Ann. For. Res. 65(1): 6238-6252, 2022
ISSN: 18448135, 20652445

ANNALS OF FOREST RESEARCH
https://www.e-afr.org/

SEQUENCING REPORT OF *COX1* GENE-BASED AMPLICONS IN TEN ISOLATES OF GRASS CARP

Maha Ali Mohammed* and Ameera Omran Hussein Aljanabi
Environmental Pollution Department, College of EnvironmentalSciences Al-Qasim Green University, Iraq
***Emai:** Mahaaljboory2017@Gmail.com

Abstract:
One specific PCR fragment partially covering the coding regions of the cytochrome c oxidase subunit I encoding gene was selected in this study. The amplified fragments were directly exposed to Sanger sequencing experiments to assess the pattern of genetic polymorphism in the collected fish samples. Then, a specific comprehensive tree was generated to assess the accurate identification of the observed variants and their phylogenetic distribution. Sequencing reactions showed the accurate identity of the investigated samples due to the presence of one species, as it was confirmed that S1-S10 belonged to grass carp (*Ctenopharyngodon idella*). Further details were also observed from the investigated sequencing reactions as a total of five nucleic acid substitutions were observed in some of the analyzed fishes. It was found that the most variable type was grass carp due to the presence of five nucleic acid variants compared with its referring sequences (GenBank acc. no. MG827396.1). The identified five nucleic acid variations (324T>C, 506T>A, 609T>A, 473T>C, and 578T>G) were found in some of the grass carp with three silent (p.135N=, p.196L=, and p.230L=) and two missense (p.185V>A and p.220F>C) effects respectively. The generated phylogenetic tree was constructed to incorporate five phylogenetic clades with distinct phylogenetic distances. It was inferred from the tree that the grass carp group was suited in the vicinity to the clade of Prussian carp. This positioning referred to the fact that both species shared the highest homology than the other incorporated outgroup species. The detected nucleic acid substitutions showed a slight effect of the observed variations on the evolutionary positioning of grass carp samples in comparison with the other investigated referring sequences. This was due to the positioning of the variant samples (S1, S2, and S6) slightly away from the other wild-type seven samples of the grass carp group.
Keywords: *COX1(cytochrome oxidase) , Polymorbhis , Sequence , Gene Diversity , Grass Carp.*

Introduction:

Grass carp is the most-studied and-used fish for controlling submerged aquatic weeds. This species lives in Asia's major rivers. In Iraq, is a popular industrial fish that grows enormous in local markets. Japan introduced it to Iraq in 1968 for pond breeding (Fisher and Lyakhnovich, 1973; Shireman and Smith, 1983). Grass carp are known for their capacity to transform plant proteins into animal proteins through direct digestion and absorption, as well as for their excellent and nutrient-rich meat, extensive environmental adaptation, and great temperature tolerance (S. Liu et al., 2013; Q. Liu et al., 2013). Ctenopharyngodon has just grass carp, there's no subspecies

(Shireman and Smith 1983, N. Bogutskaya, Zoological Institute, Russian Academy of Sciences, St. Petersburg (Shireman and Smith 1983, Chilton and Muoneke 1992). This species has a large, scaleless head, a sub-terminal or terminal mouth with simple lips, no barbells, and a very short snout less than or equal to its eye diameter. Postorbital length is more than half head length, and the body is thin and compressed with a rounded belly and large, somewhat decurved lateral line.The fins feature 7, 8, and 18 rays, respectively, Spineless dorsal, anal fins, dark-edged, black-spotted cycloid scales (35-45 lateral count), 12 unfused, lanceolate, broadly placed gill rakers, biserial pharyngeal teeth measure 2.5-4.2 to 2.4-5.2. Five biopsies revealed 49 loci on 48 diploid chromosomes (Page and Burr 1991; Eccles 1992, Opuszynski and Shireman 1995; Keith and Allardi 2001).

The present study was conducted to identify the pattern of the genetic variation of the investigated *COX1* locus in ten samples of *Ctenopharyngodon idella*. Based on the genetic variants of the investigated *COX1*, the pattern of the genetic diversity of these organisms was assessed in these samples (assigned S1 – S10) in the Middle Euphrates region in Iraq.

Materials and Method:

- **Nucleic acids sequencing of PCR amplicons**

The resolved PCR amplicons were commercially sequenced from both (forward and reverse) directions, following instruction manuals of the sequencing company (Macrogen Inc. Geumchen, Seoul, South Korea). Only clear chromatographs obtained from ABI (Applied Biosystem) sequence files were further analyzed, ensuring that the annotation and variations are not because of PCR or sequencing artifacts. By comparing the observed nucleic acid sequences of local samples with the retrieved nucleic acid sequences, the virtual positions, and other details of the retrieved PCR fragments were identified.

- **Interpretation of sequencing data**

The sequencing results of the PCR products of the targeted samples were edited, aligned, and analyzed as long as with the respective sequences in the reference database using BioEdit Sequence Alignment Editor Software Version 7.1 (DNASTAR, Madison, WI, USA). The observed variations in each sequenced sample were numbered in PCR amplicons as well as in their corresponding position within the referring genome. The observed nucleic acids were numbered in PCR amplicons as well as in their corresponding positions within the referring genome. Each detected variant within the fish sequences was annotated by SnapGene Viewer ver. 4.0.4 (https://www.snapg ene.com).

