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Molecular Barcoding of Fish: Applications and Implementation in Modern Ichthyology

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Abstract

DNA barcoding has emerged as a revolutionary molecular tool for the identification of fish species and for addressing traditional taxonomic challenges through the analysis of cytochrome c oxidase I (COI) gene sequences. This methodology, when combined with conventional morphological approaches, offers effective solutions for species identification challenges presented across various developmental stages and in degraded specimens. In this article, we examine the principles, applications, and implementation of molecular barcoding in fish identification, with a particular focus on Indian fish species. Additionally, we underscore the significance of COI-based identification systems, global database initiatives, and the critical role of institutional frameworks in fostering biodiversity conservation and managing fisheries.

Keywords: DNA barcoding, COI gene, fish identification, molecular taxonomy, biodiversity, FISH-BOL

1. Introduction

Fishes represent nearly half of the 39,900 vertebrate species known to exist, with 8411 freshwater and 11,650 marine species. Of the 2,500 fish species in India, 930 are freshwater and 1,570 are marine (Jayaram, 2010; Kar 2013; Bhakta and Saxena 2024). Fish identification has historically relied on morphological features, internal anatomy, physiology, behavior, and geographic information over the last few decades (Avise, 1994).Although morphological characteristics are essential for taxonomic classification, challenges arise when identifying fish at various developmental stages or dealing with incomplete or degraded specimens. Large-scale fishery surveys exacerbate the issue since morphological characters in adult fish can show precise taxonomic differences (Hebert et al., 2003), leading to time-consuming and costly management practices (Eschmeyer, 2003) for global assessments of fish biodiversity, conservation initiatives, and sustainable management practices. In commercial settings, mislabeling persists across markets, restaurants, and landing sites, eroding consumer confidence and market integrity. As a result, consumers pay high prices for low-grade products. Over the decades, molecular methods for identifying fish species have made significant advancements, starting with allozyme variations and later progressing to mitochondrial DNA (mtDNA) analysis (Avise, 1994). However, DNA-based methods and sequencing offer notable advantages over protein-based methods due to their increased resilience to degradation (Hanner et al., 2005) and their ability to detect synonymous mutations. Polymerase chain reaction (PCR) techniques facilitate the analysis of minimal tissue samples and allow sampling at any developmental stage. DNA barcoding comprises a curated reference library that facilitates the comparison of DNA sequences from unidentified organisms with sequences from previously identified taxa. The Barcode of Life Data Systems (BOLD) is the most extensive database available for this purpose. The development of methods for sequencing composite samples and high-throughput sequencing of PCR amplicons with generic primers (DNA metabarcoding) represents two significant advancements in DNA barcoding. The metabarcoding approach has expanded the application of DNA barcoding and associated libraries to microbiomes, nutrition, bulk sample biomonitoring, sequencing environmental samples, detecting indigenous and invasive fish species, and paleogenomics, enabling the analysis of entire communities in complex samples. DNA metabarcoding serves as a valuable tool in the molecular toolbox for investigating species dynamics in both temporal and spatial contexts (Grant et al., 2021). This article explores the transformative potential of DNA barcoding as a solution to the challenges of fish taxonomy, fisheries management, and commercial authentication, all while promoting global fisheries conservation and sustainability initiatives.

2. Principles of Molecular Barcoding

The cytochrome c oxidase I (COI) mitochondrial gene used in DNA barcoding (Hebert et al., 2003) (Fig 1) facilitates the extraction of genomic DNA through various methods (Weigt et al., 2012). Its high mutation rate and suitable effective population size are critical for studying evolutionary trends. Reliable COI amplification across diverse taxa is ensured by a range of primers and amplification techniques (Ivanova et al., 2007). The establishment of clear species boundaries using mtDNA sequences is complicated by several factors, including introgressive hybridization, ancestral polymorphism, and pseudogenes (Funk & Omland, 2003). Research shows that blended genealogy is common among closely related species, although the percentage varies by phylum (Funk & Omland, 2003). Typically, species assignment errors in many organisms remain below 5-10% (April et al., 2011). In 2005, Mayer and Pauly conducted a study estimating error rates for two purposes: (1) distinguishing samples against a well-characterized phylogeny and (2) aiding in the identification of species in partially known groups. The species identification process yields the lowest overall error rate of 4%.

