International Journal of Fauna and Biological Studies
Available online at www.faunajournal.com

ISSN 2347-2677
www.faunajournal.com
IJFBS 2020; 7(4): 72-75
Received: 04-05-2020
Accepted: 06-06-2020

Chandan Haldar
Centurion University of Technology and Management, Paralakhemundi, Odisha, India

Suchismita Nath
Department of Microbiology, Vidyasagar University, Midnapore, West Bengal, India.

Corresponding Author:
Chandan Haldar
Centurion University of Technology and Management, Paralakhemundi, Odisha, India

DNA barcoding for fish species identification: current status and future prospective

Chandan Haldar and Suchismita Nath

Abstract
DNA barcoding seems to be a promising approach for discovery of newer fish species, taxonomic identification, characterization and facilitating biodiversity studies. It supports researchers to appreciate genetic and phylogenetic associations by collection of molecular, morphological, and distributional data. Fish DNA barcoding is based on the sequencing of a uniform area of Cytochrome C Oxidase type I (COI) gene, has received significant interest as an accurate tool for species identification, authentication, and phylogenetic analysis. In recent times, taxonomic approach for fish species identification is achieved with a greater efficiency by integrating DNA sequence information with morphological traits. With the development of novel and faster sequencing techniques, DNA barcoding holds great promise in the assessment, analysis and conservation of aquatic biodiversity. The aim of this review article is to investigate recent global status, approaches, and future direction of DNA barcoding in fisheries.

Keywords: DNA barcode, aquatic biodiversity, cytochrome oxidase subunit I and fish-bol

Introduction
Among the different taxonomic methods DNA barcoding is most useful technique to identify fish species using short genetic marker. Although barcodes are sometimes used in an effort to identify unknown species or assess whether species should be combined or separated, the utility of DNA barcoding for these purposes is subject to debate. The most commonly used barcode region for animals and protists is a segment of approximately 648 base pairs of the mitochondrial gene cytochrome oxidase I (COI or COX1), is proving highly effective in identifying birds, butterflies, fish, flies and many other animal groups. This differs in the case of fungi, where part of Internal Transcribed Spacer 2 (ITS2) between rRNA genes is used, and again in plants, where multiple regions are used. The advantage of using COI is that it is short enough to be sequenced quickly and cheaply yet long enough to identify variations among species. Taxonomy, the science of classifying living things according to shared features, has always been a part of human society. Scientist formalized biological classification with different nomenclature system that assigns each organism a genus and species name. Identifying organisms has grown in importance as we monitor the biological effects of global climate change and attempt to preserve species diversity in the face of accelerating habitat destruction. Classical taxonomy falls short in this race to catalog biological diversity before it disappears. Specimens must be carefully collected and handled to preserve their distinguishing features. Differentiating subtle anatomical differences between closely related species requires the subjective judgment of a highly trained specialist.

Now, DNA barcodes allow non-experts to objectively identify species—even from small, damaged, or industrially processed material. Just as the unique pattern of bars in a universal product code (UPC) identifies each consumer product, a “DNA barcoding” is a concept in which a short nucleotide sequence of 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 works under 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 (Wong *et al*., 2008) [1]. DNA barcoding first came to the attention of the scientific community in 2003 when Paul Hebert’s research group at the University of Guelph published a paper titled "Biological identifications through DNA barcodes". In it, they proposed a new system of species identification and discovery using a short section of DNA from

a standardized region of the genome. Barcoding provides a rapid and cost-effective method for the identification of eukaryotes and is revolutionizing the application of taxonomy for taxa with validated data sets (e.g. fishes). DNA barcodes have provided new perspectives in ecology, diversity, and the taxonomy of fishes from many geographic regions.

A global initiative was taken up to develop and coordinate comprehensive DNA barcode database (Barcode of Life Database) that represents species specific markers for all living species. BOLD is an accessible database that aids in management, analysis, dissemination, and searching of DNA barcodes. An understanding of the taxonomy and systematics of fish species is a pre-requisite for sustainable management of genetic resources. DNA barcoding enhances the prospects for species-level identifications globally using a standardized and authenticated DNA based approach (Bhattacharya *et al*., 2016) [2]. The application of cytochrome c oxidase I (COI) gene for species identification in fish triggered the international initiative for barcoding all fishes (FISH-BOL; www.fishbol.org) (Ward *et al*., 2009). DNA barcoding provides an accurate and automated species identification system through the use of molecular tags based on short and standardized mitochondrial genes

The Fish Barcode of Life Initiative (FISH-BOL) is a concerted global effort to aid assembly of a standardized reference sequence library for all fish species; one that is derived from voucher specimens with authoritative taxonomic identifications. The benefits of barcoding fishes include facilitating species identification for all potential users, including taxonomists; highlighting specimens that represent a range expansion of known species; flagging previously unrecognized species; and, perhaps most importantly, enabling identifications where traditional methods are not applicable. FISH-BOL has the primary goal of gathering DNA barcode records for all the world's fishes, about 31,000 species (Kochzius *et al*. 2010) [3]. The current progress detailed by FISH-BOL region is presented in Table 1., which shows that out of the 29112 fish species only in 5334 species barcodes have been completed.

