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USING MITOCHONDRIAL GENOMES AND COI-BASED DNA BARCODING IN AQUACULTURE

ABSTRACT

Aquatic resources constitute one of the most important biological assets worldwide in terms of natural ecosystems, human nutrition, and economic sustainability. The effective management of fisheries and aquaculture activities is closely linked to accurate species identification, monitoring of biodiversity, and the elucidation of genetic stock structure. In this context, the mitochondrial genome (mtDNA) has become a widely used molecular marker in fisheries research due to its small genome size, relatively high mutation rate, maternal inheritance, and lack of recombination. The mitochondrial cytochrome c oxidase subunit I (COI) gene is recognized as the standard DNA barcode region for animals and enables rapid, reliable, and highly accurate identification of fish species in particular. COI-based DNA barcoding provides significant advantages in the identification of morphologically similar species, the detection of cryptic taxa, and the assessment of both intra- and interspecific genetic variation. Furthermore, COI barcode data are effectively utilized to investigate the population structure, phylogenetic relationships, and evolutionary history of fish species.

Keywords: Aquaculture, Mitochondrial DNA, DNA Barcoding, cytochrome C Oxidase Subunit I (COI), Species Identification

1. INTRODUCTION

The mitochondrial genome (mtDNA), which constitutes a fundamental genetic component within cells, has gained widespread use in recent years in the classification of eukaryotic organisms and in evolutionary research [1]. Studies on mitochondrial genomes began in the 1960s and initially focused primarily on their roles in energy metabolism. During the 1980s and 1990s, the scope of mtDNA research expanded considerably, and mitochondrial genomes became widely employed in species identification and taxonomic studies, particularly for the analysis of biological diversity and the investigation of speciation mechanisms. In recent years, the rapid advancement of high-throughput sequencing technologies has ushered mitochondrial genome research into a new phase [2].

The structural and functional characteristics of mitochondrial DNA render it an ideal tool for studies in biological evolution and systematics [2]. Owing to its relatively small genome size, high mutation rate, maternal mode of inheritance, and lack of recombination, mtDNA can be effectively used to trace the evolutionary history of species, elucidate phylogenetic relationships among taxa, and interpret the timing and mechanisms of speciation processes [3]. The elevated mutation rate of mitochondrial DNA is particularly advantageous for resolving

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recent evolutionary events and population dynamics, thereby providing a robust framework for understanding the historical evolution of species [4].

The application of mitochondrial genomes in species classification offers significant advantages, especially in taxonomically complex and highly diverse biological groups. Through mtDNA-based barcoding approaches, species can be identified rapidly and reliably, and phylogenetic trees can be constructed with high resolution [2]. Moreover, analyses of mitochondrial genomes from species inhabiting specific environmental conditions facilitate the detection of genetic variation associated with adaptive changes. Such studies not only elucidate how organisms respond to environmental pressures at the genetic level but also contribute to a deeper understanding of the role of selective forces in evolutionary processes [5].

In this context, the DNA barcoding approach is based on the analysis of a partial sequence of approximately 600–700 base pairs derived from the mitochondrial DNA (mtDNA) cytochrome c oxidase subunit I (COI) gene. This gene region is widely used because it enables rapid, reliable, and highly accurate species identification [6]. Aimed at revealing species-specific genetic profiles, this method facilitates species diagnosis by interpreting DNA sequence variation as a molecular "barcode." Numerous studies have demonstrated that partial COI sequences achieve high success in species identification across diverse taxonomic groups, including birds, butterflies, and fishes, and the application of this approach has expanded steadily in recent years [7]. The COI barcode region also provides valuable insights into evolutionary processes, demographic history, and the genetic diversity, structure, and connectivity of populations within a species. Many zooplankton taxa, for example, exhibit sufficient intraspecific DNA sequence variation in the COI region to allow detailed analyses of population genetic diversity and structure [8]. A substantial body of research further indicates that COI-based barcoding possesses high discriminatory power for distinguishing fish species, underscoring its effectiveness as a molecular tool for species delimitation in ichthyological studies [9].

