Fish DNA barcoding: A review

Nahavandi R.[1*]

Received: May 2024 Accepted: July 2024

Abstract

The accompanying text provides a comprehensive overview of fish DNA barcoding, a powerful molecular technique used for species identification and biodiversity assessment in ichthyology. DNA barcoding uses a standardized genetic marker, typically a segment of the mitochondrial cytochrome c oxidase subunit I (COI) gene, to accurately identify fish species. This method has revolutionized biodiversity assessment, environmental monitoring, and detection of mislabeling in commercial seafood products. The text discusses the methodology of DNA barcoding, including the use of the COI gene as a standard barcode and the development of mini-barcoding techniques for degraded DNA samples. It also highlights the importance of DNA barcode databases such as the Barcode of Life Data System (BOLD) and GenBank in facilitating species identification and research. Major applications of fish DNA barcoding include species identification and discovery, assessing mislabeling in commercial seafood products, monitoring biodiversity in marine environments, and assessing anthropogenic pressures on aquatic ecosystems. Despite its many advantages, the text also acknowledges challenges and limitations, such as taxonomic resolution beyond the species level and dealing with cryptic species. Overall, barcoding fish DNA represents a significant advance in ichthyological research and closes the gap between traditional taxonomy and modern molecular biology.

Keywords: DNA barcoding, Cytochrome oxidase subunit I, Fish, Biodiversity

1-Animal Science Research Institute of Iran (ASRI), Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran
*Corresponding author's Email: Rezanahavandi91@gmail.com

[DOI: 10.22034/INJBIR.2.2.3]

Introduction

DNA barcoding has emerged as a powerful tool in the field of ichthyology for species identification and biodiversity assessment. This molecular technique uses a st andardized genetic marker, typically a short segment of the mitochondrial cytochrome c oxidase subunit 1 (COI) gene, to accurately identify fish species. The method has gained significant traction due to its ability to overcome the limitations associated with traditional morphological identification, particularly in cases where visual features are ambiguous or inadequate. The importance of DNA barcoding in fish species identification cannot be overemphasized. It has revolutionized our ability to conduct accurate biodiversity assessments, monitor the marine environment and detect mislabeling in commercial seafood products. In aquatic ecosystems where cryptic species and juvenile forms often present challenges to identification, DNA barcoding provides a reliable means of species delimitation. One of the main advantages of DNA barcoding is its applicability in different life stages of fish, from eggs and larvae to adult fish (Comia and Morris, 2024). This is particularly valuable in studies of marine ecology, where early life stages are often difficult to identify using traditional methods. Additionally, the technique has proven useful in uncovering hidden diversity within fish populations and uncovering cryptic species and genetic variations that can have significant implications for conservation and fisheries management. The establishment of comprehensive DNA barcode reference libraries such as the Barcode of Life Data System (BOLD) has further enhanced the utility of this approach. These databases serve as repositories of DNA barcode sequences and allow researchers to compare unknown samples to a variety of known species. This has not only facilitated rapid and accurate species identification, but also contributed to our underst anding of fish phylogeny and evolution (Ephigenie *et al*., 2024).. As anthropogenic pressures on aquatic ecosystems continue to increase, the role of DNA barcoding in conservation efforts will become increasingly important. It allows researchers to conduct non-invasive sampling, assess biodiversity in protected areas and monitor the effects of environmental change on fish populations. Furthermore, in the context of fishing and seafood trade, DNA barcoding has become an essential tool to detect fraudulent labeling and ensure the authenticity of seafood products on the market. In summary, fish DNA barcoding represents a significant advance in ichthyological research, providing a st andardized, efficient and accurate method for species identification. As we continue to explore and underst and the enormous diversity of fish species in our oceans and freshwater systems, DNA barcoding represents a cornerstone technology that bridges the gap between traditional

[DOI: 10.22034/INJBIR.2.2.3]

taxonomy and modern molecular biology (Fadli *et al*., 2023).

Methodology

COI gene as the st andard DNA barcode for animals

The cytochrome c oxidase subunit I (COI) gene has been widely adopted as a st andard DNA barcode for animal species identification. This mitochondrial gene segment, typically around 650 base pairs long, has proven to be extremely effective at distinguishing between species in different animal groups. The popularity of the COI gene as a barcode is due to several key factors:

- Sufficient species-level genetic divergence
- Conserved flanking regions allow the development of universal PCR primers
- Extensive representation in DNA sequence reference databases

The effectiveness of the COI gene has been demonstrated in various animal species , including insects, birds, etc., detected fish. In a study of 64,414 insect species, researchers found that the COI gene provides significant discriminatory power for species delineation. However, it is important to note that approximately a quarter of insect species had high intraspecific genetic variation (>3%), which may complicate species identification in some cases (Habib *et al*., 2023).

