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DNA barcoding: Importance in fisheries research and food safety

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ABSTRACT

DNA barcoding gives an impression of being linked to the electronic barcoding but differ a lot in the process involved in identification. In electronic barcoding the handheld scanner "Star Trek tricorder" scans the specific product with barcodes printed on it for its identification. However in DNA barcoding we require DNA from the target life form and identification is carried out with molecular protocol. DNA barcoding have demonstrated effectiveness in recognition of wide range of taxonomic groups. This technique also received suggestions and reproach. The solution for the accuracy in the said modern tool lies in the proper sampling and data analysis. The traditional taxonomy is practical way of discriminating the good form of live or dead fish but not useful for identification of unknown, processed or mixed food samples. For such identifications we must take the help of modern tools in molecular biology to go ahead with the identification in shorter time. DNA barcoding perhaps is the modern tool which could serve the purpose. We discuss about the DNA barcoding in food safety and fisheries research with the value of classical taxonomy.

KEYWORDS

DNA barcoding;
Taxonomy;
Fisheries science;
Food safety;
COI gene.

INTRODUCTION

Taxonomy is very important branch of biology which clarifies the identity of the specimens. Correct identification of species is carried out before start of any biological experiment in order to avoid biological error. Fish identification can be challenging, especially in the tropics and this is particularly true for larval forms or fragmentary remains[1]. Species identification and classification has traditionally been the specialist domain of taxonomists, providing a nomenclatural backbone and a key prerequisite for numerous biological studies[2]. Unfortunately, over the past few decades, taxonomy is being completely overshadowed by seemingly spectacular and glamorous branches of biology[3]. This is not to say that classical taxonomy has become less important, but taxonomic identification by DNA based methods is more sophisticate way which identifies specimens in almost all forms and stages. The use of DNA-based methods for species detection presents a number of advantages over protein-based methods, including increased specificity, sensitivity, and reliable perfor-

mance with highly processed samples[4]. In this scenario, DNA barcoding is a modern tool of taxonomy which aims towards documenting all life on earth on molecular level by a simple standardized genetic tag. The tool assures perfect inspection of processed food, convinced species identity in biodiversity studies and biological research.

Nowadays the technology of DNA sequencing became better, from manual sequencing to automated sequencers. This can be achieved with the help of a single automated sequencer which gives sequences of 1000 base pairs (bp) per day. If the sequencer is not available in lab, it could be carried out by commercial labs that offer this service at a competitive price. Clearly, the development of DNA barcoding is linked to these improvements[5]. Enthusiasts aim to create a portable DNA barcoding device that will identify an anonymous specimen by species and link to a database crammed with biological information[6, 7]. Cameron et al[8] also predicted the development of portable DNA scanners at some point due to the need for scanners capable of detecting biological weapons. However he is not sure about the affordability or practicality for the kinds of mass identification users. The cost of testing a specimen for cytochrome c oxidase 1 gene variation is now about $2 without labor cost and bar coding all of life would be relatively cheap in terms of other big science projects[6, 9].

Studies on DNA barcoding suggests that it is one of the modern tools in molecular taxonomy which helps to carry fast and accurate species identification. In fisheries research this marker would be very useful for biodiversity studies, biological research as well as for food safety. Of course this tool surely needs strong base of traditional taxonomy to become universal for serving the science and society.

HISTORY AND DEFINITION

DNA sequence analysis has been used for 30 years to assist species identifications, but different sequences have been used for different taxonomic groups and in different laboratories[10]. Paul D.N. Hebert from the University of Guelph, Ontario, Canada has started DNA barcoding in 2003 with the proposal that organisms could be assigned to their correct species using a short gene sequence from a standardized position in the genome[9]. The gene region that is being used as the standard barcode for almost all animal groups is a 648 base pair region in the mitochondrial cytochrome c oxidase 1 gene ("COI"). COI is highly effective in identifying birds, butterflies, fish, flies and many other animal groups but not an effective barcode region in plants because it evolves too slowly. It becomes the useful tool for identifying the animals from egg to larval forms to the damaged or unidentified forms. Several loci have been suggested for DNA barcoding animals, eukaryotes, land plants and fungi. But a common set of standardized regions were as given in TABLE 1. The process of generating DNA barcodes from an unknown tissue sample is given in Figure1.

TABLE 1: Standardized regions for DNA barcoding[9]

Sr. No.	Group	Region
1	Animals	Mitochondrial COI gene
2	Land plants	rbcL and matK chloroplast gene
3	Fungi	Internal transcribed spacer (ITS) region

GLOBAL DNA BARCODING INITIATIVES

The barcoding work is globally carried out under the project named as iBOL, the International Barcode of Life Project. This 25-nation consortium was organized by the Biodiversity Institute of Ontario at the University of Guelph with support from Genome Canada. iBOL's goal is to create 5 million barcode records from 500, 000 species in five years. Ten Working Groups devoted to different taxonomic groups or habitat types form the core of the activity. The Consortium for the Barcode of Life (CBOL) is an international initiative devoted to developing DNA barcoding as a global standard for the identification of biological species. It is established in 2004 through support from the Alfred P. Sloan Foundation and promotes barcoding through Working Groups, networks, workshops, conferences, outreach, and trainings. CBOL has 200 Member Organizations from 50 countries and operates from a Secretariat Office located in the Smithsonian Institution's National Museum of Natural History in Washington, DC. The other consortium work-

