International Journal of Food Science and Agriculture, 2023, 7(4), 493-500
https://www. hillpublisher. com/journals/jsfa/
ISSN Online: 2578-3475
ISSN Print: 2578-3467

Application of DNA Barcode COI Sequence in the Identification of Common Fish Adulteration

Ruining Kang[1], Qingxiang Zhang[1], Qing Yang[2], Zhengpeng Wei[2], Yanbo Wang[1], Jinmei Wang[2], Lanlan Zhu[1,*]

[1]Shandong University of Technology, Zibo, Shandong, China.
[2]Rongcheng Taixiang Food Co., Ltd, Rongcheng, Shandong, China.

How to cite this paper: Ruining Kang, Qingxiang Zhang, Qing Yang, Zhengpeng Wei, Yanbo Wang, Jinmei Wang, Lanlan Zhu. (2023). Application of DNA Barcode COI Sequence in the Identification of Common Fish Adulteration. *International Journal of Food Science and Agriculture*, 7(4), 493-500.
DOI: 10.26855/ijfsa.2023.12.012

Received: November 28, 2023
Accepted: December 25, 2023
Published: January 22, 2024

Corresponding author: Lanlan Zhu, Shandong University of Technology, Zibo, Shandong, China.

Abstract

Objective: In order to reduce the adulteration of fish products and ensure the rights and interests of consumers and their lives and health, this study draws on the research results of bioinformatics and molecular biology and uses DNA barcode COI sequence for analysis. Establish a technical system for the identification of the origin of common fish varieties in the market, in order to provide technical support for the quality assurance of fish products. **Methods:** DNA barcoding technology was used in this study with the mitochondrial cytochrome oxidase subunit I (COI) gene as the target locus. DNA was extracted from samples of 7 commercially available fish species. PCR was carried out using specific COI primers followed by agarose gel electrophoresis to separate amplified products. The amplified DNA was separated using agarose electrophoresis. This allowed for the determination of the most appropriate annealing temperature for the PCR reaction system and COI primers, based on the observed electrophoretic separation effect. The amplified DNA was then manually sequenced, and subsequently, the sequences were compared and spliced manually. Multiple sequence comparison was used to analyze the homology of the related sequences. **Results:** COI sequences showed over 98% similarity, with the exception of bighead carp and mackerel sequencing, demonstrating that universal primers are not adequate for freshwater fish like bighead carp and mackerel. The Spanish mackerel, together with other fish, had indeterminate fish mixture components; DNA barcoding technology was found useful in precisely detecting single fish, but not for the identification of fish flesh mixtures. **Conclusion:** In this investigation, we examined the COI sequences of seven different fish varieties procured from the market, including Spanish mackerel, crucian carp, black carp, clear river fish, mackerel, carp, and bighead carp. Our findings indicate that not all fish items in the market can be identified through the utilization of universal primers for the COI gene. New primers should be designed to optimize the DNA streaking technique. The results from the PCR amplification of DNA extracted from mixed fish meat indicated that, simultaneously, COI was unable to identify mixed fish products using the DNA streaking technique. New universal primers for the COI gene or PCR amplification of double or multiple genes can enhance DNA barcode technology. This improvement reduces fish product adulteration, ensuring market order, and protecting consumer rights and interests.

Keywords

DNA barcoding, COI gene, Identification of fish products

1. Introduction

The 2022 report on the State of World Fisheries and Aquaculture by the World Food and Agriculture Organization

(WFAO) states that in 2020, worldwide fish production will amount to approximately 214 million tonnes, with aquatic animals accounting for 178 million tonnes. China, as a significant fishing nation, occupies one-third of the global aquatic product output. However, a series of incidents regarding the adulteration of aquatic products have arisen, significantly impacting the interests and the health of consumers [1, 2]. In this context, Paul Hebert, a taxonomist from the University of Guelph in Canada, introduced the DNA barcoding concept [3, 4]. DNA, as the fundamental genetic material, is present in the majority of living cells with a certain level of thermal stability and species-specificity. Consequently, this provides greater resolution, specificity, and sensitivity when compared to alternative techniques [5]. Since the DNA sequence of each animal is unique and stable, and the DNA sequence of different tissues and organs is identical, it is possible to extract DNA from various locations [6]. Mitochondrial genes are frequently employed as target genes for animal identification, encompassing the D-loop region, the mitochondrial cytochrome b gene (cyt b), 12S rRNA, 16S rRNA, and the three cytochrome oxidase subunit genes (CoI, CoII, CoIII) [7]. The study demonstrates that complete DNA barcoding of approximately COI regions presents significant potential for identifying fish [8, 9]. Using fish samples from the Guiyang market in Southwest China, Qian Tang [10] employed COI as a target gene in DNA barcoding technology to confirm its effectiveness in identifying fish products. This tool can enhance transparency and foster fair trade in the domestic fishing sector. Kannuchamy Nagalakshmi [11] gathered a total of 100 seafood samples from various regions in India. She analyzed the COI gene sequences against a publicly referenced classification database. The findings successfully verified that the Indian domestic market currently experiences a significant prevalence of seafood labeling errors, amounting to 22%. Kaiying Liu [12] gathered 179 fish samples from supermarkets in Henan Province's Zhengzhou and Xinxiang cities. The COI gene sequences were acquired using specific and universal primers and PCR techniques, which validated that the majority of commercial fish can be identified with the newly developed COIDNA barcode. Therefore, in this study, the COI gene was employed as a target for identifying commonly traded fish in the market including mackerel, crucian carp, black carp, clear river fish, mackerel, carp, and bighead carp.

