of climate changes or human activities on the distribution of haplotypes. In the world, and in our current study, we used, to compare with the sequences of the *Cyt B* gene of the 30 local carp fish, 83 sequence of reference copies from different locations around the world taken from the NCBI gene bank database (China, Canada, America, Greece, Taiwan, and Russia). Through the results of the network of haplotypes Fig. 3 that were designed by using Network V.10.2 Software (Bandelt et al., 1999)., it was noted that the sequences of the *Cyt B* gene of the local carp fish were distributed into three individual haplotypes that were independent of the main haplotypes belonging to the sequences of Chinese fish. The countries of Canada, America, and Greece participated with them, and the Chinese sequences of the gene were taken. Cyt B 7 independent haplotype. The ninth haplotype was shared with Russian fish, while Taiwanese fish and some American fish sequences had independent patterns derived from Chinese sequences.

IV. Conclusion

It is of great importance to conduct scientific studies on fish species in Iraq and genetic characteristics to determine the extent of genetic diversity and to conduct studies to preserve these species genetically. By reviewing the results in the current study, we found that there is no significant change in the region of the *cyt B* gene, which is somewhat similar to the international strains. To confirm this, the work requires conducting larger studies on fish groups from different locations in the country, in addition to studying the whole mitochondrial genome of common carp fish.

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