Mini-barcoding Techniques and Their Applications

Mini-barcoding has emerged as a valuable technique in DNA barcoding, particularly when dealing with degraded DNA samples or when it is difficult to obtain full-length barcode sequences. These shorter sequences, typically between 100 and 300 base pairs, have shown significant potential for species identification. Mini barcodes are particularly useful in situations where:

- Sample quality is poor or DNA is degraded.
- Rapid species identification is required.
- High-throughput sequencing methods are used.

Mini-barcodes have found application in various areas, including:

- Environmental DNA studies (eDNA studies)
- Analysis of processed food products
- Forensic investigations
- Ancient DNA research
- Comparison of different primer sets for amplification success

The choice of primer sets plays a crucial role in the success of DNA barcoding, especially when dealing with different taxonomic groups. Different primer sets may differ in their amplification efficiency and ability to capture species-specific variations. When comparing primer sets, researchers consider factors such as:

- Universality across all target taxa
- Amplification success rate
- Ability to distinguish between closely related species

[DOI: 10.22034/INJBIR.2.2.3]

Studies have shown that while st andard COI primers work well for many groups of animals, some taxa may require specialized primers to achieve optimal results. For example, when barcoding fish, researchers have developed and tested different primer sets to improve amplification success in different fish species. To evaluate the effectiveness of different sets of primers, researchers often conduct comparative studies and evaluate:

- Amplification success rates across multiple species
- Sequence quality and length
- Species discrimination power

By carefully selecting and optimizing primer sets, researchers can improve the accuracy and efficiency of DNA barcoding efforts for different animal groups (Hidayani *et al*., 2024).

DNA barcode databases

Barcode of life data system (BOLD systems)

The Barcode of Life Data System (BOLD) is a comprehensive and curated reference barcode database that plays a critical role in DNA barcoding efforts, particularly for animal species identification. BOLD serves as a central repository for DNA barcode sequences and offers several key features:

- Extensive coverage: BOLD contains a vast collection of DNA barcodes across various taxonomic groups. For example, it houses around 2500 sequences for Tetrigidae, an orthopteran family.
- Metadata integration: BOLD provides important metadata associated with barcode sequences, including taxonomic information, collection data, and photographic vouchers.
- Identification tools: The platform offers an identification engine that allows researchers to compare unknown sequences against the database for species identification
- BIN system: BOLD implements the Barcode Index Number (BIN) system, which clusters sequences into operational taxonomic units, support in species delimitation

However, BOLD has some limitations:

- Taxonomic gaps: Some regions and taxonomic groups are underrepresented in the database
- Outdated taxonomic information: The taxonomic backbone of BOLD may not always reflect the most current classifications
- Incomplete identifications: Many records in BOLD are not identified beyond the family level
- Access limitations: While BOLD offers an API for batch queries, it does not provide access to unpublished data through this interface (Singh *et al*., 2024).

GenBank and other relevant databases

GenBank, maintained by the National Center for Biotechnology Information (NCBI), is another significant database used for molecular identification:

- Broad scope: GenBank contains a wide range of genetic sequences, including but not limited to DNA barcodes
- Open access: GenBank provides free and unrestricted access to all its data, making it a valuable resource for researchers worldwide.
- Regular updates: The database is updated frequently, incorporating new sequences and revisions.
- Integration with other NCBI tools: GenBank is integrated with other NCBI resources, facilitating comprehensive genetic analyses.
- However, GenBank also has limitations:
- Less curation: Compared to BOLD, GenBank has less stringent curation processes, potentially leading to more errors or misidentifications
- Lack of standardization: Unlike BOLD, which focuses on standardized DNA barcodes, GenBank contains a variety of genetic sequences that may not adhere to specific barcoding standards.

Other relevant databases include:

- UNITE: Specialized in fungal ITS sequences.
- PR2: Focused on protist ribosomal RNA gene sequences.
- SILVA: Provides quality-checked databases of aligned rRNA sequences for Bacteria, Archaea, and Eukarya.

These specialized databases complement BOLD and GenBank and provide curated sequences for specific taxonomic groups or genetic markers. Researchers often use a combination of these databases to ensure comprehensive coverage and accurate identification in their molecular studies *(*Muhala *et al.*, 2024*)*.