2. Materials and Methods

2.1 Materials and Reagents

Spanish mackerel, crucian carp, black carp, Qingjiang fish, mackerel, carp, and bighead carp were obtained from the Zibo seafood market. The fish were packed in foam boxes with ice and transported to the aquatic products preservation laboratory at Shandong University of Technology.

Anhydrous ethanol, chloroform, isoamyl alcohol, isopropanol (all analytically pure, Sinopharm Chemical Reagent Co., Ltd.), Marine Animal Tissue Genomic DNA Extraction Kit (Centrifugal Column Type, Beijing Solepol Science and Technology Co., Ltd.), Taq DNA polymerase, dNTPs, DNAMarker (Guangzhou Ruizhen Bio-technology Co., Ltd.)

2.2 Instruments and equipment

High-speed centrifuge, Hunan Kaida Scientific Instrument Co., Ltd; PCR instrument, Nanjing Beden Medical Group; water bath, Haineng Instrument Co., Ltd; SC-315NE refrigerator, Aucma Co., Ltd; electrophoresis instrument, Thermo Fisher Scientific.

2.3 Methodologies

2.3.1 Purification method

Referring to Jiang Zhihua's [13] method for extracting DNA from samples, the freshly purchased fish meat underwent pre-treatment before being processed using the Marine Animal Tissue Genomic DNA Extraction Kit (Centrifugal Column Type). The DNA of the various fish meat types was extracted in accordance with the kit's instruction manual, and the resulting samples were stored at -20℃.

2.3.2 PCR amplification and sequencing of the COI gene

Fish universal primers were used [14]:

FishF2t1(TGTAAAACGACGGCCAGTCGACTAATCATAAAGATATCGGCAC);

FishR2t1(CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGAATCAGAA);

fishF2(TCGACTAATCATAAAGATATCGGCAC);

fishR2(ACTTCAGGGTGACCGAAGAATCAGAA);

VF2_t1(TGTAAAACGACGGCCAGTCAACCAACCACAAAGACATTGGCAC);

VR1_t1(CAGGAAACAGCTATGACTAGACTTCTGGGTGGCCAAAGAATCA).

The PCR amplification system was 50 μL: 5.0 μL of 10×PCR Buffer, 4 μL of dNTPs (2.5 mmol/L), 0.25 μL of TaKaRa Taq (5 U/μL), 0.2 μL of each primer, 1.0 μL of DNA template, and 39.35 μL of deionized water.PCR reaction program:

94℃, 2 min; 94℃, 30 s, 58℃, 40 s, 72℃, 1 min, 30 cycles; 72℃, 1 min, 16 cycles; 72℃, 10 min.

The PCR amplification product's molecular mass was identified utilizing 1% agarose gel electrophoresis according to Min Wang's [15] method. Add 1 g of agarose to 100 mL of 1×TAE buffer, melt it through microwave heating, and let it cool to 60℃. Mix 10 μL Gold view and pour it into the gel tank with the desired comb. Weigh, add, cool, mix, and pour. After solidification of the agarose gel, transfer it to the electrophoresis tank and add the appropriate amount of 1× TAE buffer. Sequentially take 5 μL of the sample into the spiked wells and use the DNA marker as a control. Electrophoresis was carried out at 120 V for 30 minutes, after which the gel was removed and observed using a gel imager and the successfully amplified COI gene was sequenced.