- **Translation of nucleic acid variations into anima acid residues**

The amino acid sequences of the targeted protein were retrieved online from the protein data bank (http://www.ncbi.nlm.nih.gov). The observed nucleic acid variants in the coding portions of the analyzed genetic loci were translated into a reading frame corresponding to the referring amino acid residues in the encoded protein using the *Expasy* online program (http://web.expasy.org/translate/). Multiple amino acid sequence alignment was conducted between the referring amino acid sequences and their observed mutated counterpart using the "align" script of the BioEdit server.

- **Comprehensive phylogenetic tree construction**

A specific comprehensive tree was constructed in this study according to the neighbour-joining protocol described by Sarhan *et al.* (2019). The observed variants were compared with their neighbour homologous reference sequences using the NCBI-BLASTn server (Zhang *et al*. 2000). Then, a full inclusive tree, including the observed variant, was built by the neighbour-joining method and visualized as a circular cladogram using the iTOL suit (Letunic and Bork, 2019). The sequences of each incorporated group in the comprehensive tree were colored in an appropriate color.

Results and Discussion :

Within this locus, ten samples were included in the present study (assigned as S1 to S10). These samples were screened to amplify the *COX1* gene sequences of one group of fish, namely grass carp (*Ctenopharyngodon idella*) (S1-S10). Thus, the variation of the *COX1* gene can be used for fish characterization due to its possible ability to adapt to variable genetic diversity as was seen in different fish organisms. The sequencing reactions indicated the exact identity after performing NCBI blastn for these PCR amplicons. Concerning the 655 bp amplicons, the NCBI BLASTn engine showed about 99% of sequence similarity between the sequenced samples and the intended reference target sequences. By comparing the observed nucleic acid sequences of these investigated samples with the retrieved nucleic acid sequences (GenBank acc. MG827396.1), the accurate positions and other details of the retrieved PCR fragments were identified. The NCBI Blastn suit identified the presence of one identity for the currently investigated S1-S10 samples. Grass carp was the identity of the first ten samples (S1-S10) with about 99% homology with the reference sequences of Grass carp (GenBank acc. MG827396.1). The total length of the targeted locus was localized in the NCBI server, and the positions of the start and end of the targeted locus were also confirmed within the most homologous target recognized (Fig. 1).

(S1-S10) grass carp (*Ctenopharyngodon idella*)

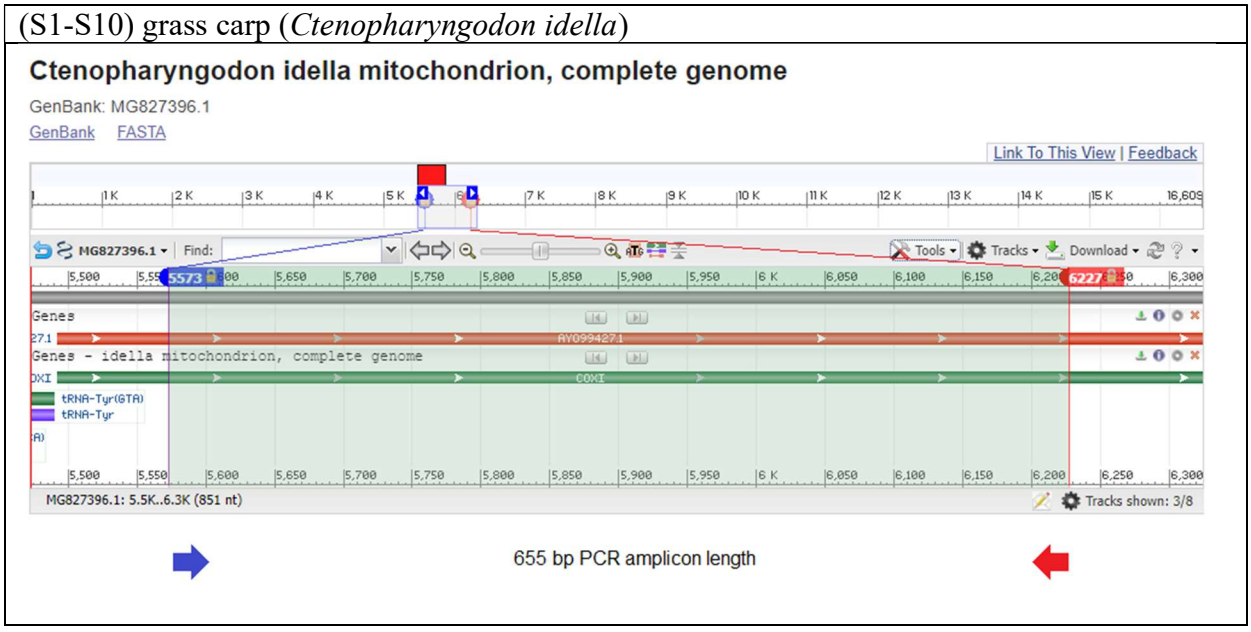

Fig. 1. The exact position of the retrieved 655 bp amplicon partially covered a portion of the *COX1* gene within ten samples of grass carb genomic sequences (GenBank acc. no. MG827396.1). The blue arrow refers to the starting point of this amplicon while the red arrow refers to its endpoint.
After positioning the 655 bp amplicons' sequences within the genomic sequences of grass carp samples, the details of its sequences were highlighted, and the total length of the amplified amplicons was also determined (Table 1).

Table 1. The position and length of the 655 bp PCR amplicons that used to amplify a portion of the *COX1* gene within the genomic sequences of grass carp samples (GenBank acc. no. MG827396.1).