Conversely, barcoding shows limited effectiveness in groups that are incompletely sampled. Thresholds are used to differentiate between intraspecific variation and interspecific divergence during species delineation. Alternatively, proponents expect a "barcoding gap" between the two, which could lead to minimal error rates of approximately 17% due to substantial overlap. Analyzing only traditionally recognized species results in a doubling of error rates. To separate introgressive hybridization from retained ancestral polymorphism, it is essential to thoroughly evaluate independent evidence, such as nuclear DNA and phenotypic traits, as mitochondrial DNA follows a maternal inheritance pattern. To address the challenges associated with DNA barcoding for species identification, it is important to recognize these issues. This can be especially difficult in groups that have recently diverged, exhibit significant hybridization, and/or have slow mutation rates compared to rates of speciation, since some species may not be easily distinguished by standard barcodes due to a lack of a clear barcode gap (i.e., maximum intraspecific distance lower than minimum interspecific distance [Hollingsworth et al. 2016]. Multiple alternative markers or approaches are recommended to improve the limited discriminatory ability of standard barcodes. Furthermore, established universal primers may bind to a wide variety of templates but fail to amplify specific target groups. As a result, there is a need for either more degenerative or target-specific primers (Lobo et al. 2013). Another challenge is the existence of barcode pseudogenes, which are non-functional duplicates of barcode regions. This can lead to misidentifications and an overestimation of species diversity (Leite et al. 2012).

DNA barcoding often involves sequencing individual specimens, while contemporary DNA metabarcoding analyzes DNA from multiple samples, allowing for the simultaneous identification of numerous species. DNA metabarcoding introduces a unique set of challenges, as study results depend on various factors and experimental design choices, such as primer selection, marker specificity, and taxonomic resolution (Taberlet et al. 2013). The demands of metabarcoding protocols have necessitated the use of alternative or additional DNA barcoding regions that are more suitable for specific taxa or applications (Miya et al. 2012).

3. Global Database Initiatives and Digital Resources

DNA barcoding initiatives like the Fish Barcode of Life (FISH-BOL) and the International Barcoding of Life (iBOL.org) aim to develop extensive sequence databases. A global effort endeavors to create a standardized DNA sequence library for around 8,000 fish species, where 98% of marine and 93% of freshwater species can be distinguished by barcodes, improving precision in species identification (Ward et al., 2012). The Fish Barcode of Life

Project, an international initiative, seeks to establish a comprehensive DNA barcode library for fish based on precise taxonomic evaluations, enabling swift and systematic identification of aquatic organisms. FISH-BOL offers public resources, including electronic DNA barcode databases, specimen images, and geographic data. BOLD organizes this information, accessible through the FISH-BOL website, and employs taxonomic frameworks from FishBase, the Catalogue of Fishes (Eschmeyer, 2003), and the Integrated Taxonomic Information System (ITIS). Digital initiatives enhance accessibility to taxonomic data for biodiversity researchers through platforms such as the Biodiversity Heritage Library and the online Registry for Zoological Nomenclature. Organizations such as the Consortium for the Barcode of Life (CBOL), Global Biodiversity Information Facility (GBIF), and GenBank leverage this data to build extensive biological collection catalogs. The ICAR-National Bureau of Fish Genetic Resources (NBFGR) is India's primary repository for fish genetic resources, mandated by the Biological Diversity Act of 2002. The bureau has created the National Fish Museum and Repository, storing collections of voucher specimens, tissues, DNA samples, cell lines, and fish milt, which are essential for researchers and stakeholders. The lab features specialized radiography equipment for detailed study of fish vertebrae counts, fin rays, and osteological traits, adhering to standardized protocols outlined by Leviton et al. (1985). DNA barcodes have been extracted from 72 species of freshwater fish across the Orders Cypriniformes, Siluriformes, Perciformes, Synbranchiformes, and Osteoglossiformes, representing 50 genera and 19 families (Lakra et al. 2015). Sahu et al. (2025) identified 37 commercially significant fish species from the Ganga Basin, spanning 8 orders, 16 families, and 29 genera. Tables 1 and 2 detail molecular barcoding information for specific species, including identification codes and corresponding GenBank accession numbers, facilitating reproducible research and database expansion. The Fish Barcode Information System (FBIS) functions as a regional DNA barcode storage and analysis system for Indian fishes, comprising 2,334 COI gene sequencing records across 472 aquatic species from 39 orders and 136 families, derived from published studies. It also catalogues phenotypic characteristics, distribution, and IUCN Red List status of fish (Nagpure et al., 2012). The barcoding process includes a methodical workflow: (1) identifying species and collecting specimens, (2) extracting DNA from tissue, (3) amplifying the target gene (COI), (4) sequencing the DNA, and (5) identifying species through database comparison (Fig 2). Specimen identification involves comparing sequences against established databases using similarity-based techniques and various alignment tools (Thompson et al., 1994). Universal primer cocktails for fish DNA barcoding have been designed to enhance taxonomic coverage and dependable amplification (Ivanova et al., 2007; Weigt et al., 2012). The success of COI-based identification systems hinges on two