2.3.3 Sequence comparison and analysis

The sequences that were sequenced were manually compared and spliced. Then, the processed sequences were entered into the databases of both BOLD (http://www.boldsystems.org) and NCBI (http://blast.ncbi.nlmm.nihgov) to enable the identification and similarity analysis of the COI sequences of the samples. Finally, the collated sequences were analyzed using DNAMAN 7.0 software to examine the homology of related sequences that were downloaded from GenBank [16].

3. Results and analyses

3.1 Effect of different annealing temperatures on PCR reaction systems

The binding kinetics between complementary individual deoxyribonucleotides and target DNA in PCR vary with temperature. As a result, the choice of annealing temperature in the reaction system has a substantial impact on the gene amplification outcomes, particularly when there is a mismatch between the template primers and the target section [17]. Additionally, it has been demonstrated that annealing temperature is more significant than primer among the single factors influencing PCR results [18]. The optimization of the annealing temperature for PCR was the first step. In this study, Spanish mackerel DNA was selected as the sample and the PCR reaction system in 1.3.2 was utilized. Eight annealing temperature ladders were established for PCR amplification. Figure 1-3 presents the electrophoresis results.

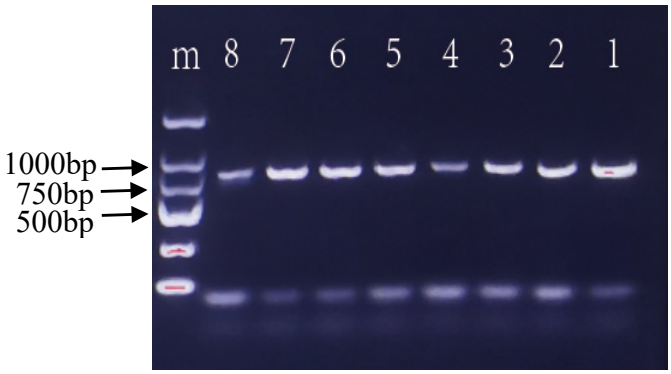


Figure 1. Electropherograms of PCR amplification products at different annealing temperatures for primers Fish-F1/ Fish-R1.

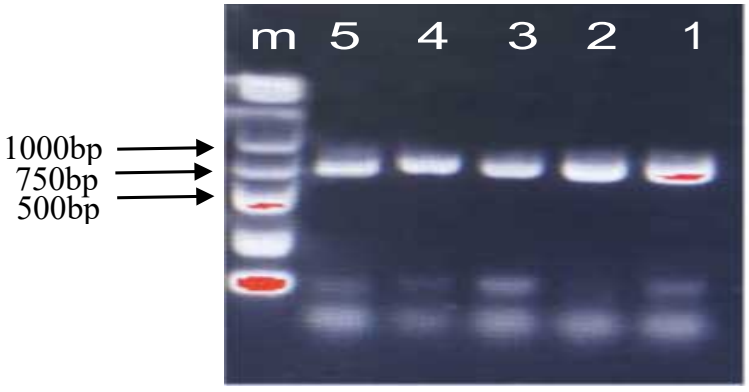
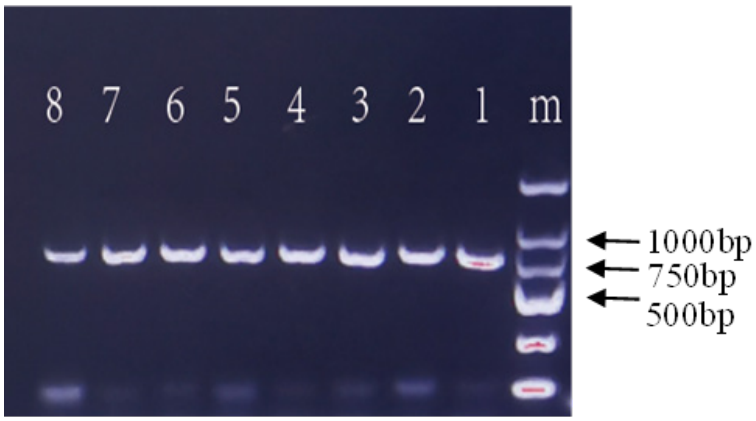


Figure 2. Electropherograms of PCR amplification products at different annealing temperatures for primers.



Note: 1-8 annealing temperatures of 58.7℃, 59.1℃, 59.6℃, 60.2℃, 60.8℃, 61.3℃, 61.9℃, 62.3℃.

Figure 3. Electropherograms of PCR amplification products at different annealing temperatures for primers VF1-t1/VR1-t1.