Applications of fish DNA barcoding

Fish DNA barcoding has a wide range of applications, significantly exp anding our underst anding of biodiversity, improving species identification, and contributing to conservation efforts. The following sections detail the major applications of fish DNA barcoding.

Species identification and discovery

One of the main applications of fish DNA barcoding is species identification. The technique uses a short, st andardized section of the mitochondrial cytochrome c oxidase subunit I (COI) gene to differentiate between species. This is particularly valuable in cases where traditional morphological identification methods are inadequate due to cryptic species, juvenile forms, or damaged specimens.

- Identification of new species: DNA barcodes have facilitated the discovery of previously unrecognized species. For instance, studies have uncovered hidden diversity within known taxa, leading to the identification of new species and subspecies. This is crucial for updating taxonomic classifications and understanding evolutionary relationships among fishes.

[DOI: 10.22034/INJBIR.2.2.3]

- Life Stage Identification: The ability to identify fish at various life stages, including larvae and eggs, is another significant advantage. This capability is essential for ecological studies that examine reproductive patterns, recruitment dynamics, and population structure.
- Environmental Monitoring: By employing DNA barcoding in environmental assessments, researchers can identify species present in a given habitat without needing to capture or observe them directly. This non-invasive approach is particularly useful in sensitive ecosystems where traditional sampling methods may be disruptive (Siddika *et al*., 2024).

Assessment of mislabeling in commercial fish products

DNA barcodes play a crucial role in combating seafood fraud and ensuring the authenticity of seafood products in the market. Mislabeling can occur for a variety of reasons, including economic incentives to substitute more expensive species for cheaper species or inadvertent errors in labeling.

- Food Safety and Consumer Protection: Studies have shown that a significant percentage of seafood products are mislabeled. DNA barcoding allows for rapid and accurate identification of fish species in commercial products, helping to verify labeling claims and protect consumers from fraudulent practices.
- Regulatory Compliance: Regulatory agencies can use DNA barcoding as a tool for monitoring compliance with fisheries regulations and labeling laws. This ensures that fish sold in markets meet legal standards regarding species identification and sustainability.
- Traceability: By establishing a reliable method for identifying fish species throughout the supply chain, DNA barcoding enhances traceability from catch to consumer. This transparency is essential for promoting sustainable fishing practices and responsible consumption (Panprommin *et al*., 2023).

Biodiversity monitoring in marine environments

Barcoding fish DNA is crucial for monitoring biodiversity in marine environments. It provides researchers with powerful tools to assess species richness, distribution patterns and ecosystem health.

- Biodiversity Assessments: Using DNA barcoding, researchers can conduct comprehensive surveys of fish biodiversity in various habitats. This information is vital for understanding ecosystem dynamics and identifying areas of high conservation value.
- Monitoring Changes Over Time: Regular monitoring using DNA barcoding allows scientists to detect changes in species composition and abundance over time. Such data can

[DOI: 10.22034/INJBIR.2.2.3]

indicate shifts in community structure caused by environmental changes or anthropogenic impacts.

- Conservation Planning: The information gathered through biodiversity assessments can inform conservation strategies by identifying priority areas for protection and management. This is particularly important for regions facing threats from overfishing, habitat destruction, or climate change (Modeel *et al.*, 2024).

Evaluation of anthropogenic pressures on aquatic ecosystems

The application of fish DNA barcoding extends to assessing the impact of human activities on aquatic ecosystems. By assessing how anthropogenic pressures affect fish populations and community structure, researchers can gain insight into the health of ecosystems.

- Impact Assessment: DNA barcoding can be used to assess the effects of pollution, habitat degradation, invasive species introduction, and overfishing on native fish populations. Understanding these impacts is crucial for developing effective management strategies.
- Restoration Efforts: In areas undergoing ecological restoration, DNA barcoding can help monitor the success of reintroduction efforts by tracking the presence and abundance of target species over time.
- Ecosystem Health Indicators: Fish communities serve as important indicators of ecosystem health. By analyzing shifts in community composition through DNA barcoding, researchers can infer broader ecological changes resulting from human activities.

In summary, fish DNA barcoding serves as a transformative tool in various fields, improving our ability to identify species, monitor biodiversity, ensure food safety, and assess anthropogenic impacts on aquatic ecosystems. As techniques continue to develop and databases expand, the potential applications of this technology are likely to continue to exp and and contribute significantly to conservation biology and sustainable fisheries management (Sure andiran *et al*., 2023).