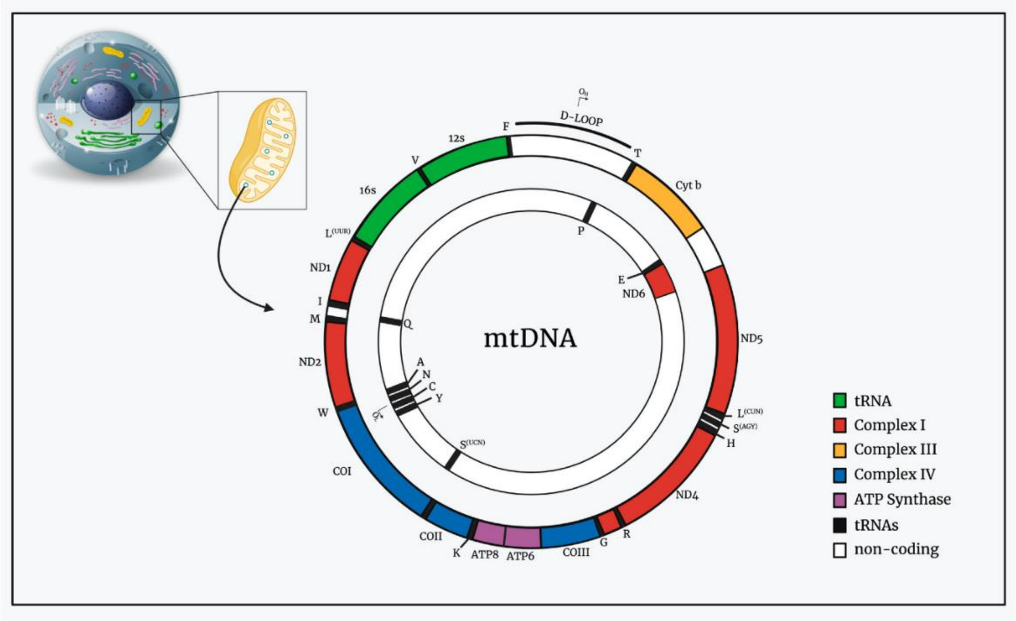

Fig 1: Position of COI gene in the mitochondrial genome (Adapted from Ferreira and Rodriguez 2024)

Table 1: A list of barcoded freshwater fish species, local names, IUCN Status (adapted from Sahu et al., 2025).

Order	**Family**	**Species**	**Local Name**	**English Name**	**IUCN Status**	**GenBank accession numbers**
Cypriniforms	Cyprinidae	*Labeo catla (Hamilton, 1822*)	Bhakur, Katla	Catla	LC	JX887593
		L. rohita (Hamilton, 1822)	Rohu	Rohu	LC	MK732923
		L. bata (Hamilton, 1822)	Bata	Bata	LC	MN080530
		L. gonius (Hamilton, 1822)	Kuria, Kurai	Kuria labeo	LC	EU417800 MH156966
		Cyprinus carpio(Linnaeus, 1758)	China Rohu	Common carp	LC	OR148070
		Cirrhinus mrigala (Hamilton, 1822)	Nain, Naini	Mrigal	LC	MG736434
	Xenocyprididae	*Hypophthalmichthys molitrix (Valenciennes, 1844)*	Silver carp	Silver carp	NT	FJ459502
	Danionidae	*Salmostoma bacaila (Hamilton,*	Chilwa, Chelhava	Large razorbelly	LC	M224244