2. RESEARCH SIGNIFICANCE

Accurate species identification is fundamental to sustainable fisheries management, biodiversity conservation, and the effective development of aquaculture practices. This study is important because it highlights the pivotal role of mitochondrial DNA, particularly the cytochrome c oxidase subunit I (COI) gene, as a robust and reliable molecular marker for species identification in aquatic organisms. By emphasizing the strengths of COI-based DNA barcoding, the study contributes to improving taxonomic resolution in cases where traditional morphological approaches are insufficient, such as in closely related species, early life stages, or processed fish products. Moreover, the integration of mitochondrial genome information into species identification frameworks enhances the accuracy, reproducibility, and global comparability of taxonomic data through standardized reference databases. The findings of this study support the broader application of molecular tools in fisheries biology, stock assessment, and conservation planning, thereby providing a scientific basis for informed management decisions. In this context, the study represents a valuable contribution to the advancement of molecular approaches for monitoring and preserving aquatic biodiversity.

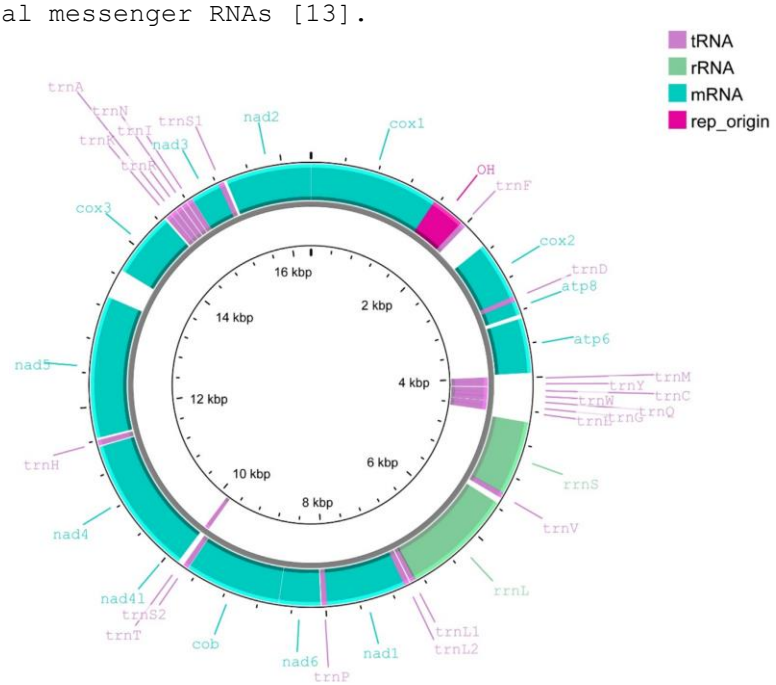
Highlights

- The mitochondrial genome, particularly the COI gene-based DNA barcoding approach, represents a reliable and universal molecular tool for species identification in aquaculture.

- The COI barcode region enables the accurate identification of fish species due to its low intraspecific variation and high interspecific genetic divergence.
- DNA barcoding provides significant advantages in identifying species that are morphologically similar or difficult to distinguish at different life stages.
- COI-based barcoding contributes not only to species identification but also to the assessment of population structure, phylogenetic relationships, and overall biodiversity monitoring.

3. STRUCTURE AND CHARACTERISTICS OF THE MITOCHONDRIAL GENOME

Mitochondrial DNA (mtDNA) is an essential genetic component located within the mitochondria of eukaryotic cells and is primarily recognized for its central role in cellular energy production through oxidative phosphorylation [10]. Unlike the linear chromosomes of nuclear DNA, the mitochondrial genome generally exhibits a circular structure, a feature that resembles the genomic organization of many prokaryotes and provides advantages in terms of genome stability and replication efficiency [11]. The mitochondrial genome contains 13 protein-coding genes that are directly involved in oxidative phosphorylation, encoding key subunits of enzyme complexes such as ATP synthase, cytochrome c oxidase, and NADH dehydrogenase. These genes play a critical role in the electron transport chain and ATP synthesis, underscoring the mitochondrion's function as the main energy-producing organelle of the cell. The compact and gene-dense organization of the mitochondrial genome reflects its evolutionary adaptation following endosymbiotic integration, enabling efficient regulation of specialized metabolic processes [12]. In addition to protein-coding genes, the mitochondrial genome encodes 22 transfer RNA (tRNA) genes that are required for mitochondrial protein synthesis. These tRNAs ensure the accurate delivery of specific amino acids to the ribosome during translation, thereby facilitating the proper assembly of mitochondrial proteins. Furthermore, mtDNA includes two ribosomal RNA genes (12S and 16S rRNA), which form the core structural and functional components of the mitochondrial ribosome and are indispensable for the translation of mitochondrial messenger RNAs [13].