Amplicon	Reference locus sequences (5′ - 3′)	length
COX1 gene nucleic acid sequences of grass carp (S1-S10)	ATAGTGGGAACCGCTCTAAGCCTTCTCATTCGAGCCGA ACTAAGCCAACCCGGATCACTTCTGGGCGATGATCAAA TTTATAATGTTATTGTCACTGCCCATGCCTTCGTAATAA TTTTCTTTATAGTAATACCAATTCTTATTGGAGGGTTTG GAAATTGACTCGTACCATTAATAATTGGAGCACCCGAC ATAGCATTCCCACGAATAAACAACATGAGTTTCTGACT TCTACCCCCTTCTTTCCTCCTACTATTAGCCTCTTCTGGT GTTGAGGCCGGAGCTGGAACAGGGTGAACAGTTTACCC ACCACTCGCAGGCAATCTTGCCCACGCAGGAGCATCCG TAGACCTAACAATTTTCTCACTCCACCTGGCAGGTGTGT CATCAATTTTAGGGGCAATTAATTTTATTACTACAACCA TTAACATGAAACCACCAGCCATCTCCCAATACCAAACA CCTCTCTTCGTTTGAGCTGTACTTGTAACAGCTGTACTC CTTCTTCTATCTCTACCAGTTCTAGCCGCCGGAATTACA ATACTCCTAACAGACCGTAATCTTAACACTACATTCTTT GACCCGGCGGGAGGAGGAGACCCAATTCTTTATCAACA CTTATTCTGATTCTTTGGTCACCCGGAAGTTTATATTC	655 bp

Interestingly, the alignment results of the 655 bp samples revealed the presence of five nucleic acid substitutions in variable positions of the grass carp samples in comparison with the most similar referring reference nucleic acid sequences (Fig. 2).

(S1-S10) Grass carp (*Ctenopharyngodon idella*)

```
          10        20        30        40        50        60        70        80        90       100
    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ref.
ATAGTGGGAACCGCTCTAAGCCTTCTCATTCGAGCCGAACTAAGCCAACCCG
GATCACTTCTGGGCGATGATCAAATTTATAATGTTATTGTCACTGCCC
```

S1 .....
S2 .....
S3 .....
S4 .....
S5 .....
S6 .....
S7 .....
S8 .....
S9 .....
S10 .....

110 120 130 140 150 160 170 180 190 200
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|

ref.
ATGCCTTCGTAATAATTTTCTTTATAGTAATACCAATTCTTATTGGAGGGTTT
GGAAATTGACTCGTACCATTAATAATTGGAGCACCCGACATAGCATT
S1 .....
S2 .....
S3 .....
S4 .....
S5 .....
S6 .....
S7 .....
S8 .....
S9 .....
S10 .....

210 220 230 240 250 260 270 280 290 300
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|

ref.
CCCACGAATAAACAACATGAGTTTCTGACTTCTACCCCCTTCTTTCCTCCTAC
TATTAGCCTCTTCTGGTGTTGAGGCCGGAGCTGGAACAGGGTGAACA
S1 .....
S2 .....
S3 .....
S4 .....
S5 .....
S6 .....
S7 .....
S8 .....
S9 .....
S10 .....

310 320 330 340 350 360 370 380 390 400
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|

ref.
GTTTACCCACCACTCGCAGGCAATCTTGCCCACGCAGGAGCATCCGTAGACC
TAACAATTTTCTCACTCCACCTGGCAGGTGTGTCATCAATTTTAGGGG
S1 .....C.....
S2 .....C.....
S3 .....
S4 .....
S5 .....
S6 .....
S7 .....
S8 .....
S9 .....
S10 .....

410 420 430 440 450 460 470 480 490 500
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ref.
CAATTAATTTTATTACTACAACCATTAACATGAAACCACCAGCCATCTCCCAA
TACCAAACACCTCTCTTCGTTTGAGCTGTACTTGTAACAGCTGTACT
S1 .....
S2 .....
S3 .....
S4 .....
S5 .....
S6 .....C.....
S7 .....
S8 .....
S9 .....
S10 .....

510 520 530 540 550 560 570 580 590 600
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ref.
CCTTCTTCTATCTCTACCAGTTCTAGCCGCCGGAATTACAATACTCCTAACAG
ACCGTAATCTTAACACTACATTCTTTGACCCGGCGGGAGGAGGAGAC
S1 .....G.....
S2 .....
S3 .....
S4 .....
S5 .....
S6 .....A.....
S7 .....
S8 .....
S9 .....
S10 .....

