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Research Article

THE EFFECT OF POLLUTION ON FISH

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Abstract

In this work, A particular PCR fragment was chosen, and it only covered a portion of the coding regions of the gene producing cytochrome c oxidase subunit I.. Sanger sequencing assays were used on fish samples collected in amplified sections in order to evaluate the pattern of genetic variation. The identification of the observed variations and their phylogenetic distribution was then evaluated using a specific comprehensive tree that was created. By using sequencing reactions, the examined Eurasian carp samples were identified with accuracy. It was confirmed that S21 to S30 belonged to Eurasian carp (*Cyprinus carpio*). As just one nucleic acid substitution was found in some of the studied Eurasian carp samples, additional information was also seen from the investigated sequencing processes. One nucleic acid substitutions (107T>C) were detected in the Eurosian carp samples with one silent (p.59T=) effect. Five phylogenetic clades with various phylogenetic distances were included in the phylogenetic tree that was created. The tree suggested that the Eurasian carp group and the clade of Prussian carp were compatible neighbors. In compared to the other analyzed wild-type sequences indicated a little impact of the observed differences on the evolutionary placement of fish samples. This resulted from the variation S21 sample being positioned somewhat apart from the other Eurasian carp group wild-type samples. This study discovered that every genetic locus that was used, COX1, was capable of accurately identifying 10 Eurasian carp isolates and differentiating them from the other outgroup samples. Additionally, among the other methods used, this study proposes the potential use of cytochrome c oxidase subunit I amplicons, which have the highest specific capacity to distinguish between phylogenetic diversity. These PCR fragments may effectively be utilized to identify the biological diversity of a larger variety of fish genomes, and can thus be investigated to learn more specifics about these discovered groupings.

Keywords: Polymorbhis, Cytochrome oxidase, Gene-Diversity, Sequence.

INTRODUCTION

Is the major species being farmed in Iraq's central and southern regions. It was introduced to various Inland waters such as lakes, dams, and streams, is the third most significant farmed freshwater species in the world (Vilizzi *et al.,* 2015; Ljubojevi *et al.,* 2016) .Furthermore, it is the most significant in Eastern Europe. Carp is a hardy fish that can survive near low oxygen conditions, extensive temperature ranges (1-35C°), and a broad pH range (5-9) in addition to having moderate halo-tolerance (0.5-5 practical salinity units). In 2016, the overall world output of common carps was 4.557 million tons, accounting for 8% of the principal species forms generated in aquaculture (Woynarovich *et al.,* 2011; FAO, 2018). Carp inhabits small ponds, cages, and being an omnivore, it consumes aquatic plants, benthic invertebrates, insects, crustaceans, and artificial food. However, aquaculture projects have failed in recent years as a result of extensive destruction following the fall of the old administration in 2003, resulting in the cancellation of the bulk of permits and a drop in the number of fish farms. In order to satisfy their families' minimal daily subsistence requirements, farmers began to seek alternate forms of revenue (Pillay and Kutty, 2005). Warm, deep, slow-moving and motionless waters, including lowland rivers and large lakes. All body types along shorelines and backwaters. Larvae only thrive in warm water and shallow vegetation. Males reproduce between 3 and 5 and females between 4 and 6. Reproduces annually and lives to 50. Latitude/altitude/maturity age May and June at 18C°. Adults spawn in backwaters and flooded meadows. Males and females spawn in dense vegetation. The adhesive eggs stick to underwater plants and things.

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Larvae and juveniles live in warm, shallow, flooded river margins and backwaters (rotifers). Reproduction is confined to years when the water level rises in May and high temperatures and flooding of terrestrial vegetation persist in May and June. Adults and juveniles feed benthic animals and plants. Dusk and morning are busy. Low-oxygen-tolerant (Freyhof and Kottelat, 2008). Carp farming has become an important feature of many nations' economies since common carp was introduced. In other wealthy nations, such as the U.S. and Australia, the species is considered a nuisance and efforts have been made to exterminate it, common carp has harmed various aquatic environments. Carp have destroyed aquatic flora and weeds. Excavating and churning the bottom layer raises the water's turbidity. Light penetration decreases, eradicating macrophyte colonies in phytophilic spawning grounds. Carp may eat other species' eggs and help sustain wetlands' ecosystem by destroying spawning substrates, eating native species' eggs, and competing with similar-feeding species, carp may reduce native species' populations (Rahman, 2015; FAO, 2019; ISSG, 2021).