		1822)		minnow		
		Amblypharyngodon mola(Hamilton, 1822)	Moa/Dhawai	Mola carplet	LC	KT896674 KX399051
Siluriforms	Siluridae	*Wallago attu (Bloch & Schneider, 1801)*	Padhani/ Pardni	Freshwater Shark	VU	JK983211 KX946584
		Ompok bimaculatus (Bloch, 1794)	Jalkapoor	Indian Butter Catfish	NT	MT654651
		O.pabda (Hamilton, 1822)	Pabda	Pabdah catfish	NT	FJ229974
	Bagridae	*Mystus cavasius (Hamilton ,1822)*	Sutahawa, tengra, Dariai	Gangetic mystus	LC	JN228946
	Ritidae	*Rita rita (Hamilton,1822)*	Belgagara Ritha, Hunna	Rita	LC	EU417792
	Heteropneustidae	*Heteropneustes fossilis (Bloch,1794)*	Singhi	Singee, Fossil catfish	LC	OP815346
	Clariidae	*Clarias magur (Hamilton,1822)*	Mangur, Deshi magur	Magur	EN	KP940355
Perciformes	Channidae	*Channa punctara (Bloch,1793)*	Girai	Spotted snakehead	LC	FJ459408 MF496889
		C. striata (Bloch,1793)	Souri	Snakehead Murrel	LC	KP842444
		C. marulius (Hamilton,1822)	Souri, Saur, Saul	Great snakehead	LC	PP26670
	Ambassidae	*P. lala (Hamilton, 1822)*	Tanbijla	Highfin Glassy perchlet	LC	NW485079
Anabantiformes	Anabantidae	*Anabas testudineus (Bloch,1792)*	Kawai	Climbing perch	LC	MZ312364
	Cichlidae	*Oreochromis niloticus (Linnaeus,1758)*	Jalebi	Nile tilapia	LC	KJ920137 KT307737
Osteoglossiforme	Notopteridae	*Chitala chitala (Hamilton,1822)*	Moya, Chital	Clown knife fish	NT	MF140393
Gobiiformes	Gobiidae	*Glossgobius giuris (Hamilton,1822)*	Bulla	Gangetic Tank Goby	LC	OR144574 MK57222
Mugiliformes	Mugilidae	*Minimugil cascasia (Hamilton,1822)*		Yellowtail mullet	LC	MK572330 PP918020

critical criteria: COI sequences from individual specimens must exhibit higher similarity within species than between species, and the species categorizations aligning with traditional taxonomic methods should derive from COI divergence patterns (Hebert et al., 2003). A major challenge in barcoding is defining species boundaries, given that different taxa show varying threshold values.

Table 2: List of DNA Barcoded Indian marine fish Species with GenBank Accession No

S No.	Order	Family	Genus	Species	GenBank Accession No
1	Perciformes	Carangidae	*Decapterus*	*russeli*	EF609507–EF609511
2	Perciformes	Carangidae	*Megalaspis*	*cordyla*	EF609548–EF609552
3	Perciformes	Scombridae	*Auxis*	*thazard*	FJ226525–FJ226528
4	Perciformes	Serranidae	*Epinephelus*	*fasciatus*	EU392207–EU392208
5	Perciformes	Scianidae	*Otolithes*	*cuvieri*	FJ347924–FJ347927
6	Clupeiformes	Clupeidae	*Dussumieria*	*elopsoides*	FJ347959–FJ347963
7	Clupeiformes	Clupeidae	*Tenualosa*	*toli*	EF609623–EF609626
8	Clupeiformes	Clupeidae	*Hilsa*	*kelee*	FJ158558–FJ158561
9	Clupeiformes	Engraulidae	*Stolephorus*	*indicus*	FJ347956–FJ347957
10	Clupeiformes	Engraulidae	*Thryssa*	*malabarica*	FJ347943, FJ347882–FJ347884
11	Mugiliformes	Mugilidae	*Liza*	*macrolepis*	FJ347967, EF609544–EF609547
12	Siluriformes	Ariidae	*Osteogeneiosus*	*militaris*	EF609562–EF609566
13	Siluriformes	Ariidae	*Netuma*	*thalassinus*	EU014251–EU014255
14	Siluriformes	Ariidae	*Arius*	*subroastratus*	EU148555–EU148556
15	Siluriformes	Ariidae	*Arius*	*arius*	EU148548–EU148552