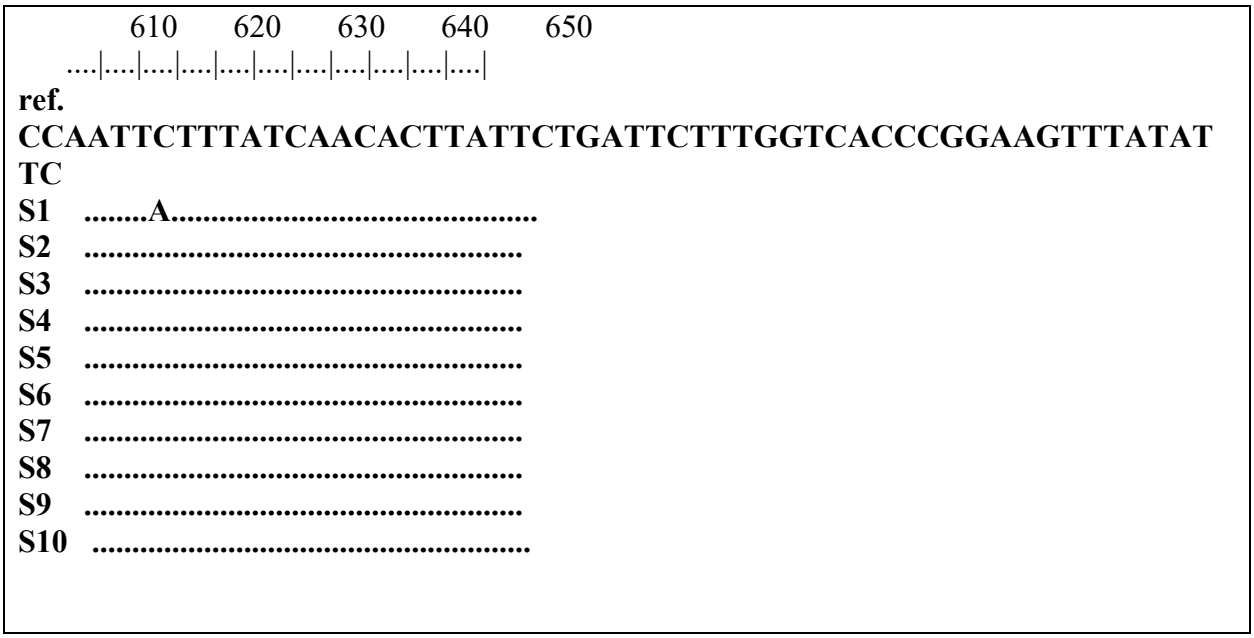

Fig. 2. Nucleic acid sequences alignment of ten samples of Grass carb with their corresponding reference sequences of the 655 bp amplicons of the *COX1* genetic sequences. The symbol "ref" refers to the NCBI referring sequence, letter "S", followed by a number refers to the sample number.

Our results indicated the presence of five nucleic acid variants. The observed variants were identified in five nucleic acid variations (324T>C, 506T>A, 609T>A, 473T>C, and 578T>G) were found in S1, S2, and S6 of the grass carp group with three silent (p.135N=, p.196L=, and p.230L=) and two missense (p.185V>A and p.220F>C) effects respectively. To confirm the observed variations, the sequencing chromatograms of the investigated samples, as well as their detailed annotations, were verified and documented, and the chromatograms of their sequences were shown according to their positions in the PCR amplicons (Fig. 3).

(S1-S10) Grass carp (*Ctenopharyngodon idella*)