Based on Figures 1-3 from the annealing temperature gradient, the amplified Spanish mackerel COI gene produced target fragments with a size of approximately 750 bp using all three primers. The brightest band was detected at an annealing temperature of 58.7℃, which was subsequently determined to be the optimal annealing temperature.

3.2 Effect of different primers on the amplification of the COI gene in different fishes

The selection of primers is crucial in PCR reactions. The success of the PCR reaction and its specificity are directly related to the quality of primers used [19]. In this study, the PCR reaction system was used to amplify the three pairs of universal primers for known COIs that were selected in 1.3.2, using the optimal annealing temperature derived from 2.1. As part of our investigation into the identification of mixed fish products using COI sequences, DNA was extracted from 14 samples. To ensure that the DNA was representative of the mixed fish product, DNA from purchased fish was mixed with Spanish mackerel in pairs. Following PCR amplification, the results of agar gel electrophoresis were compared to select the most suitable pair of universal primers. The results are presented in Figure 4-6.

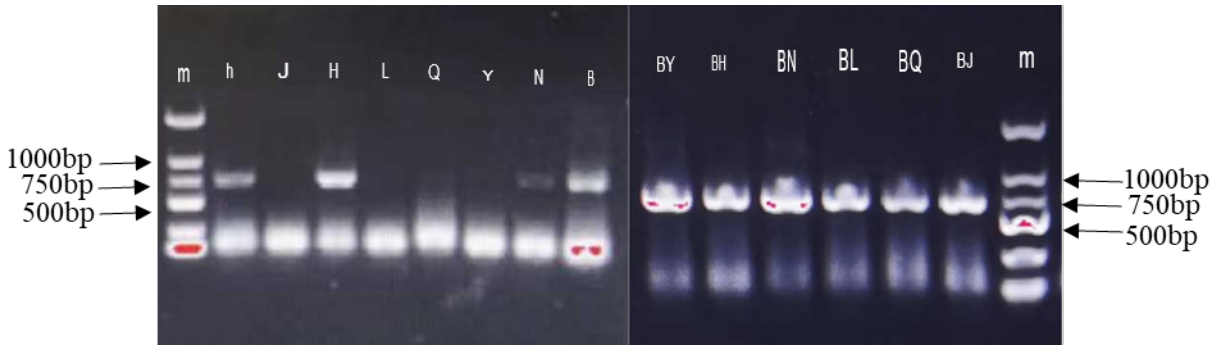


Figure 4. Electrophoresis of PCR products amplified by primers fishF2-t1/fishR2-t1 on different fish DNAs.

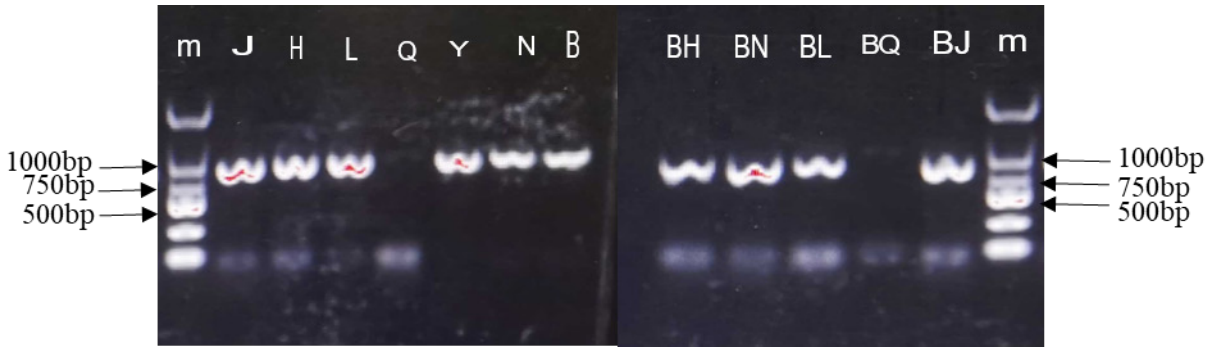
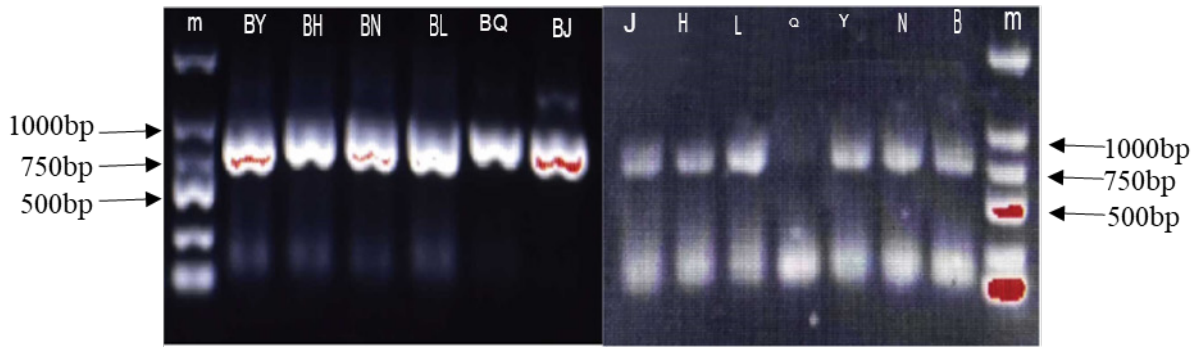