Figüre 1. Structure of a typical mitochondrial genome [2]

4. USE OF MITOCHONDRIAL DNA IN FISHERIES AND AQUATIC SCIENCES RESEARCH

4.1. The Use of Mitochondrial DNA in Biodiversity Conservation and Species Identification

Mitochondrial DNA (mtDNA), in addition to its fundamental role in cellular energy production, has long been recognized as an important genetic marker in phylogenetic and evolutionary studies [4]. Decades of research have demonstrated that mtDNA possesses several distinctive characteristics that differentiate it from nuclear DNA and render it particularly valuable for evolutionary analyses. One of its most notable features is maternal inheritance, whereby mitochondrial DNA is transmitted exclusively from mother to offspring [14].

mtDNA is widely employed in the investigation of speciation processes, the characterization of population structure, and the assessment of the effects of environmental factors on genetic diversity. Another defining property of mitochondrial DNA is its relatively high mutation rate [15]. The rapid accumulation of mutations facilitates the reconstruction of recent evolutionary events and enables the evaluation of genetic variation among closely related species [2].

In contrast to nuclear DNA, mitochondrial DNA generally does not undergo recombination. The absence of recombination allows mtDNA to follow a consistent genetic lineage across generations, thereby enhancing its reliability as a molecular marker for tracing ancestry and inferring evolutionary relationships [3].

Mitochondrial DNA barcoding is not limited to species identification alone; it also represents a critical tool for monitoring changes in biological diversity, detecting invasive species, and investigating evolutionary processes [16]. The development of comprehensive barcode reference libraries across diverse taxonomic groups provides extensive datasets that serve as essential references for future species identification studies. Overall, mitochondrial DNA contributes substantially not only to species delineation but also to a broader understanding of evolutionary dynamics, biodiversity monitoring, and the advancement of conservation strategies [17]. With ongoing technological progress, the integration of mtDNA with genomic approaches, environmental DNA (eDNA) analyses, and other molecular methods is expected to further expand its application potential in biodiversity research and conservation management [18].

4.2. Use of Mitochondrial DNA in Biodiversity Conservation and Species Identification

Mitochondrial DNA (mtDNA), beyond its central role in cellular energy production, is widely employed as an informative genetic marker in phylogenetic research [4]. Decades of investigation have demonstrated that mtDNA possesses several distinctive characteristics that differentiate it from nuclear DNA and render it particularly valuable for evolutionary studies. Foremost among these features is its mode of maternal inheritance, whereby genetic material is transmitted exclusively from mother to offspring [14].

mtDNA has been extensively applied to the analysis of speciation processes, the characterization of population structure, and the assessment of how environmental factors influence genetic diversity. Another notable property of mtDNA is its relatively high mutation rate [15]. The rapid accumulation of mutations facilitates the investigation of recent evolutionary events and enables the evaluation of genetic variation among closely related taxa [2].

In contrast to nuclear DNA, mitochondrial DNA does not undergo recombination. The absence of recombination allows mtDNA lineages to be traced consistently across generations, thereby enhancing its

reliability as a marker for lineage reconstruction and the inference of evolutionary relationships [3].

Applications of mitochondrial DNA barcoding extend well beyond species identification alone; this approach has also become a critical tool for monitoring changes in biological diversity, detecting invasive species, and examining evolutionary Dynamics [16]. The development of comprehensive barcode reference libraries across diverse taxonomic groups provides robust datasets that support future species identification efforts. Overall, mtDNA represents more than a fundamental instrument for taxonomic resolution; it contributes substantially to understanding evolutionary processes, tracking biodiversity patterns, and advancing conservation strategies [5]. As molecular technologies continue to evolve, the integration of mtDNA with genomic approaches, environmental DNA (eDNA) analyses, and complementary molecular methods is expected to further expand its application potential in biodiversity research and conservation management [18].