Challenges and limitations

Despite the significant advances in fish DNA barcoding, several challenges and limitations hinder its widespread application and effectiveness. These challenges can be divided into three main areas: taxonomic resolution beyond the species level, outdated taxonomic frameworks in databases, and dealing with cryptic species and current divergences.

Taxonomic resolution beyond species level

One of the major challenges in DNA barcoding is its limited ability to resolve taxonomic relationships beyond the species level. While DNA barcodes are useful for distinguishing species, there are often problems with:

[DOI: 10.22034/INJBIR.2.2.3]

- Intraspecific Variation: Many species exhibit considerable genetic diversity due to factors such as geographical variation, environmental influences, and evolutionary history. This intraspecific variation can complicate the interpretation of barcode data, leading to difficulties in accurately identifying populations or subspecies.
- Cryptic Species: The existence of cryptic species—groups of organisms that are morphologically indistinguishable but genetically distinct—poses a significant challenge for taxonomic resolution. DNA barcoding may identify these cryptic species at the genetic level; however, without morphological characteristics, it can be challenging to classify them accurately within existing taxonomic frameworks.
- Phylogenetic Relationships: While DNA barcoding can provide insights into species-level relationships, it may not adequately resolve deeper phylogenetic relationships among closely related species or genera. This limitation arises from the relatively short length of the COI gene used in barcoding, which may not capture enough genetic information to infer comprehensive evolutionary histories (Zhang *et al*., 2023).

Outdated taxonomic backbones in databases

The effectiveness of DNA barcoding depends heavily on the quality and completeness of reference databases such as the Barcode of Life Data System (BOLD) and GenBank. However, several problems arise from outdated taxonomic bases:

- Taxonomic Gaps: Many regions and taxonomic groups remain underrepresented in existing databases. For instance, certain fish families or geographical areas may lack sufficient barcode sequences, limiting researchers' ability to identify species accurately.
- Misidentifications: The presence of misidentified sequences in databases can lead to erroneous conclusions during species identification. Inaccurate taxonomic assignments can stem from historical misclassifications or insufficiently vetted sequences.
- Dynamic Taxonomy: Taxonomy is a continually evolving field, with new discoveries and reclassifications occurring regularly. Databases may not always reflect these changes promptly, leading to discrepancies between current scientific understanding and available data.
- Quality Control Issues: While BOLD and GenBank provide valuable resources, they also face challenges related to data quality control. Inconsistent curation practices can result in errors or ambiguities within the databases that affect downstream analyses (Huang *et al*., 2023).

[DOI: 10.22034/INJBIR.2.2.3]

Handling of cryptic species and recent divergences

The identification and classification of cryptic species and recently diverged lineages present additional challenges for fish DNA barcoding:

- Identifying and classifying cryptic species and recently diverged lineages presents additional challenges to fish DNA barcoding:
- Cryptic Diversity: Cryptic species often require more than just barcoding for accurate identification. Researchers may need to integrate molecular data with morphological characteristics or ecological information to delineate these hidden taxa effectively.
- Recent Divergences: Species that have recently diverged may exhibit minimal genetic differences due to their close evolutionary relationship. In such cases, traditional COI barcoding may not provide sufficient resolution for accurate identification, necessitating the use of additional genetic markers or longer sequences that capture more variation.
- Hybridization Events: Hybridization between closely related species can complicate species delimitation efforts using DNA barcoding alone. The presence of hybrid individuals may blur the genetic boundaries between species, making it difficult to assign individuals confidently to specific taxa based solely on barcode sequences.
- Methodological Limitations: The reliance on a single genetic marker (COI) for barcoding can limit the ability to resolve complex evolutionary histories involving hybridization or rapid speciation events. Researchers are increasingly exploring multi-locus approaches that incorporate additional genetic markers to enhance resolution capabilities.

In conclusion, while fish DNA barcoding provides powerful tools for species identification and biodiversity assessment, it faces several challenges that must be addressed to maximize its utility. Overcoming limitations related to taxonomic resolution, database quality, and cryptic diversity requires continued collaboration among researchers, improved database management practices, and the integration of complementary methods in molecular ecology and taxonomy (Mahboobeh *et al*., 2023).

Bioinformatics tools and analysis

Bioinformatics plays a crucial role in analyzing DNA barcode data, facilitating species identification, phylogenetic studies and biodiversity assessments. This section reviews key bioinformatics tools and methods used in sequence similarity analysis, phylogenetic analysis, and the meaning of barcode index numbers (BINs).