MATERIALS AND METHODS

PCR amplicon nucleic acid sequencing

Following the procedures given by (Macrogen Inc, South Korea), the PCR-amplicons were sequenced in both directions (forward and reverse). Additional analyzes performed only on the clear chromatograms obtained from the ABI sequence files. These extra steps make sure that changes and annotations aren't the result of PCR or sequence components. By comparing the nucleic acid sequences of the recovered samples with those of the surrounding samples, further information

about the recoverable PCR fragments and the possible individuals may be ascertained.

Data interpretation for sequencing

Bio Edit-Alignment Editor-Software Version 7.1 was used to edit the sequencing results of the targeted PCR products, as well as their alignment and evaluation against the matching sequences in the reference database. When a specific modification is detected in a sequenced sample it is correspondingly assigned a number that matches its position in the reference genome and its PCR amplicon. Version 4.0.4 of SnapGene Viewer was used to evaluate the fish sequences in addition to giving the nucleic acids numbers in the PCR amplicon.

Anima acid residues are created from several forms of nucleic acids

In this case, the amino acid sequence was obtained from the well-known internet sources that contain the protein data bank. DNA differences of the genetic sites that are being studied were discovered in the coding section and were processed using the Expasy tool through the Internet to a similar reading that matches the rest of the amino acids. An alignment of the amino acid sequences between the reference acids and their mutated equivalents was done using the text "align" of the BioEdit service.

The development of a thorough phylogenetic tree

Using the neighbourhood-joining approach, this work created a particular comprehensive tree (Sarhan *et al.,* 2019). Several comparisons were carried out using the NCBI-BLASTn service, especially on variants found with their closely matched reference sequence (Zhang et al. 2000). Following that, the observed variance was included into a fully inclusive tree that was produced using the neighbour-joining method and shown (Letunic and Bork, 2019). Each sequence in the comprehensive tree was assigned a distinct color to represent the group it was a part of.

RESULTS AND DISCUSSION

Ten samples (designated as S21 to S30) were included in the current analysis inside this locus. The COX1 gene sequences in Eurasian carp (Cyprinus carpio) were amplified using these samples (S21–S30). As a result, the COX1 gene variation can be utilized to characterize fish due to its potential adaptability to varying genetic variety as was shown in many fish populations. After executing NCBI blastn for these PCR amplicons, the sequencing reactions confirmed the precise identification. For the 655 bp amplicons, there was up to 99% sequence identity between the reference target sequences and the sequenced samples, according to the NCBI BLASTn engine. By contrasting the returned nucleic acid sequences (GenBank acc. KX505166.1) with the tested samples' observed nucleic acid sequences, The obtained PCR fragments' precise locations and other information were found. The NCBI Blastn tool correctly recognized the S21-S30 samples under examination when compared to the reference sequences (GenBank entry MF180230.1). The target's start and finish locations were validated inside the target with the highest degree of homology, and the targeted locus' total length was discovered in the NCBI server (Fig. 1).

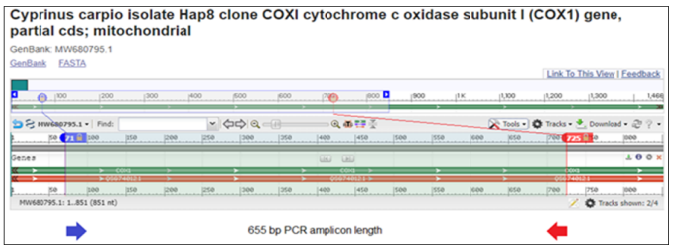

Figure 1. The precise location of the obtained 655 bp amplicaon, which partially encompassed the COX1 gene in 10 samples of genomic sequences from Eurasians (GenBank accession number MN180091.1). The blue arrow denotes the amplicon's beginning, while the red arrow designates its termination

Table 1. The genomic sequences of Eurasian carp were examined to determine the location and size a segment of the COX1 gene that was amplified using a 655 bp PCR amplicon (GenBank accession no. MN180091.1).

