

Review

## DNA barcoding of aquatic animals: A review on modern taxonomy

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### Abstract:

DNA bar-coding is regarded as a competent equipment, a system designated to provide rapid and accurate identification of species along with phylogeny of organism focused on the use of short, standardized genetic region, mitochondrial cytochrome c oxidase 1 (COI) gene fragment. At gene level the modern technique of DNA bar-coding approved to be conducive or fruitful. With the aim of setting reference segment depository and tracking of DNA bar-coding program advancement at local level global investigation alliance hub called the fish bar-code of life campaign FISH-BOL was set up as DNA bar-code library. The objective of this chapter is to examine ongoing status phase of fish species identification using DNA bar-coding, strategies and practice as well as future orientation of DNA bar-coding in fishery speciation and management science. Existing condition of taxonomical works in which DNA barcodes are used in relation with fish taxonomy in India has been assessed and a comprehensive analysis of the surviving literature has been accomplished at the local, national and international level. The research outcome clarifies that the DNA bar-coding research in aquatic marine invertebrates is quietly into juvenile phase in India.

**Keywords:** DNA Barcoding, mitochondrial DNA, taxonomy

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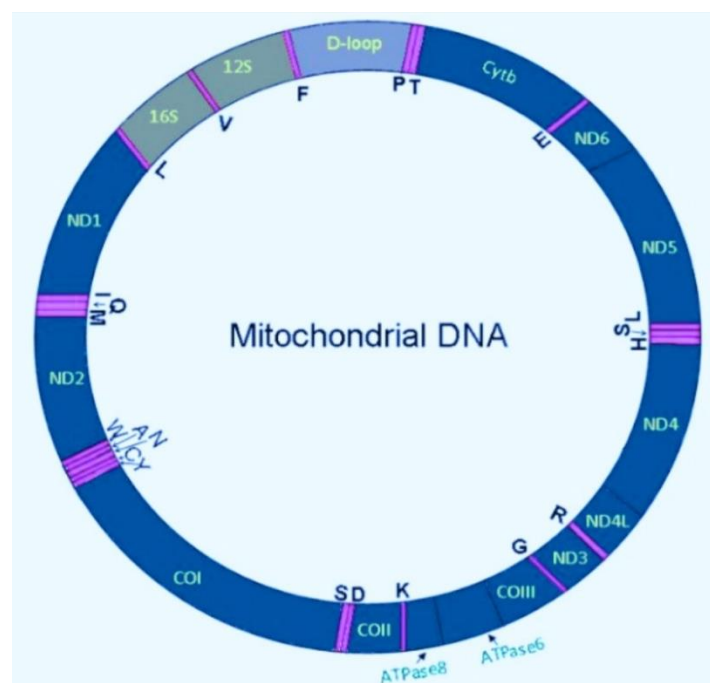
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### 1. Introduction

Till date, enormous biological variability of the globe is waiting to be completely explored. Approximately 1.6 million species have been taxonomically categorized so far, out of some another 7 million species anticipate to be discovered. For identification of organisms, DNA bar-coding is a technique which uses a compact standardized fragment of genomic DNA. Although this method speed up indexing of millions of species yet without conventional taxonomy this is strenuous labor. For the assortment of animal DNA bar-coding technique uses mitochondrial gene like 16S, COI, etc. as a exemplar gene targets. As open access data available on the public domain systems like NCBI, BOLD till date 48,000 species had been bar-coded (Wang 2017). Morphological ambiguity and extensive biodiversity of fishes results in mis-categorization of species. In case that, taxonomical identification of fishes by short genetic marker (DNA barcode) is an cost effective and fruitful methodology. Fishes are the most diverse vertebrate, practically 60% out of which 31,200 existence fish species have been documented so far (Kim et al., 2020). Till date, as per the records furnished by experts states that DNA bar-coded species of fishes were

5000, out of these 172 fishes species from Indian waters, 600 species were from New Zealand, 550 species from South Africa, 283 species from Australia and 100 fish species from Pacific Canada water (Sonet et al., 2019; Oishi et al., 2016) (Fig A1).