Sequence similarity analyses

Sequence similarity analysis is fundamental to DNA barcoding and allows researchers to compare unknown sequences with reference databases to

[DOI: 10.22034/INJBIR.2.2.3]

identify species. Various bioinformatics tools facilitate this process:

- BLAST (Basic Local Alignment Search Tool), BLAST is one of the most widely used tools for sequence similarity searching. It enables researchers to input a query sequence and find similar sequences in databases such as GenBank or BOLD. By providing a list of matching sequences along with statistical significance scores, BLAST helps in identifying the closest relatives of an unknown sample.
- MUSCLE (Multiple Sequence Comparison by Log-Expectation), MUSCLE is a tool for aligning multiple sequences to identify conserved regions and variations among them. This alignment is essential for subsequent phylogenetic analyses and helps in understanding evolutionary relationships between species.
- Profile Hidden Markov Models (HMMs), HMMs are increasingly used in sequence analysis to identify conserved motifs within sequences and filter out putative pseudogenes from datasets. This approach enhances the accuracy of species identification by focusing on biologically relevant sequences.
- Deep Learning Approaches: Recent advancements have introduced deep learning frameworks for species classification using DNA barcodes. For example, Deep Barcoding employs convolutional neural networks to analyze raw sequence data, achieving high accuracy rates (>90%) for species classification by converting DNA sequences into one-dimensional images for analysis.

These tools collectively improve the reliability of species identification through robust sequence similarity analyses, enabling researchers to detect misidentification, assess biodiversity, and monitor ecological change (Kang *et al.*, 2023).

Phylogenetic analysis methods (Maximum Likelihood, Bayesian analysis)

Phylogenetic analysis is essential for underst anding the evolutionary relationships between species based on genetic data. In this context, several methods are usually used:

- Maximum Likelihood (ML), The Maximum Likelihood method estimates the probability of observing the data given a particular tree topology and model of evolution. ML methods are widely used due to their statistical rigor and ability to handle large datasets efficiently. The approach involves constructing a tree that maximizes the likelihood of the observed genetic data under a specified model of nucleotide substitution.
- Bayesian Analysis: Bayesian methods provide a probabilistic framework for phylogenetic inference, allowing researchers to incorporate prior knowledge into their analyses. This method calculates

[DOI: 10.22034/INJBIR.2.2.3]

posterior probabilities for different tree topologies based on prior distributions and observed data. Bayesian analysis is particularly useful for estimating confidence intervals around phylogenetic estimates and handling uncertainty in evolutionary relationships.

- Other Methods: Additional approaches such as Neighbor-Joining (NJ) and UPGMA (Unweighted Pair Group Method with Arithmetic Mean) are also used for constructing phylogenetic trees. While these methods are generally faster than ML and Bayesian approaches, they may not provide the same level of statistical robustness.

Phylogenetic trees generated using these methods can provide insights into evolutionary history, speciation events, and patterns of genetic divergence among fish species, contributing to our underst anding of biodiversity and conservation efforts (Modeel *et al.*, 2024).

Barcode index numbers (BINs) and their significance

The Barcode Index Number (BIN) system is a critical component of DNA barcoding that improves species identification and biodiversity assessment:

- Definition: A BIN is a unique identifier assigned to clusters of DNA barcode sequences that share high levels of similarity, typically representing operational taxonomic units (OTUs). The BIN system was developed as part of the BOLD database to facilitate the organization and retrieval of barcode data.
- Species Delimitation: BINs help researchers delineate species boundaries by grouping closely related sequences that may represent distinct species or cryptic diversity within a taxon. This clustering aids in identifying potential new species or subspecies that may not be apparent through traditional morphological methods.
- Biodiversity Monitoring: The use of BINs allows for efficient monitoring of biodiversity changes over time. By tracking shifts in BIN composition within ecosystems, researchers can assess the impacts of environmental changes, habitat degradation, or invasive species on local fish populations.
- Conservation Applications: BINs play an essential role in conservation efforts by providing a framework for identifying priority species for protection based on their genetic distinctiveness and vulnerability to threats. They also facilitate international collaboration by standardizing species identification across different regions.
- Integration with Other Databases: BINs can be cross-referenced with other databases like GenBank or local taxonomic databases, enhancing the accessibility and usability of barcode data for researchers worldwide.