Amplicon	Reference locus sequences (5′ - 3′)	length
COX1 gene nucleic acid sequences of Eurasian carp (S21-S30)	CCTGAGCCGGAATAGTAGGAACCGCCTTAAGCCTCCTCATTCGGGCCGAACT TAGCCAACCCGGGTCACTTCTAGGTGATGACCAAATTTATAACGTTATCGTC ACTGCCCACGCCTTTGTAATAATTTTCTTTATAGTAATGCCTATCCTTATTG GAGGATTTGGAAACTGACTTGTACCACTAATAATCGGAGCCCCAGACATAGC ATTCCCACGAATAAATAACATAAGCTTCTGACTACTACCCCCATCATTCCTT CTACTCCTAGCTTCTTCTGGTGTTGAAGCTGGAGCTGGAACAGGATGAACCG TATACCCACCTCTTGCAGGGAACTTAGCCCACGCAGGAGCATCAGTAGACCT AACAATTTTCTCACTTCACCTAGCAGGTGTTTCATCAATTCTAGGGGCAATC AACTTTATTACTACAACCATCAACATGAAACCCCCAGCCATCTCTCAATACC AAACACCCCTGTTCGTCTGATCCGTGCTTGTAACCGCCGTATTGCTCCTTCT ATCATTACCTGTTTTAGCCGCAGGAATTACAATGCTCCTAACAGACCGAAAC CTTAATACTACATTCTTTGACCCGGCAGGAGGAGGAGACCCAATCCTTTATC AACACTTATTCTGATTCTTCGGCCACCCAGA	655 bp

The features of sequences of the 655 bp amplicons were emphasized, moreoverthe amplified amplifiers' whole length was ascertained. (Table 1) after the identification of the sequences within the Eurasian carp genomic sequences. The S21 sample of Eurasian carps included just one nucleic acid variation, represented by one nucleic acid substitution, according to the alignment findings of the 655 bp samples, namely 107T>C (Fig. 2). Our results showed that there was only one nucleic acid variation, as mentioned above. The S21 Eurasian carp group had the observed mutation, 107T>C, which had a little effect on cytochrome c oxidase subunit one due to p.59T=. The sequencing chromatograms of the analyzed samples were checked, documented, and thoroughly annotated to support the observed alterations. The ' sequencing chromatograms were then shown in accordance with the locations of the PCR amplicons. (Fig. 3) The detected nucleic acid variation underwent further analysis to see if the substitution would result in a change to their locations in the cytochrome c oxidase. Using the Expasy translate suite, The amplified S21 through S30 PCR products' whole nucleic acid sequences were converted to the matching amino acid sequences (Fig. 4). When these amino acid sequences were compared to their respective references, it was discovered that the identified nucleic acid variation of 107T>C had a quiet (synonymous) effect on the changed COX1 protein (p.59T=). In the current study, a thorough phylogenetic tree was created based on variations found in the amplified 655 bp of the COX1. In addition to other associated silver carp nucleic acid sequences, grass carp, This phylogenetic tree contained S21 to S30 samples of the Eurasian carps, as well as sequences from the Binni group and prussian carp. By adding our study samples to this tree along with other relevant sequences, 5 major clades of contained sequences. Along with the dominant Eurasian clade, four major clades were also represented as outgroup in the same tree. The Prussian carp, grass carp, silver carp, and Binni group sequences, which were fitted in different evolutionary clades apart from one another's sequences, represent these clades.