Molecular Identification tool, COI gene sequence is regarded as a taxonomy sorting tool, because of precision in species identification and documentation using DNA bar-coding method is gaining impulse in taxonomical assortment science for classification of fishes, independent of morphological uniqueness. All over the world employing COI gene sequence for recognition of different fauna such as bat, bird and fishes at co family level are efficiently registered. An effective demonstration of DNA bar-coding promote identification of distinguishable taxonomic category namely invertebrate mollusca (Gulf of Mexico), butterflies, Australian fish, Pacific Canada's fishes, Indian fishes and bird along with other mammals. DNA bar-coding has its application in integrative discipline, such as fish management, conservation and sustainable use. Mitochondrial DNA COI sequence allows detection of mis-categorized economically foremost species in food safety analysis (Wen et al., 2022). To permit universal taxonomic identification of fishes, the FISH-BOL was addressed to build an extensive storehouse of COI gene sequence. Accumulating fish specimen globally by researchers mastering in taxonomy (both conventional and modern approaches) and formulation of taxonomical classification initially by classical method followed by COI gene sequencing was the chief intent of the FISH-BOL. This taxonomical approach has furnished a triumph quantity of COI gene sequence outcome. From divergent sources, different studies were conducted and it was found approximately 78% fishes of Arctic and 60% of Antarctic region were bar-coded and catalogued as a result (Barman et al., 2018).



**Fig A1. Structure of Mitochondrial DNA**

According to several research attempts made to mentioned that one-fifth of total Atralian water fishes have been bar-coded so far (Zhang et al., 2019). According to Keekn & Atar (2018) DNA bar-coded data from tropic to hemitropic marine habitat including species rich

region of Africa, Asia and South America is not sufficient. Although nearly 11,023 species of fishes are taxonomically classified by conventional approaches in India, yet only 1918 species had their DNA barcode of COI gene sequence recorded which is 19.8%, and data is accumulated from figures on public platforms of NCBI and BOLD databases. These figures serve as a enormous possibilities to analyze worldwide stand, strategies and future trends of DNA bar-coding in pisciculture discipline (Galal et al., 2014). Given attempt elaborates an investigation on the status of fish bar-coding at global, national and local level.

## **2. Contemporary status of DNA bar-coding in Fishes and other Animals**

Branch of biological systematic that is concerned with the identification and classification based on reproductive behavior is called as taxonomy. At present very finite proficient individuals are pursuing the science through traditional taxonomy, which has accelerated worldwide inadequacy of skillful taxonomist and that has resulted in enormous short fall in professional manpower for the assortment of biological variability and exploration of new biological species on globe. Due to wide variety of environment conflict as well as climate change factors, it is necessary to categorize the species prior the drain of saline aquatic and terrestrial biological variability. Identification of species based on the gross morphological character had previously been decreasing as a result of downfall in financing by the government administration authorities and regional organization and additionally because of the sudden decline of the taxonomical expertise for conducting research studies on a taxonomical sector. Under these conditions for identification of 12-16 million unreported (Raharinaivo et al., 2020). It will be challenging to specify the new species which possesses morphological ambiguities and environmental identities.

The Census of Marine Life (COML) programme had undertaken courageous attempt to classify the distribution and fortune of specific species through marine territory by evaluating, including development/maturation of the guidelines database for administration and biological conservation of aquatic marine ecosystem. At present, professionals are adopting progressive advanced molecular methodology (genetic tool) for natural resources demonstration and extraction in the place of principally fundamental sciences including multiple disciplines like cytology, earth science, taxonomy, sustainable ecological sciences and elementary sciences. One after the other creation have recourse the sequencing short DNA bar-coding technique like of mtDNA, the above mention progressively acquired in taxonomical identification studies (Wang et al., 2019).

These days several approaches are being put in to extensive range of taxonomic assembly counting flittermouse (bat), pisces, invertebrate (mollusca), parasites (sponges), marine annelids (polychaetes), aves, vertebrate (mammals) etc. DNA sequencing had appreciably enhanced biological variability evaluation, willingly facilitate in the finding and uncovering of latest unique species and correspondingly to conventional assortment taxonomy, countless of which morphological ambiguous and incomprehensible. A cautious examination of scientific research literature announced that a lot of tasks had been tackled using molecular tools such as DNA bar-coding to recognize several species that involve relative species in spite of any ambiguities. For species identification, documentation and phylogenetic molecular systematic examination, diverse genetic markers like RFLP, RAPD,

isozymes etc are accessible worldwide, these comprise of two circular chromosomes (mitochondrial gene) 12S rDNA and 16S rDNA, 18S nuclear ribosome along with single nuclear gene 18S rDNA. Nevertheless, because of shortage of specific gene information, the genomic data respiratory was impotent to identify, classify the identical species from other corner utilizing these methodology. For analysis of the evolutionary relationship pattern based on their genomics and phenotypic characteristics, many researches were attempted on distinct life from using 18S ribosomal DNA and modern 16S ribosomal DNA sequence data (Ward et al., 2019).