In summary, bioinformatics tools and analytical methods are essential for the

[DOI: 10.22034/INJBIR.2.2.3]

effective application of DNA barcoding in fish identification and biodiversity studies. Sequence similarity analyzes enable accurate species identification, while phylogenetic methods provide insights into evolutionary relationships. The barcode index number system further enhances these efforts by facilitating species delimitation and biodiversity monitoring, ultimately contributing to conservation strategies and ecological research initiatives (Singh *et al*., 2024).

Future perspectives

The future of fish DNA barcoding promises to improve biodiversity assessment, improve conservation efforts, and integrate molecular techniques into traditional monitoring methods. This section discusses the integration of DNA barcoding into traditional monitoring methods, its potential to improve biomonitoring programs, and its role in maintaining and assessing ecosystem health.

Integration with traditional monitoring methods

The integration of DNA barcodes into traditional monitoring methods represents a critical advance in ecological research and biodiversity management. Traditional methods are often based on morphological identification, which can be time-consuming and requires experienced taxonomists. By combining these approaches, researchers can achieve more comprehensive and accurate assessments of fish populations.

- Enhanced Identification Accuracy: DNA barcoding provides a molecular tool to confirm species identities that may be difficult to distinguish morphologically, particularly in cases of cryptic species or juvenile forms. This integration allows for more reliable data collection in ecological studies.
- Complementary Data: Integrating molecular data with traditional ecological metrics (e.g., abundance, biomass) can yield a more holistic understanding of fish communities. For example, combining DNA barcoding with environmental assessments (such as habitat quality or water chemistry) can help elucidate the factors influencing biodiversity patterns.
- Training and Capacity Building: As DNA barcoding becomes more integrated into ecological monitoring programs, it will necessitate training for researchers and practitioners in both molecular techniques and bioinformatics analysis. This capacity building will enhance the overall skill set within conservation biology and fisheries management.
- Cost-Effectiveness: Although initial investments in sequencing technology may be high, the long-term benefits of integrating DNA barcoding into monitoring programs could lead to cost savings by reducing the need for extensive fieldwork and expert taxonomic identification (Hidayani *et al*., 2024).

Potential for improving biomonitoring programs

DNA barcoding has the potential to revolutionize biomonitoring programs by enabling rapid and accurate assessments of biodiversity in aquatic ecosystems. The following aspects illustrate this potential:

- Environmental DNA (eDNA) Applications: The use of eDNA—DNA extracted from environmental samples such as water—has emerged as a powerful tool for biomonitoring. By analyzing eDNA samples using barcoding techniques, researchers can detect the presence of species without needing to capture or observe them directly. This non-invasive approach is particularly valuable in sensitive habitats where traditional sampling methods may disturb ecosystems.
- Rapid Assessment Capabilities: Advances in high-throughput sequencing technologies allow for the simultaneous analysis of multiple samples, enabling rapid assessments of fish diversity across large spatial scales. This capability is essential for timely responses to environmental changes or anthropogenic impacts.
- Detection of Invasive Species: Biomonitoring programs utilizing DNA barcoding can effectively identify invasive species that may threaten native fish populations. Early detection is critical for implementing management strategies to mitigate the impacts of invasive on local ecosystems.
- Longitudinal Studies: Integrating DNA barcoding into long-term biomonitoring programs can facilitate tracking changes in biodiversity over time, providing insights into population dynamics and ecosystem health (Ephigenie *et al*., 2024).

Role in conservation and ecosystem health assessment

Fish DNA barcoding plays a critical role in conservation efforts and ecosystem health assessment by providing important data on species distributions, genetic diversity and population structures:

- Conservation Planning: Accurate species identification through DNA barcoding informs conservation planning by identifying priority species or populations that require protection. Understanding genetic diversity within populations is essential for developing effective management strategies that enhance resilience to environmental changes.
- Ecosystem Health Indicators: Fish communities serve as important indicators of ecosystem health. Changes in species composition or abundance can signal shifts in environmental conditions or anthropogenic pressures. By employing DNA barcoding to monitor these communities, researchers can assess the overall health of aquatic ecosystems and identify areas requiring intervention.
- Restoration Efforts: In restoration projects, DNA barcoding can help

[DOI: 10.22034/INJBIR.2.2.3]

evaluate the success of reintroduction efforts by confirming the presence of target species and assessing their genetic diversity post-restoration. This information is crucial for ensuring that restoration efforts contribute positively to ecosystem recovery.

- Policy Development: The data generated from DNA barcoding studies can inform policy decisions regarding fisheries management, habitat protection, and biodiversity conservation initiatives at local, national, and international levels.
- Public Engagement: As awareness of biodiversity loss grows among the public, utilizing DNA barcoding in outreach efforts can enhance engagement by demonstrating the importance of species identification and conservation efforts through tangible examples and findings (Comia and Morris, 2024).