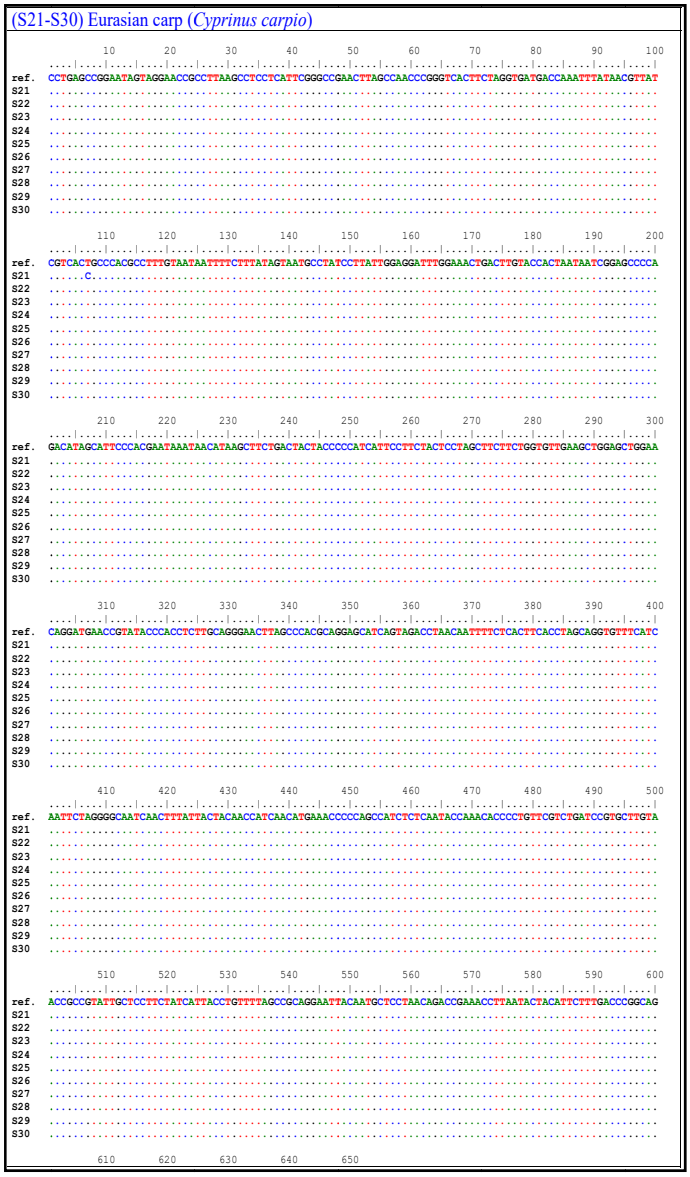

Figure 2. The nucleic acid sequences from 10 Eurasian carp samples were aligned with the pertinent reference sequences for the COX1 sequences' 655 bp amplicons

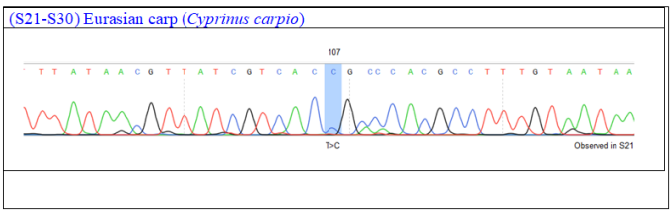

Figure 3 shows a chromatogram of the DNA sequences from Eurasian carp. The analyzed samples in this study that have this variation have the letter "S" as their code.

This finding suggested that the five integrated species within the same tree had an intermediate degree of similarity. Apart from this revelation, the results showed that COX1 amplicons could identify these fish without showing any obvious resemblance with other sequences of related species or outgroup sequences. 51 nucleic acid sequences in all were aligned and included in this extensive tree. As indicated above, Within the integrated sequences of Eurasian carp, 5 phylogenetic clades, each with known phylogenetic distances, were formed from the investigated samples, silver carp, Prussian carp, and Binni.

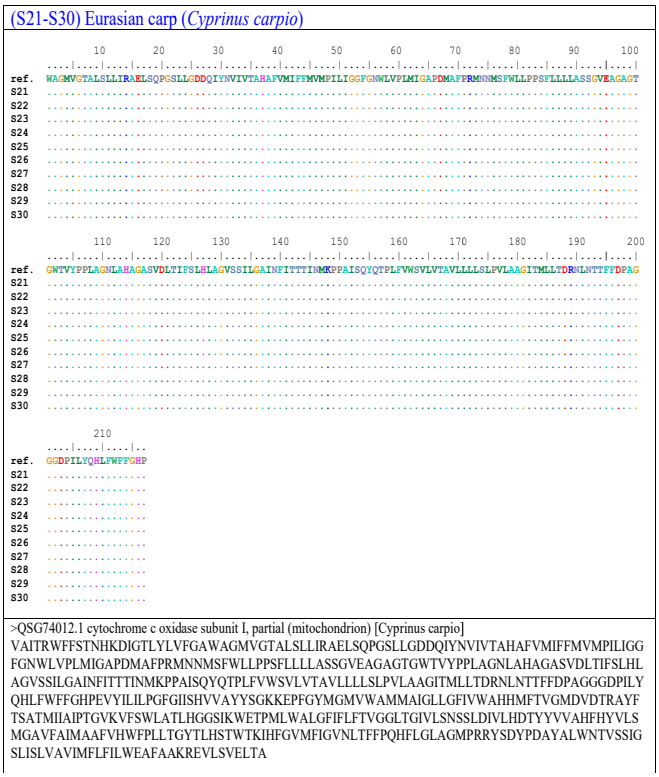

Figure 4. The amino acid residue alignment of the cytochrome c oxidase subunit I variations that were found in the samples of Eurasian carp that were under investigation. The differences are noted based on where they are located both inside the amplified 655 bp segment and across the full protein. The gray highlights indicate the increased area of cytochrome c oxidase subunit I, which is encoded by COX1. The yellow tones in the alignment chart indicate the silent mutation