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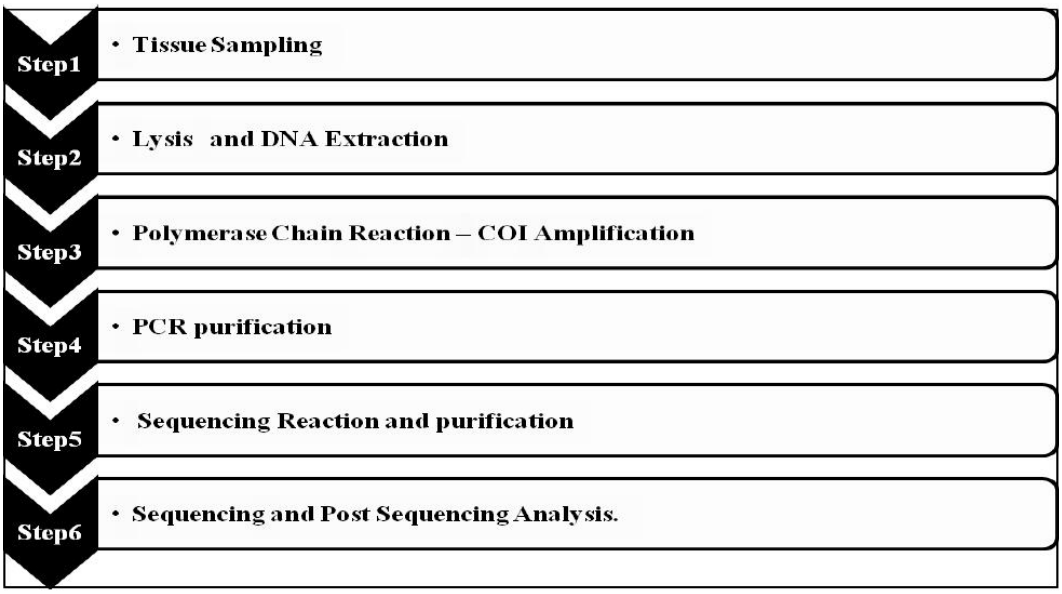

Figure 1 : Schematic representation of DNA barcoding protocol

ing on DNA barcoding is European Consortium for the Barcode of Life (ECBOL) which was established as part of the research infrastructure efforts of European Distributed Institute of Taxonomy (EDIT).

The work on use of DNA barcoding in fisheries was started globally with the launch of Fish Barcode of Life (FISHBOL) campaign in 2005 to create a global reference library of all 30, 000+ species of cartilaginous and bony fishes from marine, estuarine and freshwater ecosystems[11]. Ten Regional Working Groups have been established for the FAO regions with participation of 160 researchers. All FISHBOL data are being integrated in a single database and will be made available to the public without charge.

DNA BARCODING STUDIES IN FISHERIES RESEARCH

For species identification and database creation:

The sequencing of two hundred and seven species of Australian marine fish was carried out for a 655 bp region of the mitochondrial cytochrome oxidase subunit I gene with conclusion that DNA barcoding can be effective to identify fish species[10]. They studied the specimens of three species of chimaerids, 61 species of sharks and rays and 143 species of teleosts for the barcode region of COI. Interestingly all the species which has been sequenced are easily classified and discriminated. Hebert et al[12] sequenced barcode region of COI for 1360 Individuals belonging to 190 Canadian freshwater fish species and evidenced that freshwater fish species can be efficiently identified through the use of DNA barcoding. They found that the mean genetic distance between conspecifics was generally much smaller than the average distance between individual from distinct species. Ward et al[13] bar-coded a 650 base pair region of the mitochondrial cytochrome c oxidase I gene of Asian sea bass, *Lates calcarifer* from Australia and from Myanmar which suggested that both are different species. However he recommended further examination on genetic and morphological level for confirmation. This study put light on the misidentification mistakes carried out while identifying the fishes. A reference collection of COI barcode (650 bp) for coral reef fishes (22 species of Acanthuridae and 16 species of Holocentridae) has been constituted by Hebert et al[14]. This study revealed that all larvae sequenced could be identified to species level using DNA-barcodes. Nwani et al[1] revealed that DNA barcoding is very effective for identification of Nigerian freshwater fishes. Studies on 229 DNA sequences of COI gene from 158 marine fishes of Japan were carried out by Zhang and Hanner[15]. They also studied hybridization phenomena in two species (*Kyphosus vaigiensis* and *Pterocaesio digramma*) through searches in Barcode of Life Data Systems (BOLD). They found this study as useful and new way of discriminating the fishes for identification. Vincent et al[16]