Figure 5. Electrophoresis of PCR products amplified by primers fishF2/fishR2 on different fish DNAs.



Note: The above letters are for Spanish mackerel (B), crucian carp (J), black carp (H), qingjiang fish (N), mackerel (Q), carp (L), bighead carp (Y), Spanish mackerel and crucian carp (BJ), Spanish mackerel and black carp (BH), Spanish mackerel and qingjiang fish (BN), Spanish mackerel and mackerel (BQ), Spanish mackerel and carp (BL), and Spanish mackerel and bighead carp (BY).

Figure 6. Electrophoresis of PCR products amplified by primers VF1-t1/VR1-t1 on different fish DNAs.

Figures 4-6 illustrate that the primers fishF2-t1/fishR2-t1 successfully amplified COI genes for Spanish mackerel, blackfish, and Qingjiang fish, as well as a mixture of Spanish mackerel and various fishes. However, the genes for other fishes were not amplified. Additionally, primers fishF2/fishR2 failed to amplify COI genes for mackerel and Spanish mackerel mixed with mackerel. Finally, primers VF1-t1/VR1-t1 could not amplify the COI gene from mackerel. Various fish and mixed COI sequences of Spanish mackerel were amplified using the universal primers fishF2-t1/fishR2-t1, fishF2/fishR2, and VF1-t1/VR1-t1. However, the fishF2/fishR2 primers were unable to amplify the COI gene of the Spanish mackerel and mackerel mixed COI gene. Primers VF1-t1/VR1-t1 were determined to be the most successful and hence chosen for COI gene amplification in fish. This aligns with Cermakova E's [20] account of DNA-based methods used for identifying fish species, which enable swift and precise identification of single fish, but not of blended fish flesh.

3.3 COI gene sequence comparison and analysis

Primers VF1-t1 and VR1-t1 were chosen for amplifying the COI gene of fish. The amplified DNA fragments were subsequently forwarded to Sangon Biological Engineering Co. for sequencing and comparison purposes. The sequences that were sequenced were manually compared and spliced. The resulting sequences were added to the BOLD (http://www.boldsystems.org) and NCBI (http://blast.ncbi.nlmm.nihgov) databases to identify the COI sequences of the samples and analyze their similarity. Finally, the compared sequences were matched against the sequences downloaded from GenBank. The sequences were compared with those obtained from GenBank using DNAMAN 7.0 software to analyze their homology. The resulting data is presented in Figures 7-11.