The genetic DNA bar-coding tool is a widely acknowledged method, which use a short gene sequence (COI) to assist cataloging of a specific species precisely, suitable for amateurs who lack formal training in conventional taxonomy, by the mean of biotechnology method. DNA bar-coding is a compliment for ongoing investigation attempted in classificatory studies, by offering comprehensive data valuable for taxonomic identification in marine aquatic environment. Zemplak et al., (2019) clarified the performance of DNA bar-coding joints with sequence heterogeneity within the species (Intra specific) and species to species level (Inter specific level) from distinct environmental niche. Lara et al., (2010) demonstrated the significance of revaluation of physical structure data in concrete cases where lack of clarity remain for clarification/identification of species, this had the potential to be accomplish using additional molecular data, obtained through techniques like gene expression analysis, DNA sequencing. Ali et al., (2020) performed biological studies using mini bar-coding, preserved in formalin for protracted time frame, DNA bar-coding fulfilled a novel land mark achievement .Failure in classification in classification by conventional approach due to morphological ambiguity was overcome by adoption of COI gene sequence for recognition of feature for some group of organisms. This distinctly signifies that bar-coding is an ultimate tool for taxonomical identification of species. For divergent group of species taxonomy has been furnished in Table 1.

**Table 1 Cumulative view of DNA barcoding by using COI gene to identify animal taxa**

S.No.	Name of Taxa	Number of species targeted	Identification stage (%)	Ref.
1	Arthropod, Chordate, Mollusca, Nematode	546	64	DeSalle & Goldstein 2019
2	Gastropod Species	72	89	Hebert et al., 2020
3	Avian species	279	81	Dahrudin et al., 2016
4	Daphnia species	4	97	Ward 2019
5	Arachnid species	211	73	Arida et al., 2021
6	Marine fishes	230	88	Sheraliev & Peg 2021
7	Crustacean species	5	96	Brown et al., 2016
8	Lepidoptera species	8	69	Zhang et al., 2013
9	Cladoceran	5	90	Ran et al., 2020
10	Elasmobranches	4	91	Velzen et al., 2012

11	Marine reptiles	8	80	You et al., 2012
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In cytoplasmic mitochondrial DNA, screening analysis was performed and mitochondrial DNA found in the egg in passed down to both son and daughter from their mother for this reason each copy in indistinguishable. Maternal mtDNA significantly made a contribution to facilitate the foundational bases for ancestral geographic information, in addition furnished valuable understanding of demographic makeup. Use of polymers chain reaction (PCR) for DNA amplification of COI gene sequence and direct sequencing of large number of phyla was made practically feasible by the development of universal primers. In addition, the sequence of cytoplasmic mtDNA deviate instantaneously by it information and gene order whose structural configuration are relatively uniform. The circular chromosomes spotted inside the cellular organelles called organelles called mitochondria are beneficial to study, the species that bifurcate lately, in view of the fact that they possess a high level of alternation. Moreover whether the diverging state was not latest, nuclear genome are exemplary for study of evolutionary relationship out of biological entities. The barcode system was derived from the COI sequence heterogeneity in a single gene region, i.e. a segment of cytoplasmic mitochondria DNA cytochrome C oxidase subunit 1 (COI) gene about 650 base pairs Chakraborty et al., (2017). These COI sequence exhibited advance level genetic associated and restricted divergence, observed at arising between species (inter specific level) in addition with relative same species variability (intra specific level). In this technique of DNA bar-coding a short genetic marker in an organisms DNA is utilize to identify species in large taxonomic collection and particularity. The aptitude of employing COI gene sequence to recognize species willingly facilitates, the identification of morphologically variable taxa and cryptic as well as identify and connect individuals of sequential development stage apart from adult, supported by their accurate classified species. For discreetly segregated fish roe, larvae plus fins as well as fillets from various offshore locations, COI sequence is applied as a robust instrument for species classification and supplemental in genetic ancestry insights details Eberle et al., (2020). Galimberti et al., (2020) reported NJ trees species collection and correspondence was the consequence of decreasing sequence length (development of concept of mini bar-coding) that had intense effect of accuracy, but short sequence remarkably, persistently provide precise species. Breman et al., (2016) propose to belief that the bar-coding of DNA suggestion is the arising appliances to analyze the saltwater creature (marine biodiversity) estimation at the species level in the specified time frame. Along with this, an attempt for recognition and cladistics of polychaete (marine annelid worm) was made, utilizing molecular tool COI gene. For study of genetic distance techniques or baseline of species genetic distinctness (inter species and intra species level) and evaluation of K2P values a software application for DNA bar-coding sequence was explained by Hou et al., (2018). Another experiment for genetic classification and cladistic study of leech by DNA bar-coding was performed by Nneji et al., (2020). This research outcome furnish a broad array of information a specific species which may potentially be benefited in examining species richness index. Improving the performance for regular categorization, standard fragment (such as bar-coding region) appraises to be favorable. Under specific circumstances, for taxonomical identification and conservation of biological diverse species, DNA barcode (bar-coding