5. DNA BARCODING APPROACH

The DNA barcoding approach gained widespread recognition in the scientific community after the cytochrome c oxidase subunit I (COI) gene was proposed as a standard target region for all animal groups in 2003 [19]. This method, which is based on the use of standardized short DNA sequences, is regarded as a modern molecular identification technique that enables rapid, reliable, and accurate species identification [20]. DNA barcoding relies on the principle that sequence variation within short, species-specific DNA fragments can function as a biological "barcode," thereby allowing organisms to be identified at the species level. The methodological framework includes DNA extraction from tissue samples, amplification of the target gene region by polymerase chain reaction (PCR), and comparison of the resulting sequences with reference barcode databases [21].

The principal feature distinguishing DNA barcoding from other species identification methods is its reliance on a single standardized gene region to provide a universal and comparable identification system across a broad taxonomic range. In this context, an approximately 655 base pair fragment from the 5′ end of the COI gene has been accepted as the standard DNA barcode region for animals, as it contains sufficient genetic variation to discriminate between intra- and interspecific differences, possesses conserved flanking regions that enable amplification with universal primers, and has a short sequence length that facilitates routine laboratory analyses [22].

6. REASONS FOR THE PREFERENCE OF THE CYTOCHROME C OXIDASE SUBUNIT I (COI) GENE

The genetic discriminatory power of the COI barcode region, which is widely employed in DNA barcoding studies [23 and 24], has been clearly demonstrated by numerous investigations focusing on metazoan mitochondrial genomes. These studies consistently indicate that intraspecific genetic variation within the COI region generally remains below 3%, whereas interspecific divergence is markedly higher, typically ranging between approximately 10% and 25% [2].

A key factor underlying the effectiveness of the COI gene is the high substitution rate observed at the third codon positions, which results in a molecular evolutionary rate that is nearly three times higher than that of mitochondrial rRNA genes. In addition, the evolutionary rate of the COI gene is well suited to resolving closely related species and to revealing intraspecific genetic variation associated with geographic structure [16].

In efforts to identify a universal DNA barcode region for metazoan taxa, the mitochondrial genome has been shown to offer several advantages over the nuclear genome. These include the absence of introns, limited exposure to recombination, a high copy number per cell, a haploid organization, and strictly maternal inheritance. By contrast, mitochondrial 12S and 16S rRNA genes, which were commonly used in earlier systematic studies, often present substantial challenges in sequence alignment and comparison due to high rates of insertions and deletions. Most of the 13 protein-coding genes in the metazoan mitochondrial genome exhibit fewer such structural complications. Among these genes, COI has emerged as particularly advantageous because it can be reliably amplified across a wide range of metazoan taxa using universal primer sets and because it provides robust phylogenetic signal across multiple taxonomic levels [25].

The primary rationale for adopting the COI gene as the standard DNA barcode marker lies in its strong discriminatory capacity across diverse taxa and its characteristic variation pattern, in which intraspecific genetic differences rarely overlap with interspecific divergence [2]. Nevertheless, it has also been reported that COI barcode data may offer limited resolution for distinguishing certain groups of closely related species, resulting in reduced discriminatory power in specific taxonomic contexts [26].

Despite these relatively few exceptions, a large body of studies encompassing geographically widespread species and evaluating both interspecific and intraspecific genetic variation has produced highly consistent datasets, which have played a decisive role in establishing COI as the standard marker in DNA barcoding research. The impact of this standardization is clearly reflected in major public biological databases such as NCBI GenBank and the Barcode of Life Data Systems (BOLD), where a substantial increase in the number of deposited COI sequences has been observed following its adoption as a barcode gene. In the context of molecular species identification, no taxon has been reported to consistently yield erroneous results when analyzed using the COI gene, and it is therefore regarded as one of the most suitable molecular markers available. Nevertheless, to minimize potential biases associated with analyses based solely on mitochondrial markers, it is widely recommended that phylogenetic and population genetic studies incorporate at least one nuclear gene region alongside COI [6].

Research conducted to date has demonstrated that the use of an approximately 655 base pair fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene enables the accurate identification of 98–100% of species belonging to taxonomically complex groups such as birds, fishes, and butterflies [6]. Fishes constitute nearly half of all vertebrate diversity, comprising an estimated 15.700 marine and 13.700 freshwater species worldwide [27]. Owing to recent advances in molecular biology, DNA barcoding has become increasingly employed at a global scale for the taxonomic classification of adult fish species and for the assessment of both intra- and interspecific genetic diversity. In this context, numerous studies have been carried out across a wide range of geographical regions, from Australia to Africa [28].