Score	Expect	Identities	Gaps	Strand
1249 bits(1384)	0.0	701/707(99%)	0/707(0%)	Plus/Plus

```
Query  27    TCAACCAACCACAAAGACATTGGCACCCACTATCTAGTATTCGGTGCATGAGCTGGAATA  86
Sbjct  5504  TCAACCAATCATAAAGACATCGGCACCCTCTATCTAGTATTCGGTGCATGAGCTGGAATA  5563
Query  87    GTTGGCACAGCCCTAAGCCTGCTTATCCGAGCTGAACTAAGCCAACCAGGTGCCCTTCTT  146
Sbjct  5564  GTTGGCACAGCCCTAAGCCTGCTTATCCGAGCTGAACTAAGCCAACCAGGTGCCCTTCTT  5623
Query  147   GGAGACGACCAGATTTATAACGTAATCGTTACAGCCCATGCCTTCGTCATGATTTTCTTT  206
Sbjct  5624  GGAGACGACCAGATTTATAACGTAATCGTTACAGCCCATGCCTTCGTCATGATTTTCTTT  5683
Query  207   ATAGTAATACCAATCATGATTGGAGGTTTTGGAAACTGACTTATCCCCCTAATGATCGGA  266
Sbjct  5684  ATAGTAATACCAATCATGATTGGAGGTTTTGGAAACTGACTTATCCCCCTAATGATCGGA  5743
Query  267   GCCCCCGACATAGCATTCCCTCGAATGAATAACATAAGCTTTTGACTTCTACCCCCTTCC  326
Sbjct  5744  GCCCCCGACATAGCATTCCCTCGAATGAATAACATAAGCTTTTGACTTCTACCCCCTTCC  5803
Query  327   TTCCTCCTACTCCTCGCCTCTTCCGGCGTTGAAGCCGGGGCTGGGACTGGTTGAACAGTC  386
Sbjct  5804  TTCCTCCTACTCCTCGCCTCTTCCGGCGTTGAAGCCGGGGCTGGGACTGGTTGAACAGTC  5863
Query  387   TATCCTCCCCTTGCCGGCAATCTGGCTCACGCTGGAGCATCCGTCGACTTAACTATTTTC  446
Sbjct  5864  TATCCTCCCCTTGCCGGCAATCTGGCTCACGCTGGAGCATCCGTCGACTTAACTATTTTC  5923
Query  447   TCTCTTCACCTGGCAGGGATTTCTTCAATCCTTGGGGCAATCAACTTCATTACGACAATC  506
Sbjct  5924  TCTCTTCACCTGGCAGGGATTTCTTCAATCCTTGGGGCAATCAACTTCATTACGACAATC  5983
Query  507   ATTAATATGAAACCCCCAGCTATCTCCCAATACCAAACACCCTTATTTGTGTGGGCTGTC  566
Sbjct  5984  ATTAATATGAAACCCCCAGCTATCTCCCAATACCAAACACCCTTATTTGTGTGGGCTGTC  6043
Query  567   CTAATTACAGCTGTCCTTCTTCTATTATCACTTCCAGTTCTTGCCGCTGGTATTACAATA  626
Sbjct  6044  CTAATTACAGCTGTCCTTCTTCTATTATCACTTCCAGTTCTTGCCGCTGGTATTACAATA  6103
Query  627   CTTCTTACAGACCGTAACCTAAATACAACCTTCTTCGACCCGGCAGGCGGAGGAGACCCA  686
Sbjct  6104  CTTCTTACAGACCGTAACCTAAATACAACCTTCTTCGACCCGGCAGGCGGAGGAGACCCA  6163
Query  687   ATCCTTTACCAACACGTATACTGATTCTTTGGCCACCCAGAAGTCTA  733
Sbjct  6164  ATCCTTTACCAACACTTATTCTGATTCTTTGGCCACCCAGAAGTCTA  6210
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Figure 7. Spanish mackerel sequence comparison results.

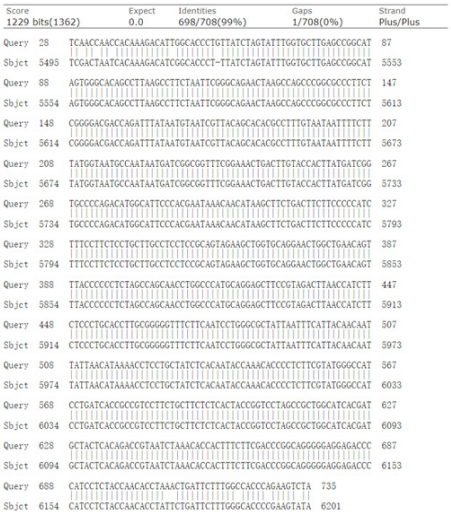

Figure 8. Black carp sequence comparison results.

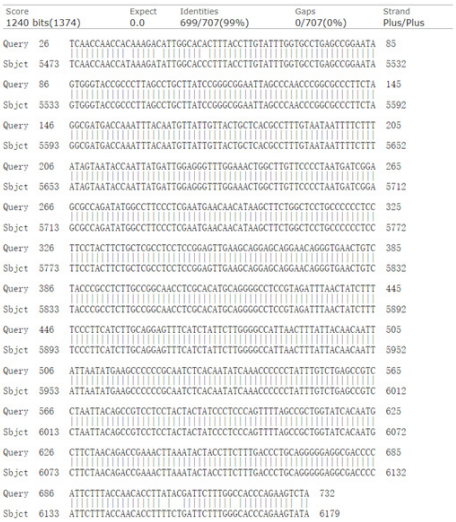

Figure 9. Qingjiang river fish sequence comparison results.

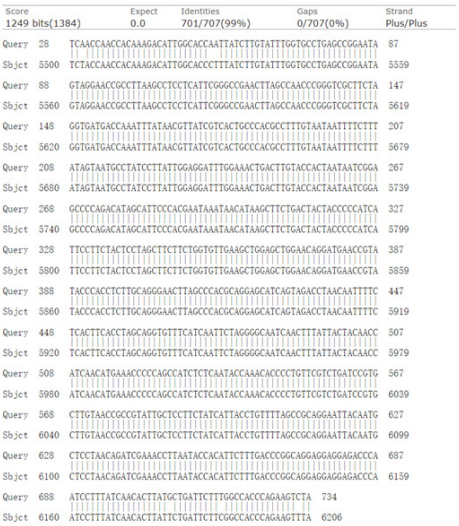

Figure 10. Carp sequence comparison results.

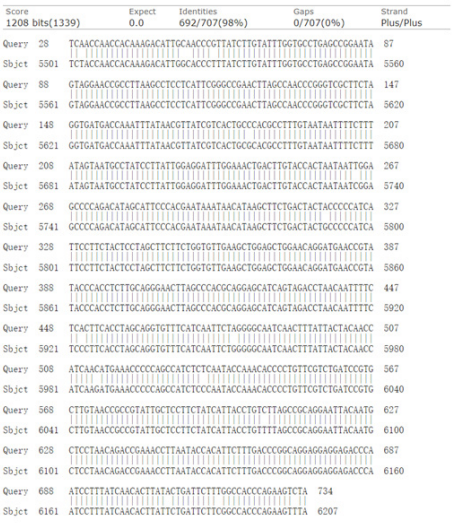

Figure 11. Crucian carp sequence comparison results.

3.4 Effects of different cold stress modes on oxidative stress in the milky way oyster (Crassostrea gigas)

Based on the sequence comparison of each fish, Table 1 displays the results: the similarity after COI sequence identification exceeded 98%, with the exception of bighead carp and cyprinid sequencing, which failed. This suggests that universal primers are not effective for freshwater fish like bighead carp and cyprinids. To enhance barcoding technology, new COI primers or dual mitochondria can be implemented to label the DNA barcodes of fish products [20, 21].

Table 1. Results of identification of different fish