Conclusion

Summary of the importance and effectiveness of fish DNA barcoding

Fish DNA barcoding has emerged as a transformative tool in the fields of ichthyology, conservation biology and fisheries management. By using a st andardized segment of the mitochondrial cytochrome c oxidase subunit I (COI) gene, DNA barcoding allows accurate identification of the species, even in cases where traditional morphological methods are inadequate. This technique has proven particularly useful for:

- Species Identification: DNA barcoding facilitates the identification of fish species across various life stages, including larvae and juvenile forms, which are often challenging to classify morphologically. Studies have demonstrated high success rates in identifying species from diverse habitats, contributing to our understanding of biodiversity.
- Food Safety and Fraud Detection: The application of DNA barcoding in commercial fisheries has highlighted its importance in detecting mislabeling and fraud in seafood products. Research has shown that a significant percentage of fish products are mislabeled, and DNA barcoding provides a reliable method for verifying species authenticity, thereby protecting consumers and promoting ethical fishing practices.
- Biodiversity Monitoring: By employing DNA barcoding in environmental assessments, researchers can monitor changes in fish diversity over time. This capability is crucial for assessing the impacts of anthropogenic activities on aquatic ecosystems and informing conservation strategies.
- Conservation Efforts: DNA barcoding contributes to conservation by identifying priority species for protection and assessing genetic diversity within populations. This information is vital for developing effective management plans aimed at preserving aquatic biodiversity.

[DOI: 10.22034/INJBIR.2.2.3]

- Integration with Other Technologies: The advancement of high-throughput sequencing technologies and environmental DNA (eDNA) methodologies has further enhanced the effectiveness of DNA barcoding, allowing for rapid assessments of biodiversity and non-invasive monitoring of aquatic ecosystems *(*Muhala *et al.*, 2024*)*.

Recommendations for future research and applications

To maximize the potential of fish DNA barcoding, several recommendations for future research and applications can be made:

- Expansion of Reference Databases: Continued efforts should be made to expand and curate comprehensive DNA barcode reference libraries like BOLD and GenBank. This includes increasing representation from underrepresented regions and taxonomic groups to enhance species identification accuracy.
- Integration with Environmental Monitoring: Future research should focus on integrating DNA barcoding with traditional ecological monitoring methods to provide a more holistic understanding of fish communities. This integration will improve data quality and inform management decisions.
- Addressing Cryptic Diversity: Research should prioritize the identification and characterization of cryptic species using multi-locus approaches that combine DNA barcoding with additional genetic markers. This will enhance our understanding of biodiversity and evolutionary relationships among fish species.
- Enhancing Public Awareness: Efforts should be made to raise public awareness about the importance of fish biodiversity and the role of DNA barcoding in conservation efforts. Engaging local communities can foster support for sustainable practices and contribute to citizen science initiatives.
- Policy Development: Policymakers should consider incorporating DNA barcoding data into fisheries management regulations and conservation strategies. This will ensure that management decisions are based on accurate species identification and genetic diversity assessments.
- Training Programs: Establishing training programs for researchers, practitioners, and policymakers on the use of DNA barcoding techniques will build capacity within the field and promote its application in various contexts.

In conclusion, fish DNA barcoding represents a powerful tool to advance our underst anding of aquatic biodiversity, improve food security, and support conservation efforts. As research continues to evolve, integrating molecular techniques with traditional methods will provide valuable insights that will contribute to the sustainable

[DOI: 10.22034/INJBIR.2.2.3]

management of fish populations and the conservation of aquatic ecosystems worldwide (Singh *et al*., 2024).

References

Comia, C. and Morris, M., 2024. Beyond BOLD: using DNA barcoding to identify Prussian carp (*Carassius gibelio* Bloch, 1782) in southern Alberta, Canada. *BioInvasions Records*, 13(**2**), 497–513. https://doi.org/10.3391/bir.2024.13.2.15.

Fadli, N., Jumiati, S., Razi, N. M. Damora, A., Muchlisin, Z.A., Dewiyanti, I., Ramadhaniaty, M., Harnelly, E., Habib, A. and Siti-Azizah, M.N., 2023. DNA barcoding of six commercially important groupers (Epinephelidae) from Langsa, Aceh, Indonesia. *HAYATI Journal of Biosciences*, 31(**2**), 328-335. https://doi.org/10.4308/hjb.31.2.328-335.