sequence), hold the capability to be employed self-sufficiently without any assistance of taxonomic professionals. Standard bar-coding came forward as a quality control measure to authenticate the specification of specimens. The economic application in DNA bar-coding in farming facet of detection of infests species, non-native species identification and pisciculture administration (without physical character), is merited consideration. Specific kinetics of larval dispersion mechanism in accordance dependent on the current water flow and the ecological determinants are the predominant constituents of the taxonomy of Pisces, marine creatures. Numerous unresolved matters related to aquatic marine flora and fauna and adaption as well as development of fish population revolve around to what extend the planktonic larvae (veliger larvae) disperse away from their maternal and paternal figures (Papa et al., 2021). Efficient administration of these population is hampered due to incapability by unequivocal taxonomic categorization of juvenile stages of many taxa nonetheless, significance of ecological operations influenced by larval fish dynamics. Differentiating larval classification at genus and species level by several scientist collectives conducting early development investigation were hindered with substantial complication. To overthrow aforementioned complication the molecular tool (COI gene sequence) also known as identification tool allowing a clarification of larvae, was pin out lacking morphological features.

The methodology of DNA bar-coding has been authenticated to be contradictory by scientific fraternity regardless of its bright prospects. Recently achieved outcomes by many professional researches exemplified the prospective gains with the utilization of efficient systematized molecular strategy for the identification of biological species. Classification purely based on morphological feature turned out to be erroneous due to intermixing of phenotypically diverse characters within a species of sister taxa in nature (Rabaoui et al., 2019). Newly implemented methodology in biodiversity research and conservation, next generation sequencing technology, possess inherited capacity to further expand the practicality of DNA information. Asserted the great practical application of DNA technology can be employed to accumulate barcodes sequence within minutes and utilize integrated barcode information repository to produce categorization by the development of a handheld apparatus. For administration and commerce of economically significant resources the accurate classification and taxonomic vagueness present for few fish genera/species were crucial. This issue was settled along with assistance provided in the revelation of ambiguous species details by the approaches of DNA bar-coding reported Han & Liu (2018). Taxonomic classification based on visible features demanded a significant number of taxonomists, which in reality is gradually declining due to it being a difficult task. Therefore, methodology of short standardized DNA bar-coding may authorize new life scientist – identification stationed on mitochondrial cytochrome c oxidase I (mtDNA COI) gene.

A wide variety of barcode studies, further provide an expertise of ecological diversification, distinctly for the marine fauna diversity, that is impossible to be supervised uninterrupted by convention taxonomy. Additionally due to the deficiency of the fundamental detail for innumerable ray fishes (cartilaginous fish) and shark (super order- selachimorpha), the fishery supervisors and research specialist are facing a lot of challenges. These conceivably recognized through bar-coding technique include guide to the seizure of

matter like shark fins (dorsal fin- prominent fin), meat, bones etc (Luo et al., 2017). The present techniques come up with elaborative data that creditably aimed for administration and scientific preservation.

Pre-juvenile fishes were consistently not recognized by applying conventional taxonomic practice, up to species level because of restricted structural advancement and maturation as well as their compact size. This challenge brings about complication in interpreting the fish life cycle histories at the post larval stages, particularly ocean water fish. For taxonomic identification of marine fishes found in diverse habitat like coral reef, near shore environment, evolutionary lineage and connections (*Lutjanus griseus*, *Epinephelus lanceolatus*) and early developmental period, a handful of genera had been surveyed (in the context of economically significant marine species); these investigations were considerable distance away from the comprehension and have been consistently evaluated (Ning 2014). Examined the barcode closely and comparative taxonomic investigation of five Tilapiine species and the outcome represented COI gene sequence differentiating species characterization free from any obscurity. For us, to assess the precise biological variability diagnostic survey of gonadactylid (*Mahitis shrimpete*), the stomatopod larvae, group of marine crustaceans were researched by the DNA barcode methodology (Yao et al., 2009).