sample	Latin name	scientific name	Chinese Common Name	species similarity
B	*Scomberomorus niphonius*	Scomberomorus niphonius	Ba yu	99%
H	*Channa argus*	ophiocephalus argus	Hei yu	99%
N	*Ictalurus punctatus*	channel catfish	Qing jiangyu	99%
Y	*Aristichthys nobilis*	bighead carp	Yong yu	Sequencing failed
Q	*Mylopharyngodon piceus*	black carp	Qing yu	Sequencing failed
L	*Cyprinus carpio*	carp	Li yu	99%
N	*Carassius auratus*	crucian carp	Ji yu	98 %

4. Conclusion

Through conducting single and mixed COI sequence analysis of seven different types of fish meat, including Spanish mackerel, crucian carp, black carp, clear river fish, mackerel, carp, and bighead carp, which were all procured from the market, this study concluded that the universal primers for the COI gene failed to identify all of the fish products available in the market. New primers were designed to optimize the DNA streaking technique, while DNA extracted from mixed fish meat was used for PCR amplification. Results of PCR amplification from the extracted DNA showed that COI failed to identify mixed fish products in the DNA streak technique. In the future, it may be possible to develop universal primers for the COI gene, or potentially use multiple genes for PCR amplification, to enhance DNA barcode technology. These improvements could further address the issue of fish product adulteration, ensuring market order and protecting consumer rights and interests.

Funding

Major Scientific and Technological Innovation Project of Shandong Province: Development of key technologies for targeted and efficient preparation of marine active peptides (2022CXGC020414).

References

[1] Ahmed MU, Hasan Q, Mosharraf Hossain M, Saito M, & Tamiya E. Meat species identification based on the loop mediated isothermal amplification and electrochemical DNA sensor [J]. Food Control, 2010, 21: 599-605.

[2] Cermakova E, Lencova S, Mukherjee S, Horka P, Vobruba S, Demnerova K, & Zdenkova K. Identification of Fish Species and Targeted Genetic Modifications Based on DNA Analysis: State of the Art [J]. Foods, 2023, 12(1): 228.

[3] Hebert P D N, Cywinska Aball S L, et al. Biological identifications through DNA barcodes [J]. Proceedings of the Royal Society of London B: Biological Sciences, 2003, 270: 313-321.

[4] Hebert P D, Nratnasingham S, & Dewaard Jr. Barcoding animal life: cytochrome C oxidase subunit I divergences among closely related species [J]. Proceedings Biological Sciences, 2003, 270(1): 96-99.

[5] Ran-Ran Xing, Ran-Ran Hu, Jian-Xun Han, Ting-Ting Deng, & Ying Chen. DNA barcoding and mini-barcoding in authenticating processed animal-derived food: A case study involving the Chinese market [J]. Food Chemistry, 2020, (309) 125653, ISSN 0308-8146.

[6] Yat-Tung Lo & Pang-Chui Shaw. DNA-based techniques for authentication of processed food and food supplements [J]. Food Chemistry, 2018, 767-774.

[7] Liu Jun. Exploring vertebrate systematic evolution and species identification of fish products based on mitochondrial DNA polymorphism [D]. Yantai: Yantai University, 2018.