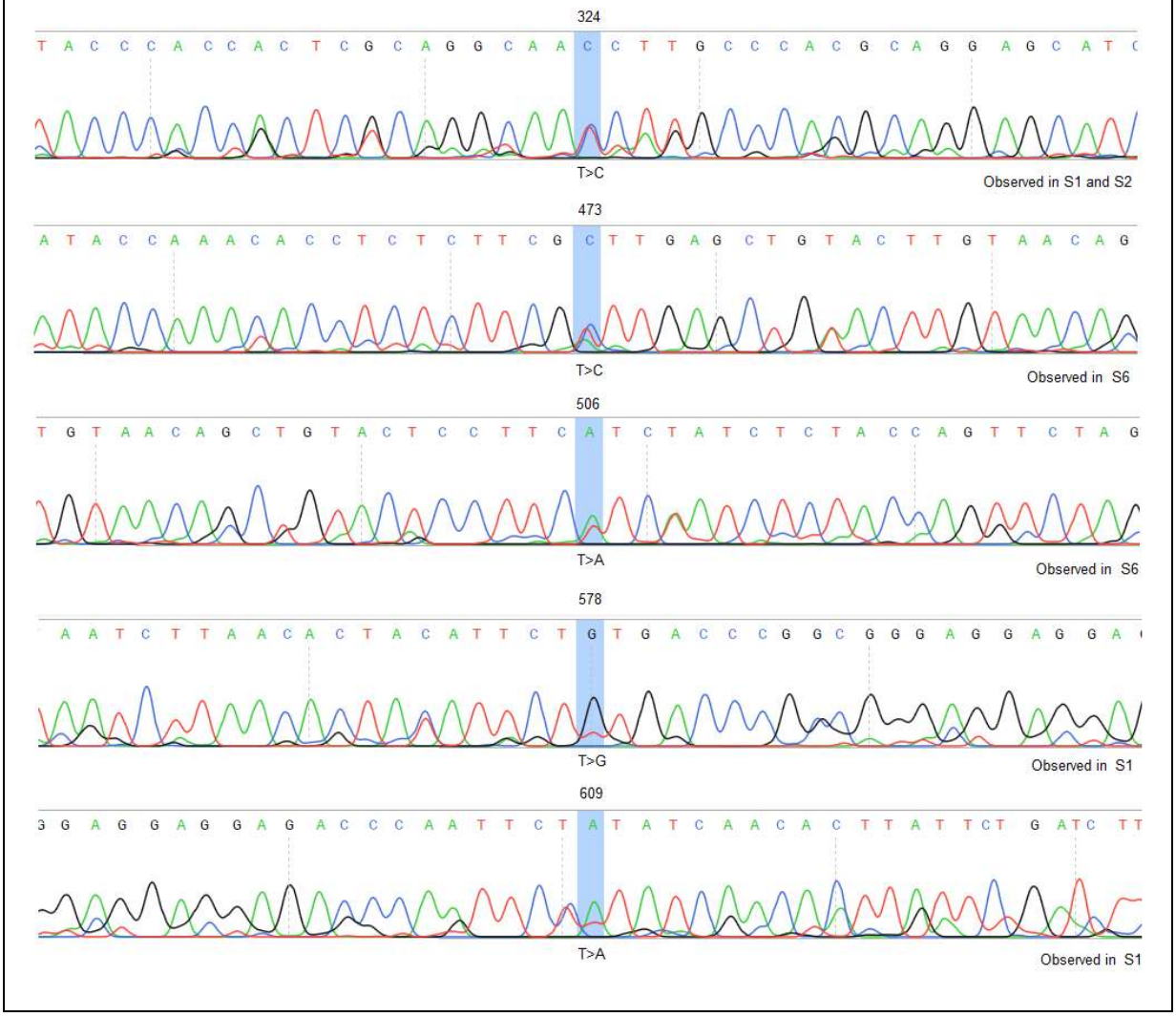

Fig. 3. The chromatogram of the grass carp sequences. The letter "S" refers to the code of the investigated samples having this variant in this study.

The observed nucleic acid variations were further analyzed to identify whether such substitution induces a possible alteration in their corresponding positions in the cytochrome c oxidase subunit I. All nucleic acid sequences of the amplified S1 to S10 PCR products were translated to their corresponding amino acid sequences using the Expasy translate suite (Fig. 4). As was indicated above, the amino acid alignment of these amino acid sequences with their references showed that two of the observed nucleic acid variants (473T>C and 578T>G) caused respectively two missense (non-synonymous) mutations (p.185V>A and p.220F>C) in the cytochrome c oxidase subunit I. Meanwhile, the other three variants caused a silent (synonymous) effect on the altered COX1 protein (p.135N=, p.196L=, and p.230L=).

(S1-S10) grass carp (*Ctenopharyngodon idella*)

```
          10        20        30        40        50        60        70        80        90        100
    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ref.
MVGTALSLLIRAELSQPGSLLGDDQIYNVIVTAHAFVMIFFMVMPILIGGFGNWL
VPLMIGAPDMAFPRMNNMSFWLLPPSFLLLLASSGVEAGAGTGWT
S1  ..................................................................................................
S2  ..................................................................................................
S3  ..................................................................................................
S4  ..................................................................................................
S5  ..................................................................................................
S6  ..................................................................................................
S7  ..................................................................................................
S8  ..................................................................................................
S9  ..................................................................................................
S10 ..................................................................................................

          110       120       130       140       150       160       170       180       190       200
    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ref.
VYPPLAGNLAHAGASVDLTIFSLHLAGVSSILGAINFITTTINMKPPAISQYQTPLF
VWAVLVTAVLLLLSLPVLAAGITMLLTDRNLNTTFFDPAGGGD
S1  ..................................................................................C.....
S2  ..................................................................................................
S3  ..................................................................................................
S4  ..................................................................................................
S5  ..................................................................................................
S6  ..........................................................A.......................................
S7  ..................................................................................................
S8  ..................................................................................................
S9  ..................................................................................................
S10 ..................................................................................................

          210
    ....|....|....|...
ref.  PILYQHLFWFFGHPEVYI
S1  ..................
S2  ..................
S3  ..................
S4  ..................
S5  ..................
S6  ..................
S7  ..................
```

S8 .....
S9 .....
S10 .....

>AYO99427.1 cytochrome c oxidase subunit I (mitochondrion) [Ctenopharyngodon idella]
MAITRWFFSTNHKDIGTLYLVFGAWAGMVGTALSLLIRAELSQPGSLLGDDQIYNVIV
TAHAFVMIFFMVMPILIGGFGNWLVPLMIGAPDMAFPRMNNMSFWLLPPSFLLLLAS
SGVEAGAGTGWTVYPPLAGNLAHAGASVDLTIFSLHLAGVSSILGAINFITTTINMKPP
AISQYQTPLFVWAVLVTAVLLLLSLPVLAAGITMLLTDRNLNTTFFDPAGGGDPILYQ
HLFWFFGHPEVYILILPGFGIISHVVAYYAGKKEPFGYMGMVWAMMAIGLLGFIVWA
HHMFTVGMDVDTRAYFTSATMIIAIPTGVKVFSWLATLHGGSIKWETPMLWALGFIF
LFTVGGLTGIVLANSSLDIVLHDTYYVVAHFHYVLSMGAVFAIMAAFVHWFPLFTGY
TLNDTWTKIHFGVMFIGVNLTFFPQHFLGLAGMPRRYSDYPDAYALWNTVSSIGSLIS
LVAVIMFLFILWEAFAAKREVSSVELTMTNAEWLHGCPPPYHTFEEPAFVQVQSN

Fig. 4. Amino acid residues alignment of the detected variations of the cytochrome c oxidase subunit I within the investigated grass carp samples as they are highlighted according to their corresponding positions within the amplified 655 bp fragment and according to their corresponding positions within the entire protein. The grey highlights refer to the amplified region of the *COX1*-encoded cytochrome c oxidase subunit I. The cyan and yellow colors refer to the missense and silent mutations in the alignment chart respectively.

All the investigated *COX1* sequences were deposited in the NCBI web server, and unique accession numbers were obtained for all analyzed sequences, starting from GenBank OP456552 which was deposited to represent the S1 sample, to the GenBank OP456561which was deposited to represent the S10 sample.

A comprehensive phylogenetic tree was generated in the present study according to nucleic acid variations observed in the amplified 655 bp of the *COX1* gene amplicons. This phylogenetic tree contained S1 to S10 samples alongside other relative nucleic acid sequences of silver carp, Eurasian carp, Prussian carp, and Binni group sequences.