The zoological research for the taxonomical identification of diverse life form utilized the COI sequence (Mitochondrial cytochrome c oxidase subunit I) rapid and powerful tool for precise identification). In comparison with the other groups such as invertebrate (97% of all animal species) sponges, corals, sea urchins etc, fishes are the most researched life forms and presently undergoing DNA bar-coding by two worldwide campaigns namely FISH-BOL and SHARK-BOL (Yang et al., 2022). A gap was conferred globally when current bar-coding of fish studies were analyzed across the globe. For molecular DNA bar-coding analysis in India, marine fishes are limited and inadequate and particularly for grouper species, there is dearth of detailed information accessible from the Indian shoreline.

Previously done taxonomical studies of grouper fishes was to enhance the knowledge of the correlation of common ancestry assay inter species investigation with associated families undertaken. Despite that morphological uncertainty, there are substantial differences on grouper species (sea bass, snowy grouper) taxonomy authentication cladistic examination within a specific region. Limited researches undertaken for identification of grouper species are as listed:- Mitochondrial DNA cytochrome b (cyto b) and 16S rDNA (ribosomal DNA) segment assay for species phylogenetic assembly between the correlated genus (*Epinephelus* and *Mycteroperca*) from Atlantic groupers was done by Wu (2010). The scientific research outcome recommended that genetic variability noticed in a proximate genus (*Epinephelus* and *Mycteroperca*). Paradis (2019) Conducted molecular procedure, to perceive the evolutionary ancestry within the fishes community in the perciform tribe Epinephelinae (serranidae) being inadequately known from Indian aquatic ecosystem.

### **3. Fish Diversity and DNA barcode status in India**

India has a submarine ledge with 8000 km long shoreline, a treasure repository of vast fishery resources and a broad coastal territory which has significant ecological, economic

and social importance. Indian shoreline water are acknowledged for their wealthy and heterogeneous aquatic fish species. From 18 century significant contributions to Indian fish lore had been made by revolutionary work.

Andaman and Nicobar Island lie at the junction of Andaman Sea with Indian Ocean and western Pacific Ocean for this reason species variability of fish in the region becomes of additional significance of saltwater biogeography and coral reef ecosystem. 1510 species falling in 610 genera of 188 family are entirely characterized from these region out of which 425 species stand with economic importance, 26 of non-saline waters, 291 from marshy ecosphere, 161 of seagrass meadows, 95 from offshore (below the sunlit surface water) habitat and 1126 of coral reef ecosphere, out of total fish species, are reported (which constitute 80.58%) whereas generally noted number of species from mangrove, littoral, seagrass and coral reef ecosphere is 161. 8 species of amphibian, 16 fish species and 31 reptilian species, documented from the island are endemic Ratnasingham & Hebert (2013). Existence of some new species documented analogous to their closest equivalent which formerly seemed different for a traditional taxonomist was exposed when research study of waters of south-west coast of India was conducted. Eastern coastal India includes species rich region of Bay of Bengal and the cluster of Andaman and Nicobar Island. The region remain as insulated and untouched in the terms of investigation and consumption of fishery resources. In order to develop impeccable and instant tool for economics as well as to get the knowledge of genetic alterations and evolution, an initiative to barcode the fishes from Andaman water region was undertaken. On both coasts of India, east as well as west, DNA bar-coding of saline water fishes was carried out. The barcode outcome explored very limited and documented that mean K2P value on intra-species level, order, family and genera were 10.31%, 18.30%, 9.50% and 1.80% proportionately. From Kakinada coastal area on Eastern seaboard of India Carangid fish for genetic variability and recognition productiveness was examined by applying the molecular approach of COI gene sequence by Puillandre et al., (2018).

Reid & Carstens (2016) conducted DNA barcode researches on the fishes of coral reef were found to be very restricted, specifically the groupers of Andaman archipelago. In an alternate investigation research undertaken using the random amplified polymorphic DNA (RAPD) technique detailed the analysis of Indian mackerel (*Rastrelliger kanagartha*) in the eastern and western coastal region of India and the research demonstrate remarkable genetic dissimilarity between species. In the Porto Nova coastal region, DNA bar-coding of *Lates calcarifer* (family latidae) was examined using COI gene for the cladistic study analysis and genetic distance were equated with species around the world. DNA bar-coding of ambiguous species of Mugilidae fishes (Mulletts) for their cladistic analysis and revelation of their haplotype variability as a result of their detected genetic distances within the species was done by the work of Fujisawa & Barraclough (2013).