By 2009, more than 5.000 fish species had been barcoded based on the COI gene, with an average of five individuals analyzed per species, and the resulting sequences were archived in the Barcode of Life Data Systems (BOLD) database [29]. In Türkiye, relatively recent DNA barcoding studies based on the mitochondrial COI gene have been conducted on adult individuals representing 89 commercially important marine and freshwater fish species [6].

COI-based DNA barcoding studies performed on fish samples collected from different ocean basins have further revealed that this

gene region exhibits a high capacity for detecting genetic differentiation among populations. In a study analyzing 149 individuals from 15 fish species sampled across northern (Atlantic and Mediterranean) and southern (Australian and Asian) hemisphere seas, two species were reported to display pronounced genetic divergence between northern and southern populations [30]. Specifically, genetic differentiation of approximately 2.75% was observed between northern and southern populations of *Lepidopus caudatus*, while this value reached 7.44% in *Zeus faber*. The authors emphasized that individuals clustered accurately according to their geographic populations, highlighting the effectiveness of the COI gene in resolving population-level structure. Similarly, another investigation examined population genetic structure in 35 fish species representing both coastal and pelagic lifestyles, sampled from opposing shores of the Indian Ocean using a COI barcoding approach [31].

Additional evidence supporting the discriminatory power of the COI gene at both species and population levels in fishes comes from a comprehensive study involving 207 fish species sampled from Australian waters [28]. The results indicated that intraspecific genetic variation was generally low and that COI sequences could be reliably used to distinguish both species and geographically structured populations within species. The authors concluded that COI-based DNA barcoding represents a valuable tool for fish biology and systematics.

The effectiveness of DNA barcoding in freshwater fishes has also been demonstrated in a study analyzing approximately 200 freshwater fish species from the Canadian fauna, including economically important salmonids and sturgeons [32]. In this work, genetic distances among species were found to be substantially greater than variation within species, supporting the utility of COI-based barcoding for species identification as well as for the evaluation of intraspecific genetic structure. Moreover, the authors highlighted that the resulting COI barcode reference library would serve as a critical resource for future studies in population genetics, ecology, and systematics.

Overall, these studies, encompassing fish species sampled from diverse geographic regions and ecological contexts, collectively demonstrate that COI-based DNA barcoding constitutes a robust, consistent, and broadly applicable molecular tool for species identification and the assessment of population genetic structure in fisheries and aquatic biodiversity research.

7. CONCLUSIONS

Accurate species identification in fisheries science is a fundamental requirement for sustainable fisheries management, the conservation of biological diversity, and the advancement of aquaculture practices. Mitochondrial genomes, particularly DNA barcoding approaches based on the cytochrome c oxidase subunit I gene, provide a reliable and effective molecular tool to meet this need. The low level of genetic variation within species combined with pronounced genetic differentiation among species enables the COI gene to achieve high accuracy in fish species identification.

Numerous studies reported in the literature demonstrate that COI based DNA barcoding achieves identification success rates reaching up to 98 to 100 percent in both marine and freshwater fishes. This high level of accuracy represents a significant advantage, especially for species that exhibit strong morphological similarity or for taxa that are difficult to identify across different life history stages. Although limited resolution has been reported for the discrimination of closely related species within certain taxonomic groups, the vast majority of

studies encompassing fish species with broad geographic distributions strongly support the use of the COI gene as a standard barcode marker.

The widespread implementation of the COI barcoding approach in international databases such as GenBank and the Barcode of Life Data Systems has substantially enhanced the global comparability of genetic data related to fisheries resources. This standardization has contributed significantly to research in fish biology, stock assessment, and conservation biology. In conclusion, mitochondrial genome analysis and COI based DNA barcoding represent indispensable tools for species identification, the elucidation of phylogenetic relationships, and the monitoring of biological diversity in fisheries research. In the future, the integration of this approach with nuclear genetic markers and environmental DNA analyses is expected to enable more comprehensive and holistic outcomes in aquatic biodiversity and fisheries studies.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

FINANCIAL DISCLOSURE

The authors received no financial support for the research.

DECLARATION OF ETHICAL STANDARDS

The authors of the article declare that the materials and methods used in this study do not require ethics committee approval and/or legal special permission.

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