[8] Xiong Xiong, Fangying Yuan, Manhong Huang, Lixia Lu, Xiaohui Xiong, & Jing Wen. DNA barcoding revealed mislabeling and potential health concerns with roasted fish products sold across China [J]. Journal of Food Protection, 2019, 1200-1209.

[9] A.B. Adibah, S. Syazwan, M.Z. Haniza Hanim, M.Z. Badrul Munir, A.G. Intan Faraha, & M.N. Siti Azizah. Evaluation of DNA barcoding to facilitate the authentication of processed fish products in the seafood industry [J]. LWT, 2020, 109585, ISSN 0023-6438.

[10] Qian Tang, Qi Luo, Qian Duan, Lei Deng, & Renyi Zhang. DNA barcode identification of fish products from Guiyang Markets in Southwestern People's Republic of China [J]. Journal of Food Protection, 2022, 583-590.

[11] Kannuchamy Nagalakshmi, Pavan-Kumar Annam, Gudipati Venkateshwarlu, Gireesh-Babu Pathakota, & Wazir Singh Lakra. Mislabeling in Indian seafood: An investigation using DNA barcoding [J]. Food Control, Volume 59, 2016, 196-200.

[12] Kaiying Liu, Shan Zhao, Zichen Yu, Yuejuan Zhou, Jinyi Yang, Rui Zhao, Changxing Yang, Wenwen Ma, Xi Wang, Mengxia Feng, Yongtao Tang, Kui Li, & Chuanjiang Zhou. Application of DNA barcoding in fish identification of supermarkets in Henan province, China: More and longer COI gene sequences were obtained by designing new primers [J]. Food Research International, Volume 136, 2020, 109516, ISSN 0963-9969.

[13] Jiang ZH. Construction of DNA barcodes of four marine fishes of the family Stenoglossidae [D]. Hangzhou: China University of Metrology, 2019.

[14] Ward R D., Zemlak T Sinnes B H, et al. DNA barcoding Australia's fish species [J]. Philosophical Transactions of the Royal Society Part B, 2005, 360: 1847-1857.

[15] Wang Min, Liu Herb, Huang Hai, et al. Application of DNA barcoding technology in the identification of fish and meat products in Shenzhen [J]. Food Science, 2015, 36(20): 247-251.

[16] Mosa KA, Soliman S, El-Keblawy A, et al. Using DNA barcoding to detect adulteration in different herbal plant-based products

in the United Arab Emirates: proof of concept and validation [J]. Recent Pat Food Nutr Agric, 2018, 9(1): 55-64.

[17] Kousuke Ishii. Manabu Fukui optimization of annealing temperature to reduce bias caused by a primer mismatch in multitemplate PCR [J]. Applied and Environmental Microbiology, 2001, 3753-3755.

[18] Li XG. Research on authentication technology of fish fillet (meat) based on DNA barcode [D]. Shanghai: Shanghai Ocean University, 2014.

[19] Qiqi Yang, Junwei Zhang, Jian Zhu, Jianping Liu, & Qiang Huang. DNA polymerase binding to the primer/template duplex affects the efficiency of PCR [J]. China Biotechnology, 2014, 34 (5): 6-13

[20] S Z O, S N P J, S K S, et al. DNA barcoding of commercial fish products using dual mitochondrial markers exposes evidence for mislabelling and trade of endangered species [J]. IOP Conference Series: Earth and Environmental Science, 2021, 736(1).

[21] Mst. Afifa khatun, Arzina Hossain, Md. Shakhawat Hossain, M. Kamruzzaman Munshi, & Roksana Huque. Detection of species adulteration in meat products and Mozzarella-type cheeses using duplex PCR of mitochondrial cyt b gene: A food safety concern in Bangladesh [J]. Food Chemistry Volume 2, 2021, 2666-5662.