16	**Pleuronectiformes**	**Cynoglossidae**	***Cynoglossus***	***macrostomus***	**FJ347954– FJ347955, FJ347911– FJ347912**
17	**Pleuronectiformes**	**Cynoglossidae**	***Cynoglossus***	***dubius***	**FJ347907– FJ347908**
18	**Beloniformes**	**Hemiramphidae**	***Hemiramphus***	***far***	**EU148546– EU148547**
19	**Beloniformes**	**Hemiramphidae**	***Hyporhamphus***	***xanthopterus***	**EU148544– EU148545, FJ237601– FJ237602**
20	**Beloniformes**	**Belonidae**	***Strongylura***	***strongylura***	**EU014256– EU014257**
21	**Aulopiformes**	**Synodontidae**	***Trachinocephalus***	***myops***	**EF609630– EF609633**
22	**Aulopiformes**	**Synodontidae**	***Saurida***	***tumbil***	**EF609599– EF609603**
23	**Aulopiformes**	**Synodontidae**	***Saurida***	***undosquamis***	**FJ347930– FJ347932**
24	**Aulopiformes**	**Synodontidae**	***Harpadon***	***nehereus***	**EU148582– EU148584**

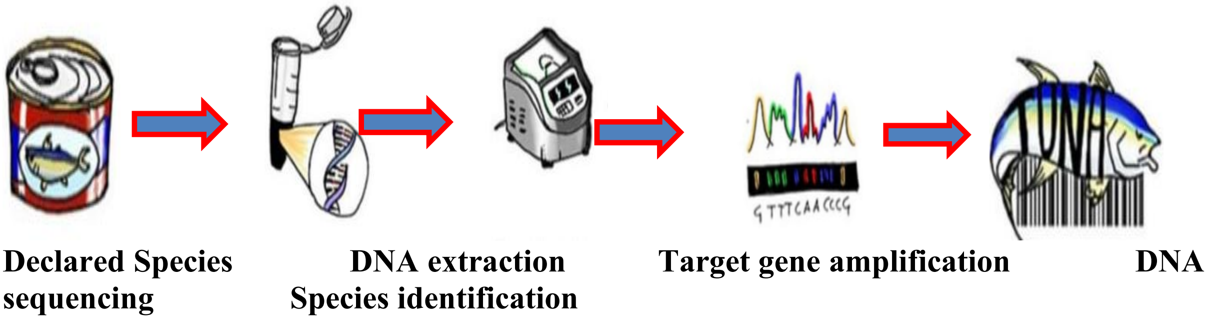

Fig 2: DNA Barcoding for species identification

4. Commercial Applications and Market Authentication

DNA barcoding has become an essential tool for tackling the mislabeling of fish species, highlighting significant differences between labeled and actual species in various markets. The regulatory use of DNA barcoding has been proved through blind testing protocols, where COI sequences obtained from muscle samples of unidentified verified specimens are accurately identified using established databases. These applications enhance market integrity by providing dependable methods for identifying species substitution and mislabeling in commercial contexts. Research shows that mislabeling is widespread across different regions, underscoring the necessity for enhanced regulatory measures and consumer awareness. In Guangzhou, testing of the mitochondrial cytochrome C oxidase subunit I (COI) gene showed that 21.58% of the 139 samples were incorrectly labeled, with all items marked as “Snapper” actually being “Tilapia” (Liu et al., 2024). A survey in South Texas found a mislabeling rate of 20.6% among 63 fish samples, with 38.1% incorrectly using suitable market names (Kaneko & Ehsan, 2024). In Mexico, the mislabeling rate was 30.8%, with local commercial names reaching rates as high as 94.4% (Munguia-Vega et al., 2022). Mislabeling may distort fish population statuses and influence consumer choices, leading to potential health risks and economic fraud. DNA barcoding precisely identifies species and promotes stricter regulations in seafood labeling (Liu et al., 2022; 2024). It proves effective for both fresh and processed fish products. A study in China using mini-DNA barcode primer sets achieved a 90.2% amplification success rate, revealing a 36.4% mislabeling rate in processed fish products. The combination of full-length and mini-length DNA barcoding methods provides a comprehensive approach for species identification, even in processed items where DNA degradation presents a challenge (Zhao et al., 2024). Ongoing monitoring and dissemination of regulatory standards are vital for combating seafood mislabeling and restoring consumer trust.