From the other outgroups in this phylogenetic distribution, which include Prussian carp, silver carp, and Binni sequences. The Eurasian carp group's placement next to the Prussian carp group is one of the most intriguing features of the tree as it is currently constructed. This indicated that these two groups shared a closer homology with a certain level of genetic relations (Fig. 5A). On the contrary, grass carp as they were placed farthest from the present tree's roots. The clade of Eurasian carp (S21-S30), 12 sequences of the species were also incorporated within this clade. Within the same main clade, our S21–S30 sequences showed two different distribution patterns. This is because the S21 samples have one nucleic acid substitution compared to the reference sequences of the Eurasian carp group that correspond to them. This mutation caused the variant S21 sample to move somewhat. As indicated in the grass carp clade, another slight deviation was also notified within this clade, which showed a little phylogenetic effect of the observed variant in inducing any remarkable alteration. The samples that were examined within the Eurasian carp group demonstrated how different strains of the Eurasian carp sequences aligned with these samples close to different strains that were derived from Chinese origin (GenBank acc. no. MW680795.1). From the created tree, it was deduced that the changes discovered in the fish samples, as compared to the other analyzed wild-type fish samples, had a modest evolutionary influence due to the nucleic acid substitutions that were detected. This pattern of sample placement suggested that the discovered genetic mutation may have had a minor evolutionary impact, leading to a potential departure in the samples' evolutionary placing.

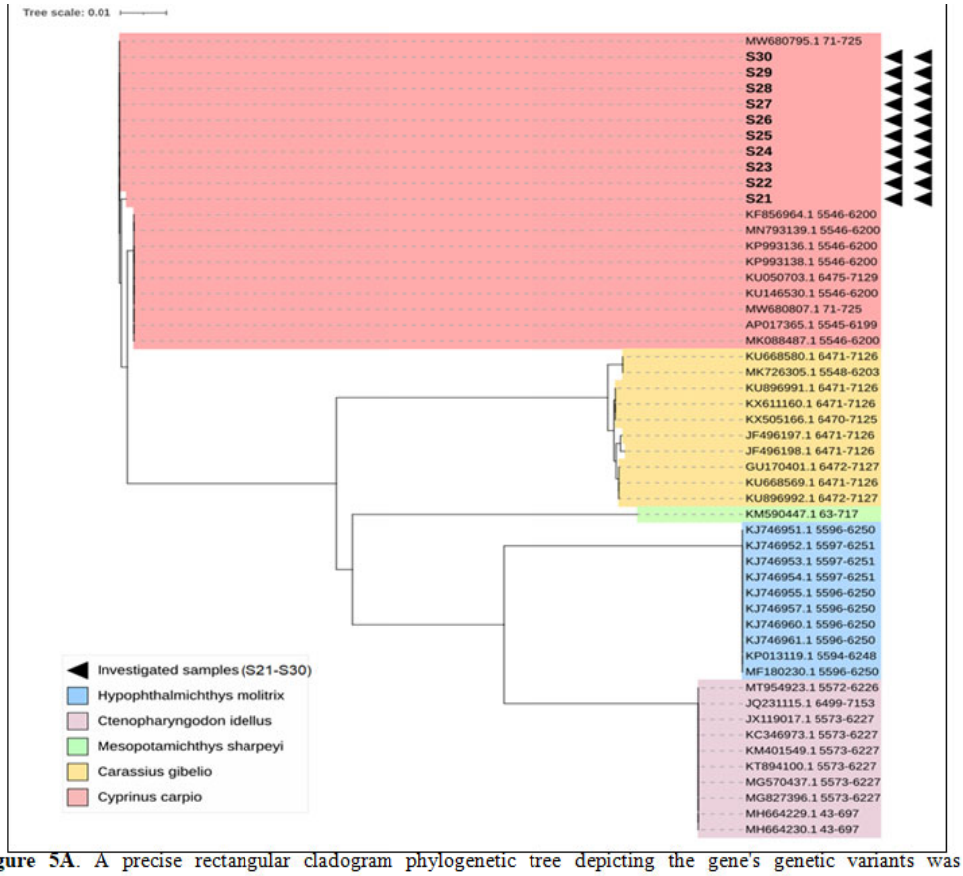

Figure 5A. A precise rectangular cladogram phylogenetic tree depicting the gene's genetic variants was generated using ten samples of Eurasian carp. The triangle in black depicts the different fish species that were investigated. All of the numbers corresponded to the linked species' GenBank entry numbers. The number "0.01" at the top of the tree indicates the scale range for each of the creature classes. "S#" stands for the code for the samples that are the subject of the inquiry.

