

## Review Article

# DNA Barcoding: A Promising Tool for Diverse Ichthyoplankton Conservation in Mangrove Ecosystem

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## Abstract

Among different ecosystems, mangroves are very exclusive that they are marginal ecosystems and distinctively well-defined by marked boundaries with high and low tide levels. A better understanding of the interaction between ichthyoplankton and its nursery ground is essential for protecting the threatened fish stocks and this information could provide insights on the impact of coastal degradation on fish nursery ground. However, identification of fish eggs up to species level is very difficult due to the lack of distinguishing visible characters. The cytochrome c oxidase subunit 1 (COI), a mitochondrial gene has been standardized to discriminate all the animal species. Further, ichthyoplankton diversity in mangroves can be a useful indicator of the state and health of an aquatic ecosystem. Ichthyoplankton samples can reflect their spawning output and provide an index of relative population size for the fish. Variation in the size of fish stocks can be detected more rapidly and sensitively by monitoring the ichthyoplankton associated with them. Despite the importance of mangroves as a nursery area for aquatic fauna, research on the early stages of aquatic fauna, their biology and ecology in the mangrove ecosystem have not been studied so far.

## INTRODUCTION

Ecosystems are dynamic complexes of biotic communities and their associated abiotic environments which interact together and act as functional units [1]. Mangrove ecosystems are one among the most productive ecosystems on the earth. They serve as custodians of aquatic animal juvenile stock and form most valuable biomass [2]. Mangrove ecosystem forms one of the ecological sensitive marine habitats (ESMH) at the niche between freshwater and marine environment. Due to its ecological and economic importance as a coastal resource, this ecosystem needs to be conserved and properly managed [3]. The flora and fauna inhabit in this ecosystem experiences tolerable to extreme environmental factors. Fish life cycle often involves various developmental stages that require different habitats for survival. Mangrove ecosystems with shallow waters, high turbidity, high habitat complexity and abundant planktonic food provide favorable conditions for fish larvae growth and survival [4,5]. Enumeration and identification of fish eggs and larvae are very useful for studying the egg and larval distributions that could provide information about both the dispersal mechanisms [6], and the magnitude of different habitat connectivity [7]. A better understanding of the interaction between ichthyoplankton and its nursery ground is necessary for preserving the endangered

fish stocks and this information could provide insights on the impact of coastal degradation on fish nursery ground [8].

DNA barcoding is a technique in which a short nucleotide sequence of the mitochondrial genome will act as a DNA barcode for species identification of eukaryotes, in particular, animals and it is proven to be a rapid tool for precise identification of biological specimens. DNA barcoding is based on the principle that inter-species variations are greater than the intraspecies variations, allowing one to distinguish the species using nucleotide sequences. Six-fifty nucleotide bases of 5' cytochrome c oxidase subunit I gene (COI) have been accepted as a universal barcode to delineate animal life of this planet. The inception of the Fish Barcode of Life Initiative (FISH-BOL) in 2005 launched a concerted effort to barcode all fishes in a standardized manner [9].

## Mangrove ecosystem and its importance in fisheries

Mangroves are salt-tolerant plants of tropical and subtropical inter-tidal regions of the world. Mangrove traps and accretes sediment material to reduce the erosion. Mangrove ecosystem act as breeding and nursery grounds for several wildlife species comprising fishes, crustaceans and mollusc population. Thus the habitat loss of this unique ecosystem has direct impacts in the fishery of the particular region. A better understanding

between different developmental stages of fish life cycle and diverse habitats has significant role in their survival. A mangrove ecosystem, with shallow water, high habitat complexity, high turbidity and abundant planktonic food, provide favourable conditions for fish larvae growth and survival [4,5], and hence is favourable nursery ground for many fish species. A proper knowledge about interactions between ichthyoplankton and its nursery ground has become a necessity to preserve the threatened fish species and it provides insight on the influence of coastal degradation [8]. Sheridan et al. [10], has reviewed literature about survival, growth and density of juvenile nekton (fishes and decapod crustaceans) in mangrove habitat. They conducted meta-analyses for density and survival data of nekton. The study reported that mangrove roots and debris provide refuge for small nekton from predators, thus enhancing overall survival [11], have demonstrated ichthyoplankton surveys of anchovy (*Engraulis encrasicolus*) and sardine (*Sardinops sagax*) in southern Benguela upwelling ecosystem using morphological characters. Their study made a substantial involvement toward recognising key mechanisms impacting on recruitment success and hence management of these small pelagic fish species [12], have characterized Ichthyoplankton of Swartvlei Bay and their results showed PL of Gobiidae, Soleidae, Sparidae and Mugilidae.