The ideal barcoding gene for DNA barcoding

A gene from mitochondria, called cytochrome c oxidase subunit 1 (CO1), was selected by the Consortium for the Barcode of Life (CBOL) as the ideal gene for DNA barcoding animal species. The gene region that is being used for almost all animal groups, a 648 base-pair region in the mitochondrial cytochrome c oxidase 1 gene (COI), is proving highly effective in identifying birds, butterflies, fish, flies and many other animal groups. The advantage of using COI is that it is short enough to be sequenced quickly and cheaply yet long enough to identify variations among species (Clare *et al*. 2010) [4]. This differs in the case of fungi, where part of Internal Transcribed Spacer 2 (ITS2) between rRNAgenes is used, and again in plants, where multiple regions of chloroplast genes (rbcL and matK) are used.

What makes the CO1 gene an ideal barcoding gene?

It's an essential gene -The CO1 gene codes for a protein that has an essential role in cellular respiration.

It's present in many living things -The CO1 gene is present in most eukaryotes and highly conserved across species

Each cell contains many identical copies - Each cell has many mitochondria (from 1-1000 copies depending upon how much energy the cell needs), each one containing multiple copies of mitochondrial DNA. When tissue sample is limited, this means that you've got a good chance of extracting enough mitochondrial DNA for successful PCR.

Close enough but not too close - CO1 gene can be used to identify individuals belonging to the same species, as well as to distinguish between individuals from different species. This is because the rate that the gene sequence changes over time is slow enough so that it's likely to be identical in the same species, but fast enough so that it's different between species.

The barcode production pipeline/methodology

- Species identification using DNA barcodes starts with the specimen. Barcoding projects obtain specimens from a variety of sources. In the laboratory, technicians use a tiny piece of tissue from the specimen to extract its DNA.
- The barcode region (rbcL, COI and ITS gene) is replicated using a process called PCR amplification.
- Analyze PCR products by gel electrophoresis.
- The amplified sequence (amplicon) is submitted for sequencing (Multiplex Illumina or NGS).
- Once the barcode sequence has been obtained, it is placed in the Barcode of Life Data Systems (BOLD) database – a reference library of DNA barcodes that can be used to assign identities to unknown specimens.
- BOLD is a searchable repository for barcode records, storing specimen data and images as well as sequences and trace files.
- It provides an identification engine based on the current barcode library and monitors the number of barcode sequence records and species coverage.
- The Fish Barcode of Life Initiative (FISH-BOL), is a global effort to coordinate an assembly of a standardized DNA barcode library for all fish species.
- However, some barcodes will be entirely new, and identification may rely on placing the unknown species in a phylogenetic tree (after multiple sequence alignment) with near relatives. Novel DNA barcodes can be submitted to GenBank® (http://www.ncbi.nlm.nih.gov).

The utility of DNA barcoding

DNA barcode have applications in various fields like, ecology, biomedicine, epidemiology, evolutionary biology, biogeography, conservation biology and in bio-industry. The low cost and rapidity makes the process easier for enabling automated species identification. DNA barcoding will help in large surveys, aiming at the unknown species detection and identification of pathogenic species with medical, ecological and the agronomical implications. It is also important into distinguish, detect and trace the distribution of patented organism in agro- biotechnology (Kim *et al*., 2010) [5]. DNA barcodes can also help assign specimens to known species in those cases where morphologic features are missing (in the case of immature, partial or damaged specimens) or misleading (as in sexually dimorphic species). DNA barcodes can also be used as a supplement to other taxonomic datasets in the process of delimiting species boundaries. Identifying the diet of an animal, based on its stomach contents or faeces. Verification of herbal medicines/foodstuffs: DNA barcoding helps in identifying adulterated products from original

components. Biosecurity and trade in the controlled species: In case of illegal import and export of economically valuable things, DNA barcoding aid as a cue to promote authorized trade and monitors illegal trade of products made of natural resources (like herbal supplements, wood, or skins and other animal parts.