Habib, K.A., Islam, M.J., Sakib, M.N., Brishti, P.S. and Neogi, A,K., 2023. DNA barcoding of reef-associated fishes of Saint Martin's Isl and, Northern Bay of Bengal, Bangladesh. *Ecology and Evolution.* 22, 13(**10**), e10641. DOI:10.1002/ece3.10641. PMID: 37877103; PMCID: PMC10590961.

Hidayani, A.A., Tassaka, A.C.M. A.R., Kadir, N.N., Yasir, I., Jompa, J., Umar, W., Litaay, M., Parenrengi, A., Ndobe, S., Gani, A., Putri, A.P., Iqram, M., Omar, S.B.A. and Moore, A.M., 2024. First DNA barcodes of two endemic fishes from the Maros karst region, South Sulawesi, Indonesia. *Cybium*, 48(**3**), 247-250.

Huang, W.C., Evacitas, F.C., Balisco, R.A., Nañola, C.L. Jr, Chou, T.K., Jhuang, W.C., Chang, C.W., Shen, K.N., Shao, K.T and Liao, T.Y., 2023. DNA barcoding of marine teleost fishes (Teleostei) in Cebu, the Philippines, a biodiversity hotspot of the coral triangle. *Scientific Rep*orts, 13(**1**), 14867. DOI:10.1038/s41598-023-41832-9. PMID: 37684303; PMCID:PMC10491795.

Kang, R., Zhang, Q., Qing Yang, Q. Zhengpeng Wei, Z., Yanbo Wang, Y., Jinmei Wang, J. and Lanlan Zhu, L., 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.

Mahboobeh, A., Sourinejad, I., Shahdadi, A. and Vera, M., 2023. DNA barcoding for identification and discovery of fish species in the protected mangroves of Hormozgan, Iran. *Estuaries and Coasts*, 47. https://doi.org/10.1007/s12237-023-01306-7.

Modeel, S., Negi, R.K., Sharma, M. D, P., Yadav, S., Siwach, S., Yadav, P. And Negi, T., 2024. A comprehensive DNA barcoding of Indian freshwater fishes of the Indus River system, Beas. *Scientific Reports*, 14, 2763.

[DOI: 10.22034/INJBIR.2.2.3]

https://doi.org/10.1038/s41598-024-52519-0.

Muhala, V., Guimarães-Costa, A., Macate, I.E., Rabelo, L.P., Bessa-Silva, A.R., Watanabe, L, Damasceno d.S., Sambora,L., Vallinoto, M. and Sampaio, I., 2024. DNA barcoding for the assessment of marine and coastal fish diversity from the coast of Mozambique. *PLoS ONE*, 19(**2**), e0293345. https://doi.org/10.1371/journal.pone.0293345.

Panprommin, D., Soontornprasit, K., Tuncharoen, S., Pithakpol, S., Kannika, K. and Wongta, K., 2023. DNA barcoding for fish species identification and diversity assessment in the Mae Tam reservoir, Thail and. *Fisheries and Aquatic Sciences*, 26(**9**), 548-557. eISSN: 2234-1757. https://doi.org/10.47853/FAS.2023.e47.

Siddika, M.A., Ahmed, K.A., Alam, M.S. Bushra, L and Begum, R.A.., 2024. Complete mitogenome and intra-family comparative mitogenomics showed distinct position of Pama Croaker *Otolithoides pama*. *Scienctific Reports*, 14, 13820. https://doi.org/10.1038/s41598-024-64791-1.

Sure andiran, B., Karuppasamy, K., Sundaramanickam, A., Badhul Haq, M.A., Arumugam, U. and Kumar, T.T., 2023. First record and DNA barcode of bigeye numbfish, *Narcine Oculifera Carvalho*, Compagno & Mee, 2002 (Elasmobranchii: Torpediniformes: Narcinidae) from Indian Coastal Waters. Thalassas: An International *Journal of Marine Sciences*, 39, 1215–1221. https://doi.org/10.1007/s41208-023-00569-y.

Zhang, J., Xu, L., Du, F., Tang, Q., Wang, L., Ning, J., Huang, D., Li, Y., Liu, S. and Wang, X., 2023. DNA barcoding of marine fish species in the waters surrounding Hainan Isl and, northern South China Sea. *Frontiers in Marine Science*, 10. https://doi.org/10.3389/fmars.2023.1249073.

[DOI: 10.22034/INJBIR.2.2.3]