### DNA barcoding: why and what for?

The insight of the paucity of our understanding about the world's biodiversity, together with the limitations of current methodologies to biodiversity analysis, are the main driving forces behind new approaches to species identification. The numbers of eukaryotic species worldwide are estimated at 3.6 million to 100 million, with approximately only 1.5 to 1.8 million species having been described to date [13]. Multiple taxonomic experts are ordinarily required to identify specimens from even a single biotic survey, and the identification is dependent on the knowledge held by the taxonomists whose work cannot cover all taxon identification requested by non-specialists. Assembling teams of appropriate experts, or distributing specimens to them for identification, are both time-consuming and expensive tasks. Moreover, accessing existing literature and assessing the validity and priority of various taxon names can be a challenge even for the expert taxonomist. Another problem is that many taxonomic protocols rely on phenotypic characters, and require detailed inspection of the specimens [14]. These traditional methods of identifying, naming and classifying organisms are largely based on visible morphology. There are limitations to this method when attempting to identify organisms during various stages of their development not considered in original treatments, or when examining fragmentary or processed remains [15].

Therefore, to overcome these complications [16], introduced the concept of a DNA barcode and proposed a new approach to species identification. DNA barcoding offers several advantages compared to conventional taxonomic identification techniques. One obvious advantage comes from the rapid acquisition of molecular data. As a contrast, morphological data gathering can be time consuming and difficult. The efficiency of DNA barcoding has also been reported in the recognition and description of cryptic species [17-22], and of sibling species [23].

The main objectives of DNA barcoding are to identify unknown specimens to species level, and the discovery of new species and facilitate identification, particularly in cryptic, microscopic and other organisms with complex or inaccessible morphologies [16,24]. DNA barcoding has major goal to develop comprehensive barcode libraries for all species on earth. The access to a public reference database of taxa allowing identification of a wide range of species will be valuable whenever accurate taxonomic identification is required [25]. Therefore, a project called the DNA Barcode of Life has developed a standardized, rapid and inexpensive identification method accessible to non-taxonomists. This project also aims to create a universal system for a eukaryotic species inventory based on a standard molecular approach. To this end, the Barcode of Life Data System (BOLD, <http://www.boldsystems.org>) enables the acquisition, storage, analysis and publication of DNA barcode records [26].

### Principle of DNA barcoding

The concept of DNA barcoding is the use of a sequence standard that corresponds to a single homologous gene region, amplified by the polymerase chain reaction (PCR) with universal primers, enabling distinguishing of species across a broad range of taxa. This is based on the premise that a short standardized sequence can distinguish individuals of a species because genetic variation between species exceeds that within species [27]. For a barcoding approach to species identification to succeed, however, within-species DNA sequences need to be more similar to one another than to sequences in different species. This "matching hypothesis" [14], constitutes the key starting point for launching and implementing the new bio-identification system where a database linking a given species and respective DNA barcode array will be constructed.

Species identification through barcoding is usually achieved by the retrieval of a short DNA sequence, the 'barcode', from a standard part of the genome from the specimen. The barcode sequence from each unknown specimen is then matched with a library of reference barcode sequences resulting from individuals of known identity. A specimen is recognized if its sequence closely matches one in the barcode library. Otherwise, the new record can lead to a novel barcode sequence for a given species, or it can recommend the existence of a new encountered species [27].

### DNA barcoding in fisheries perspective

Although fishes constitute the largest vertebrate group, they are still a manageable group for demonstrating the utility of DNA barcoding, with approximately 20,000 marine and 15,000 freshwater species (FishBase: [www.fishbase.org](http://www.fishbase.org)). They are systematically diverse, ranging from ancient jawless species (Agnatha: lampreys and 32 hagfish) through to cartilaginous fishes (Chondrichthyes: sharks and rays) and to bony fish (Osteichthyes) [28]. FAO, 2008 showed that fisheries provided more than 2.9 billion people with at least 15% of their average per capita consumption of animal protein, and these products have become significant contributors to human food security.

DNA barcoding deals with accurate and unambiguous identification of not only whole fish, but fish eggs and larvae, fish fragments, fish fillets and processed fish [14]. Identification of fish

eggs and larvae is a challenge because it requires an experienced taxonomist, and involves lengthy examination of samples using microscopy to identify species-specific characteristics. A study by [29], tested the role of molecular techniques in species identification of fish eggs discovered that over 60% of the eggs were misidentified. Some larvae can be particularly problematic if they have few morphologically distinguishable characteristics and show developmental variability [29]. Phenotypic plasticity [30], is a common phenomenon, and many larvae are easily damaged during collection, leading to a large degree of uncertainty in identification. Misidentification could mislead understanding on speciation, diversity, niche partitioning, and many other features of ecosystems. [29], have shown that it is possible to identify larvae of fish using DNA barcoding techniques, but the resolution is currently limited by the availability of comparative adult sequences in the DNA sequence database.