Advantages of DNA barcoding in fisheries perspective

One main aim of DNA barcoding initiative is the discovery of new species. Effective tool in assessment of cryptic species (DNA barcoding is useful not only for the identification of whole fish but also for the identification of larvae, eggs, fillets, fins or other fragments of the body which are difficult to identify based on morphology). Link the larval stages of a species in order to unravel the life cycle of different marine species. DNA barcoding can be used to quickly and accurately identify the invasive alien species (IAS) and prompt preventive measures with subsequent regulatory control can be initiated. Barcoding of indicator species can be fruitful in the monitoring and abatement of marine pollution including coastal pollution (Kyle *et al*., 2007) [6]. DNA barcoding can be used as an important tool for identification, authentication and safety assessment of sea food, particularly for processed, cooked or smoked products. DNA barcodes are also used to detect food fraud and products taken from conserved species. A study conducted on the Japanese delicacy tuna sushi from different restaurants in USA, revealed the presence of endangered species, fraud and also a health hazard. DNA barcoding is an important tool in wildlife forensics and conservation. It can be used to identify endangered sea turtles by assessing turtle meat, carcasses or eggs that are illegally traded. (Hubert *et al*., 2008) [7]. It is the key challenge for the DNA barcoding initiative to monitor the existing species between the boundary and population. To solve this issue, widespread intraspecific sampling should be integrated in the reference database. The relevance of the reference DNA barcode database depends on the exhaustiveness of intra-taxon sampling.

Criticisms of DNA barcoding / Limitations

DNA barcoding has some limitations, like low resolutions in the cases of recently diverged species, High rates of intra-specific divergence reported in geographically isolated populations, species complexes and hybrids. Some researchers highlighted the presence of pseudogenes and mitochondrial introgression. In the marine ecosystem, Reproductive isolation of the biological species is difficult to investigate. A study involving cosmopolitan marine bryozoan revealed that divergent barcode clusters corresponded to reproductively isolated groups, thereby establishing a link between biological species concept and DNA barcoding (Moritz *et al*., 2004) [8]. The integration of morphological, ecological and physiological data with DNA barcode data will improve species discovery and identification process (Taylor *et al*., 2012) [9].

Future of fish DNA barcoding

A taxonomic approach of integrating DNA sequences with morphological characters will achieve higher efficiency in species identification. With the development of newer and faster techniques, DNA barcoding holds great promise in the assessment, analysis and conservation of marine biodiversity.

We have completed more than 10 years of DNA barcoding and till fish DNA barcoding is in infancy period. International Barcode of Life previously stated that ‘‘DNA sequence can be used to identify different species, in the same way, a supermarket scanner can use a familiar black strip of the UPC barcode to identify your purchases’’. It provides an objective that we need to build up a digital barcode hologram ultimately and this digital barcode hologram needs to develop for all fishes. We can identify the fish species quickly with the help of the barcode reader. However, it is not an easy task. The next-generation digital information storage system may also be developed for fishes to understand the barcode sequences. Other than the COI barcode marker, different marker gene (like ND gene) should be developed from fish mitochondrial DNA for the diversification of marker gene of fishes. It may help to develop fish DNA barcoding more easily as well as quickly. However, more researches are needed in this direction to fulfill this goal (Dasmahapatra *et al*., 2006) [10].

Conclusions

In the current scenario, organization and optimization of global efforts to generate and share taxonomic knowledge of organisms are very much required. DNA barcoding is revealing itself as an immensely valuable tool in this direction. For fishes, it promises the unambiguous identification of the vast majority of species including eggs, larvae, and even processed material (April *et al*., 2011) [11]. It employs a technology that is both inexpensive and suits to high throughput procedures. About 5000 fish species have already been barcoded through FISH-BOL, but its goal to barcode all known fish species will be achieved only by building broad community participation. While the completion of this mission promises an effective identification system for fishes, it might be associated with few drawbacks. There will be some taxonomic groups for which COI barcoding cannot provide species-level resolution. However, existing results suggest that these cases will be rare and are flagged in various database search engines. The barcode generated identification errors require immediate corrections and are being removed by ongoing curtain of the database by members of the taxonomic community. Because the development of a DNA barcoding system for fish species identification lies on a foundation of accurate taxonomic identification of the reference specimens, the success of mt-DNA barcoding will demonstrate the ongoing need as well as application for investments in collections and in the broader taxonomic support system in near future. Despite of some limitations, DNA barcoding approach can be used for survey of marine biodiversity and prioritizing conservation strategies. 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.

Table 1: Status and progress of DNA barcoding in Fishes:

Class	Barcode	Species	Progress (%)
Actinopterygii	4942	27984	18
Elasmobranchii	353	968	36
Myxini	8	70	11
Cephalaspidomorphi	14	42	18
Holecephali	15	37	36
Sarcopterygii	2	11	11

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