DNA barcode work information for the grouper subfamily Epinephelinae species of Andaman water was introduced. In view of the foregoing assessment of literature, recommended that the research on monophyletic clade of fish barcode work was not conducted systematically in Indian fishery and aquaculture science. Consequently, objective of this section was to explore worldwide status, strategies and future orientation of fishery science by utilizing DNA bar-coding. The publications from Antecedent literature, which



have been undertaken in the local levels, and to highlight the shortcoming in specific community, the researchers were undertaken in coastal areas of India, that have been evaluated in depth. In accordance with the review of published survey data, it evidently demonstrate to appraise flora and fauna of Indian Marine habitat, further it is essential to research the molecular DNA bar-coding in more organized manner, with significant emphasis to invertebrate for the biological diversity evaluation.

#### **4. Focus on DNA Bar-coding**

In the sphere of fish preservation and administration utilization of DNA bar-coding approach is gaining more and more significance, this assist in approximate of marine bio resource proportion, analysis of unwanted fish or creature captured and feasible fishery tracking science. DNA bar-coding was acknowledged as and proficient approach in food safety industry as it exposed one-fourth of miscategorized or replaced specimen collected from the restaurant and market of Canada and USA as perceived from COI gene sequence Meyer (2015).

#### **5. DNA Bar-coding Progress**

Production and collection of DNA barcode data of all the fishes on global level constituting 30,800 species is the chief objective of FISH-BOL. Developing COI gene sequences for all the fishes globally was the purpose of FISH-BOL. Individually 74% fishes from Arctic region and one-half were from Antarctic region were bar-coded (Shen et al., 2019). Apart from this, from different locations like Oceania, Australia and America the documented data is suggested to be one-fifth. From the locations, although, well flourished with distinct species like Africa and Asia, the illustrated bar-code data found is very limited. This evidently signifies that there had been inclination toward the generation of abstracts of saline species. 4200 fishes (65.3%) out of 6600 bar-coded under the initiative of FISH-BOL were of marine environment, testified this fact Bagley et al., (2019).

Outlined that from Indian waters 12,412 species of fishes have been documented phenotypically out of these only 2002 species are till now bar-coded that constitute of about 21.45% of total. Hence, future investigations should focus on the gathering marine as well as freshwater specimen from Southeast Asian and Indian regions and researching on them. In forthcoming times, bar-coding specimen campaigns for the neglected families and orders of Indian fish fauna need to be performed was clearly suggested by Phillips et al., (2019).

#### **6. DNA Bar-coding Success Rate**

The bar-coding technique accomplishment rate arose to be 80% predominantly in the fish species classification executed in the saline or brackish water of estuaries but not generally set up in fresh water streams. It is achievable to recognize species using a short genetic marker in an organism's DNA advanced by Fishbowl uses a MySQL database. DNA bar-coding involves sequencing a short fragment of the mitochondrial cytochrome c oxidase subunit 1 (COI) gene specified that haplotype genetic difference and regional genetic difference are a result of changes in surrounding as well as dissimilarity in habitation (Wang et al., 2015).

#### **7. Limitation and Cautions**

Mitochondrial DNA COI gene sequence, usually derived from the maternal parents in fishes and suggested that the success of DNA bar-coding in based on erroneous

morphological systematic, recent bio-speciation and (species hybridization) inter-specific mating where bar-coding is incompetent to characterize interspecies variations. The need of scientific research collectives to be vigilant in acknowledging the aforementioned factors and use of extra supplementary nuclear gene along with the mitochondrial DNA further arose due to numerous disadvantages in the utilization of DNA bar-coding tool for taxonomic species identification. In accordance to the zoological group and data collections the biological phenomena of hybridization between closely relative species, nature introgression (natural hybridization), inaccuracy in the area of a species identification utilizing established taxonomic approaches and present speciation (development of new species) process take place at diverse extend at dissimilar magnitude and are recognized to be intervene forcefully with DNA bar-coding process. In order to generate optimum outcomes from DNA bar-coding methodology for precise species identification destitute of inconclusiveness it is well regarded to accumulate more information repositories on distinct species COI sequence from diverse geographical ecosystem.

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