Recently, a few studies have shown that barcoding can identify a large range of fish species [19,28,31-35]. In the [28], proof-of-concept study, barcoding effectively discriminated between 207 species of Australian fish including 143 species of teleosts and 61 species of sharks and rays, [32] analysed COI barcodes for 35 putative fish species collected in the Scotia Sea, and compared the resultant molecular data with field-based 33 morphological identifications, and additional sequence data obtained from GenBank and the Barcode of Life Data System (BOLD). They found that there was high congruence between morphological and molecular classification, and COI provided effective species-level discrimination for nearly all putative species. For two families, including the Liparidae and Zoarcidae, for which morphological field identification was unable to resolve taxonomy, DNA barcoding revealed significant species-level divergence [32].

### Ichthyoplankton identification using DNA barcoding

Identified fish larvae of coral reefs using DNA barcodes [36]. Among the 505 individuals DNA barcoded, 372 larvae (i.e. 75%) were recognized to the species level. A total of 106 species were identified, among which 11 corresponded to pelagic and bathypelagic species, while 95 corresponded to species observed at the adult stage on neighboring reefs. Thirumaraiselvi et al. [37], identified larvae from Vellar estuary, Tamil Nadu, India, at

the species level by comparison with GenBank data base using MtDNA sequence data. Results showed that four species namely, *Mugil cephalus*, *Terapon jarbua*, *Arothron nigropunctatus* and *Scomberomorus commerson* were dominant in that estuary. Wong et al. [38], identified wild-caught barnacle cyprids from Matang Mangrove Forest Reserve (MMFR), Malaysia, based on mitochondrial 12S-rRNA gene sequences. Sarpedonti et al. [39], have compared fish larvae diversity and abundance between two mangrove creeks (C1 and C2) of the Curuçá Estuary, state of Pará, Brazil. The study results showed that the engraulids were the most abundant, while the carangids exhibited the highest diversity. 39 Burghart et al., used DNA barcoding to compare the community compositions of planktonic fish eggs and larvae within a coastal embayment. Table 1 is highlighting important research works on the aspect of ichthyoplankton conservation in mangrove ecosystem. The clear disparities observed between the species compositions of the egg and larvae highlight the need for directly identifying eggs when studying habitat connectivity or performing stock assessment with egg production model-based methods. In conclusion it can be said that DNA barcoding can play a very significant role in assessment and conservation of biodiversity in the massive and diverse marine ecosystem.

### Benefits of DNA barcoding over other methods for assessing ichthyoplankton diversity in the mangrove ecosystem

Morphological identification of larval fishes in any mangrove ecosystem can be a huge challenge with the minimal amount of taxonomic key available and the rapid development of larval to juvenile stage. Other than that, it also requires considerable skills and taxonomic expertise. Traditionally, larval identification has always used morphological characters such as body shape, pigmentation, meristic count and measurements. The major limitation of traditional method (like morphological identification) is having limited ability to identify rare and cryptic species which are morphologically similar but genetically distinct. Furthermore, the different levels of expertise and capabilities among larval fish taxonomist has make it as a dependent variable in larval fish morphological identification process. Therefore, the limitation in morphological-based identification systems and the dwindling pool of taxonomist signal the need for a new approach

**Table 1:** Important works on the ichthyoplankton conservation in mangrove ecosystem.

Research work	Author (year)
Seasonal dynamics of the juvenile fish community structure in the Maowei Sea mangroves	Wu et al. (2018). [42-44]
The role of seagrass meadows, mangrove forests, salt marshes and reed beds as nursery areas and food sources for fishes in estuaries.	Whitfield et al. (2017). [45]
Estuarine ecoclines and the Associated fauna: ecological information as the basis for ecosystem conservation. In Coastal Wetlands: Alteration and Remediation	Barletta et al. (2017). [46]
Identification of larval fish in mangrove areas of Peninsular Malaysia using morphology and DNA barcoding methods	Azmir et al. (2017). [47]
Community structure of fish larvae in mangroves with different root types in Labuhan coastal area, Sepulu-Madura.	Muzaki et al.(2017). [48]
Composition and diversity of larval fish in the mangrove estuarine area of Marudu Bay, Sabah, Malaysia.	Rezagholinejad et al. (2016) [49]
Are mangroves nursery habitat for transient fishes and decapods?	Sheridan et al. (2003). [50-52]

to taxon recognition, for instance DNA-based identification [16]. DNA barcoding presented several advantages compared to other species identification methods including intraspecific phenotypic variation often overlaps that of sister taxa in nature, which can lead to incorrect identifications or species delineations, another DNA barcodes are effective whatever the life stages under scrutiny or available biological materials for identification. The successful rate for species identification using barcoding approach based on proper identification prior producing barcode databases has proved to be high among fish taxa varying between 80% and 100% [40], and has also helps in the assessment of cryptic species [41].

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