5. Challenges and Future Directions

The continual expansion of DNA barcode libraries requires ongoing collaboration across institutions and regions. Major challenges include standardizing collection protocols, ensuring the taxonomic precision of reference samples, and developing cost-effective strategies for regular identification tasks. Integrating molecular barcoding with traditional morphological methods represents the best approach for identifying fish and conducting taxonomic research. As sequencing technologies improve and costs decrease, molecular barcoding is anticipated to become more widely used in fisheries management, conservation biology, and commercial verification. The effectiveness of DNA barcoding initiatives depends on sustained international collaboration, established protocols, and the creation of detailed databases. Quality assurance must address concerns such as contamination, misidentification of voucher specimens, and sequence quality control. Relying on public databases for reference sequences risks misidentification since many sequences might not accurately reflect the species they represent. This underscores the need for a dependable local sequence database to address identification challenges and improve accuracy (Chan et al., 2024). Technical challenges in DNA barcoding fish include limited reference databases, the absence of universal primers, hybridization complicating species identification, and difficulties distinguishing recently diverged species due to minimal genetic variation in the COI barcode region (Pavan-Kumar et al., 2016). Technical issues like cross-contamination, parasites introducing errors, and the necessity for thorough bioinformatics processing arise from high-throughput sequencing outputs, which contain numerous insertions and deletions, complicating the precise extraction of barcode sequences (Mu et al. 2023). Future developments should focus on increasing taxonomic representation, particularly for underrepresented regions and species groups. Employing next-generation sequencing technologies and multi-gene approaches may enhance resolution for closely related species and overcome the limitations of single-gene barcoding. Portable, real-time identification systems are quickly emerging as the next frontier in fish barcoding; miniaturized sequencing devices such as the Oxford Nanopore MinION can identify species in the field within hours (Tyler et al. 2018). Complex taxonomic relationships and hybridization events will be clarified through multi-gene barcoding that combines nuclear markers with COI, improving accuracy in sequence analysis and automated species delimitation via artificial intelligence and machine learning techniques. Environmental DNA metabarcoding will revolutionize biodiversity monitoring by detecting entire fish communities from water samples (Hanfling et al. 2016). AI and machine learning are increasingly being integrated with DNA barcoding to enhance fish identification, classification, and studies of biodiversity. These technologies facilitate the analysis of DNA sequences, improve accuracy, and effectively handle large data sets. Machine learning algorithms, including SVMs, Random Forests, and deep learning models like DNABERT, classify fish species based on their DNA barcodes by analyzing nucleotide patterns, even for morphologically similar or cryptic species (Nanni et al. 2024). The combination of cloud databases and smartphone applications will expand access to species identification for researchers, conservationists, and citizen scientists worldwide, transforming the landscape of ichthyological research.

6. Conclusions

Molecular barcoding transforms fish identification, addressing critical issues in taxonomy, fisheries management, and commercial validation. The COI gene serves as a reliable species marker, bolstered by expanding databases and established protocols. Integrating molecular tools with traditional taxonomy deepens our understanding of fish biodiversity, aiding conservation, management, and trade. Different fish populations, treated as separate entities for quota assessments in commercial fishing (Stransky et al. 2008), can benefit from enhanced reference databases that include more populations per species. As technological advancements occur, their impact on aquatic biodiversity science and sustainable resource management will grow. Despite some implementation challenges, molecular barcoding significantly benefits biodiversity assessment, conservation strategies, and market integrity. Investing in barcode library development and technical skills bolsters global fish identification and sustainable management efforts. Community initiatives introducing DNA barcoding can improve genetic quality in aquaculture. Institutions should prioritize training programs for local researchers in DNA barcoding, fostering sustainable fisheries practices (Sasmita et al., 2024). Ongoing efforts are vital to expand barcoding for more species, especially in under-explored regions like India, where research is nascent (Sachithanandam & Mohan, 2020). Future studies should focus on building comprehensive barcode libraries for underrepresented groups, standardizing collection and analysis protocols, and integrating molecular data with ecological and morphological insights to enhance biodiversity conservation, sustainable fisheries management, and food security. The scalability, accessibility, and automation of DNA (meta)barcoding methods improve biodiversity research. In addition to monitoring, DNA barcoding offers insights that help mitigate threats to global biodiversity by enhancing environmental management and conservation efforts (Grant et al. 2021).

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