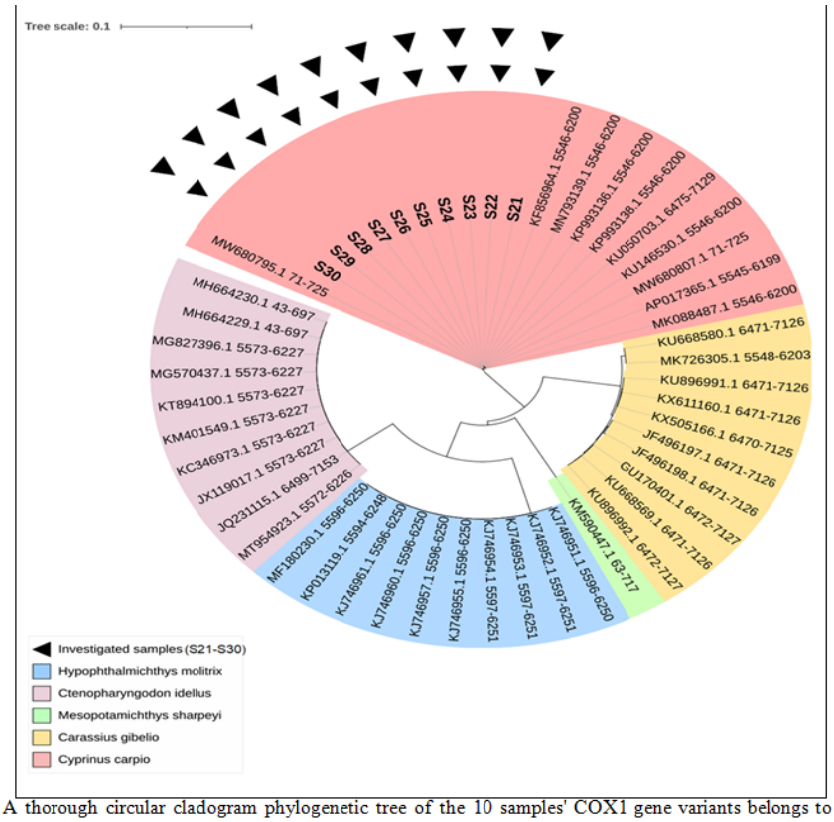

Figure 5B. A thorough circular cladogram phylogenetic tree of the 10 samples' COX1 gene variants belongs to Eurasian carp. The triangle in black depicts the different fish species that were investigated. All of the figures corresponded to the linked species' GenBank entry numbers. At the top of the tree, the value "0.1" represents the complete range of degree of scale for animals according to the tree's classification. The letter "S#" stands for the code for the samples under inquiry.

The Eurasian carps and the other samples of grass carp, silver carp, Prussian carp, and Binni groups showed notable evolutionary distances, which suggested that the COX1 sequences used in the currently used PCR products had a high resolution for effective detection and discrimination with related organisms. Because it indicated how the real neighbour-joining-based placement in these reported alterations was determined, the present observation of this tree has corroborated sequencing reactions. Interestingly, we were unable to overlook the Asian origin of our samples. Intriguingly, the use of COX1 gene sequences in this work has provided more evidence for the existence of the precise identification of these kinds of fish sequences. This COX1 gene-based comprehensive tree has given detailed proof of the great ability of such genetic segments to accurately detect this type of phylogenetic distribution. The capacity of the COX1 gene-specific primers now in use to characterize the examined Eurasian carps and their accurate phylogenetic differentiation from its related species is further demonstrated by this.

Conclusion

This research confirmed that all of the COX1 genetic loci used were able to distinguish between five different groups of fish samples and determine their common ancestor. Furthermore, the study provides support for the use of cytochrome c oxidase subunit I amplicons, which, when compared to the other approaches already in use, offer the best specific capacity to distinguish between phylogenetic diversity. By utilizing these PCR segments to investigate these newly identified groups, it is possible to effectively identify the biological diversity across a wider range of fish genomes. The kind of fish feed has a significant impact on the quantity and quality of meat minerals, which are correlated with cholesterol, triglycerides, and omega-3 fatty acids. Research into minerals is important for understanding the results of human fish eating. The highest protein concentration, iron and zinc content recorded in H. molitrix, highest Omega-3 and carbohydrate, cholesterol and phosphorus content were in Prussian carp, total calcium content highest in C. carpio.

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