Within this tree, our investigated samples were incorporated alongside other relative sequences to constitute five major clades of incorporated sequences within the cladogram. in addition to the grass carp clade, other four major clades were also incorporated to represent the silver carp, Eurasian carp, Prussian carp, and Binni group sequences. These four clades were used as outgroups to compare the ratio of phylogenetic homology between grass carp and the other related organisms in the same area of study. Phylogenetic results showed that all five clades were suited in distinct phylogenetic clades away from each other's sequences. This observation indicated an intermediate homology between the grass carp and the other four incorporated organisms within the same tree. Other than this observation, these data indicated the ability of *COX1* gene-based amplicons to detect these fish identities without including any noticeable homology with other sequences of other species whether being in the same genus or other outgroup sequences. The total number of the aligned nucleic acid sequences in this comprehensive tree was fifty-one.

As indicated above, the investigated samples were clustered into five phylogenetic clades of highly recognized phylogenetic distances within the incorporated sequences of silver carp compared with the other species of silver carp, Eurasian carp, Prussian carp, and Binni. The most interesting fact

observed in our investigated fish isolates is correlated with the ability of the utilized *COX1* gene-based amplicons to categorize the grass carp, silver carp, Eurasian carp, Prussian carp, and Binni sequences into this observed phylogenetic distribution.

Noteworthy, the grass carp group was positioned beside the roots of this tree. This sort of positioning indicated that the grass carp group was the oldest one in the evolutionary phylogenetic categories. Whereas the other groups had exhibited less old evolutionary positioning than that found in the grass carp group. This indicated that these four groups can be descended from the silver carp group with variable levels of genetic relations. After the grass carp, both rectangular and circular cladograms had clearly shown that the silver carp exerted the highest homology the Eurasian carp compared with the other incorporated outgroups (Fig. 5A and B). On the contrary, the most recent descendent of the incorporated five groups were represented by the Prussian carp since it was positioned at the utmost distance away from the roots of the current tree. However, the Binni clade followed the Prussian carp by its descendant phylogenetic positioning.

Concerning the clade of grass carp (S1-S10), twenty sequences of the same species were incorporated. Our investigated sequences of S1-S10 exhibited two sorts of distribution within the same major clade. This is due to the presence of five nucleic acid substitutions in the S1, S2, and S6 samples with respect to their corresponding reference sequences of the grass carp group. These variations induced a slight repositioning of the variants samples within the same clade of the grass carp group. However, the investigated samples within the grass carp group showed the alignment of these samples various strains of the grass carp sequences have been deposited from variable Chinese origins (GenBank acc. no. MT954923.1, JX119017.1, KC346973.1, KM401549.1, KT894100.1, and MH664229.1), Indian (GenBank acc. no. JQ231115.1), and American (GenBank acc. no. MG570437.1) origins.

It was inferred from the constructed tree that the detected nucleic acid substitutions showed a slight evolutionary effect of the variations observed in the fish samples in comparison with the other investigated wild-type fish samples. This style of sample positioning indicated the presence of a slight evolutionary effect of the observed genetic variant in inducing a possible deviation in the evolutionary positioning of these fish samples.

The presence of remarkable evolutionary distances among grass carp and the other outgroup organisms of silver carp, Eurasian carp, Prussian carp, and Binni groups indicated the high resolution of the currently utilized PCR products of the *COX1* sequences in the efficient detection and discrimination with the related organisms.

The current observation of this tree has confirmed sequencing reactions because it explained the actual neighbour-joining-based positioning in such observed variations. Interestingly, the Asian - American origins of our investigated samples could not be ignored.

Interestingly, the utilization of the *COX1* gene sequences in this study has given further proof for the presence of the accurate identification of the actual phylogenetic positoining of these types of fish sequences. This *COX1* gene-based comprehensive tree has provided comprehensive evidence about the high competency of such genetic fragments to efficiently identify this sort of phylogenetic distribution. This, in turn, gives a further indication of the ability of the currently utilized *COX1* gene-specific primers to describe the investigated grass carp isolates and their accurate phylogenetic positions.

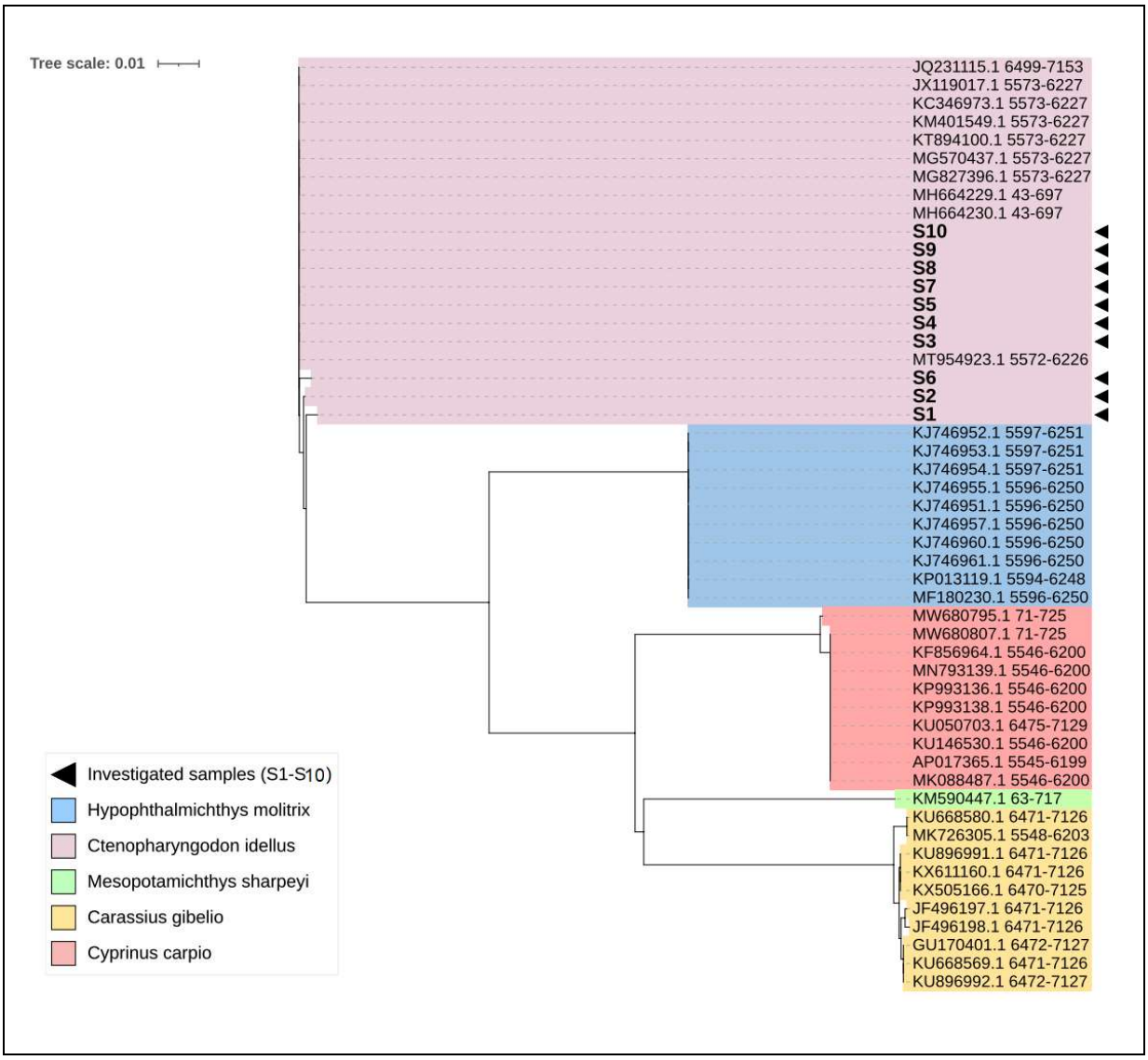

Fig. 5A. A comprehensive rectangular cladogram phylogenetic tree of genetic variants of the *COX1* gene fragment of ten samples belonging to grass carp. The black-colored triangle refers to the analyzed fish variants. All the mentioned numbers referred to GenBank accession number of each referring species. The number "0.01" at the top portion of the tree refers to the degree of scale range among the comprehensive tree-categorized organisms. The letter "S#" refers to the code of the investigated samples.

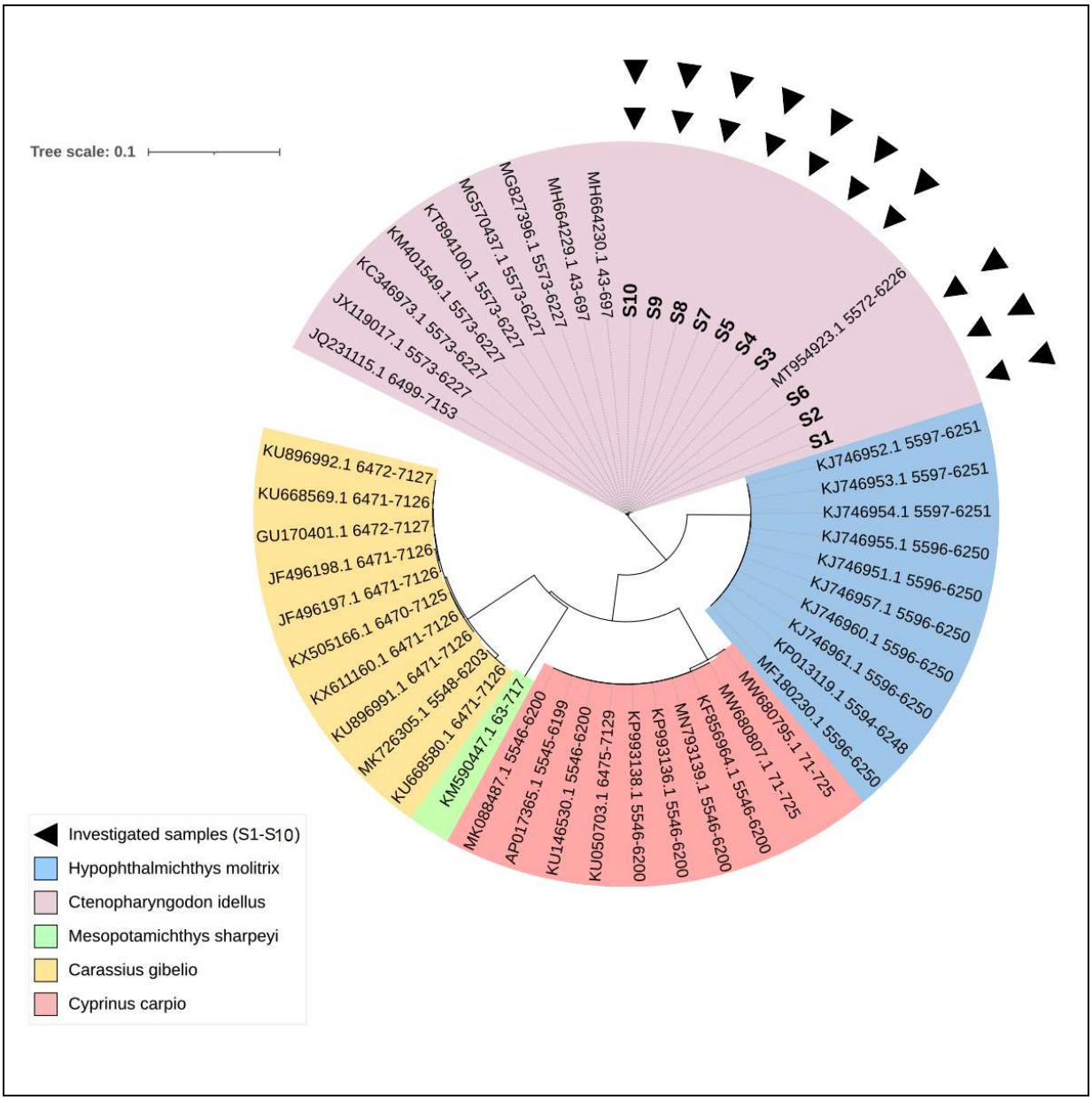

Fig. 5B. A comprehensive circular cladogram phylogenetic tree of genetic variants of the *COX1* gene fragment of ten samples belonging to grass carp. The black-colored triangle refers to the analyzed fish variants. All the mentioned numbers referred to GenBank accession number of each referring species. The number "0.1" at the top portion of the tree refers to the degree of scale range among the comprehensive tree-categorized organisms. The letter "S#" refers to the code of the investigated samples.

Conclusion:

This study found the ability of all the utilized genetic locus *COX1* to identify the identity of the investigated samples of *Ctenopharyngodon idella* and discriminate between this species and the other related organisms. Moreover, this study suggests possible employment for the amplicons of

cytochrome c oxidase subunit I of having the most specific power to discriminate between the phylogenetic diversity among the other implemented tools. These PCR fragments can efficiently be used to detect the biological diversity of a wider spectrum of fish sequences, and can therefore be explored to discover further details within these identified species.

References:

- LetunicI,Bork,P.2019.Interactive Tree Of Life (iTOL) v4: recent updates and new developme nts. Nucleic Acids Res 2;47(W1): W256-W259.
- Sarhan SR, Hashim HO, Al-Shuhaib MB. The Gly152Val mutation possibly confers resistance to beta-lactam antibiotics in ovine Staphylococcus aureus isolates. Open Veterinary Journal. 2019 Dec 22;9(4):339-48.
- Zhang Z, Schwartz S, Wagner L, Miller W. 2000. A greedy algorithm for aligning DNA sequences. J Comput Biol. 7(1-2):203-14.
- Fisher, Z.; Lyakhnovich, V. P. (1973). Biology and Bioenergetics of Grass Carp (Ctenopharyngodon Idella Val.). Polish Archieves of Hydrobiology, V. 20, N. 2, P. 521-557, 1973.
- Shireman, J.V. and Smith, C.R. (1983). Synopsis of biological data on the grass carp Ctenopharyngodon idella FAO. Fish Synopsis (135).86 pp.
- Q. Liu et al. (2013). Molecular cloning, characterization and expression analysis of coagulation factor VII gene in grass carp (Ctenopharyngodon idella) fish Shellfish Immunol (2013)
- Borkenhagen, K. (2014). A new genus and species of cyprinid fish (Actinopterygii, Cyprinidae) from the Arabian Peninsula, and its phylogenetic and zoogeographic affinities. Environment Biological Fish. 2014; 97(10):1179-1195.
- Cassani, J.R., W.E. Caton and B. Clark. (1984). Morphological comparisons of diploid and triploid hybrid grass carp, Ctenop.
- Chapman, D.C. (2010). Facts about invasive bighead and silver carps. Columbia, Missouri, USA: USGS Columbia Environmental Research Center.2 pp. https://pubs.usgs.gov/fs/2010/3033/pdf/FS2010-3033.pdf (Fact Sheet 2010–3033)
- Allen, J.r.; S.K. and Stanley, J.G. (1983). Ploidy of hybrid grass carp X bighead carp determined by flow cytometry. Trans. Am. Fish. Soc. 112: 431- 435.
- Cudmore, B. and Mandrak, N. E. (2004). Biological Synopsis of Grass Carp (Ctenopharyngodon Idella). Burlington: Fisheries and Oceans Canada/Great Lakes Laboratory for Fisheries and Aquatic Sciences, 2004. 44 P. (Canadian Manuscript Report of Fisheries and Aquatic Sciences, 2705).
- Dibble, E. D., Kovalenko, K. (2009). Ecological impact of grass carp: a review of the available data. Journal of Aquatic Plant Management, 47, 1-15.
- Freeze, M. and Henderson, S. (1983). A comparison of two year classes of hybrid grass carp and grass carp for aquatic plant control. Proceedings of the Arkansas Academy of Science, 37:25-30

- Opuszynski, K.; Shireman, J.V. and Aldridge, F.J. (1985). Intensive culture of grass carp and hybrid grass carp larvae. J. Fish. Biol. 26: 563-573.
- Page, L. M., and Burr, B. M. (1991). A field guide to freshwater fishes. Houghton Mifflin Company, Boston.