analyzed 1570 bp of mitochondrial and nuclear sequence data (cytochrome b, cytochrome c oxidase subunit I, and internal transcribed spacer 2) to assess the validity of spotted eagle ray *(Aetobatus narinari)* as a single cosmopolitan species and infer its evolutionary history. They collected specimens from the Central Atlantic, Eastern Pacific, Western Pacific, and Central Pacific and marked out at least 2 distinct species of spotted eagle ray of which the latter species is further divided into 2 subspecies. Even the technique can be easily used in early life stages of fishes in which identification with morphological characters is difficult. Research work carried out by Victor et al[17] identified the larvae and newly-settled juveniles of the Cubera Snapper, *Lutjanus cyanopterus* from the Caribbean coast of Panama with the help of DNA barcoding. Aquino et al[18] carried out DNA barcoding of 18 fish species of Laguna de Bay, Philippines and found it as fast and accurate method for species identification on the basis of COI sequences. An analysis of the COI gene sequences of 500-652 bp in length was performed on 820 individuals from 67 species of the Czech ichthyofauna[19] which revealed that as per taxonomical opinions some species which actually are in different genus clustered together in phylogenetic tree. Zhang and Hanner[20] stated DNA barcoding as a biodiversity monitoring tool on the basis of characterization of 242 species of fishes from the South China Sea with (COI) gene. The DNA barcoding was used by Adriana et al[21] for species identification in many metazoan groups including some crustaceans with a case study involving 80 malacostracan species from the Estuary and Gulf of St Lawrence. Ma et al[22] confirmed the utility of DNA barcoding for identification of genus *Scylla*, which includes four species: *Scylla paramamosain, Scylla serrata, Scylla tranquebarica* and *Scylla olivacea*. In India the national programme of DNA barcoding was carried out by Lakra et al[23] in which 115 species of marine fish covering Carangids, Clupeids, Scombrids, Groupers, Sciaenids, Silverbellies, Mullids, Polynemids and Silurids representing 79 Genera and 37 Families from the Indian Ocean have been barcoded for the first time using cytochrome c oxidase I gene (COI) of the mtDNA.

For authentication of seafood products and food safety:

The detection of commercial fraud by mislabeling is difficult, especially in processed products, where all morphological characters suitable for species identification have been eliminated. Furthermore, the large number of traded species from all over the world is making it impossible for the inspection authorities to control for correct labeling[24]. The results of the study conducted by Marko et al[25] showed that 77% of fish sold as red snapper in the United States were in fact other species. Sajeela et al[26] and Cubelio et al[27] used the COI gene sequences for biological identification of the whale shark and crabs respectively. In the study on whale shark the flesh suspected as that of the Wildlife protected whale shark was tested with this method using COI sequences. The study revealed that the suspect was true. This was the first time in India that modern tools were used in identifying meat of an aquatic organism which is enlisted in the Wildlife Protection Act. The application of the DNA barcoding in fish food industry is better tool for knowing the mislabeled food in fisheries industry for the benefit of consumers. Wong and Hanner[28] conducted study to detect the market substitution in North American sea food and found mislabeling in the 25 % of sea food samples collected in the study. This is effectively studied in crustaceans in the study carried out by Pilar et al[29] where commercial crab-meat was authenticated by DNA Barcoding a partial sequence of the Cytochrome Oxidase I (COI) gene of seven commercialized brachyuran species in Chile. The study revealed that most commercial crab packages contained more than one species of crab. DNA barcoding of smoked products from fish in 10 families in four orders was carried out by Smith et al.[30] for identification and tracking out the possibility of mislabeling of the fish fillets and found that COI sequences of fish fillets were matched against COI sequences taken from reference specimens held in BOLD and GenBank.

DISCUSSION

Apart from the assurance of DNA barcoding, there have been some different views on this method by some scientists. Will and Rubinoff[31] stated that DNA-based data should not be seen as a substitute for understanding and studying whole organisms when determining

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identities or systematic relationships. Ebach and Holdrege[32] said that the work of taxonomists provides knowledge of the organism, not a few possibly unique nucleotides. He expressed that every barcode must be linked with a known, described specimen stored somewhere. However Gregory[33] believe that the DNA barcoding would benefit and not compromise the taxonomic science As per Song et al[34] the presence of COI numts (especially when they are prevalent) makes difficult to achieve the accuracy level in identification which creates serious ambiguity into DNA barcoding. He suggested a careful examination of sequence characteristics before barcoding analyses in order to reduce the possibility of incorrect inferences. Moritz and Cicero[35] suggested that large-scale and standardized sequencing, when integrated with existing taxonomic practice, can contribute significantly to the challenges of identifying individuals and increasing the rate of discovering biological diversity.

The work on DNA barcoding is on its way as the researchers are trying to be more accurate in the collection of data and analysis. Ward et al[14] also recommended the genetic and morphological examination of the samples of sea bass *Lates calcarifer* from Australia and Myanmar which he found different by DNA barcoding. There is also the need to examine groups with frequent (possibly cryptic) hybridization, recent radiations, and high rates of gene transfer from mtDNA to the nucleus[35]. Meyer and Paulay[36] revealed that DNA barcoding holds promise for identification in taxonomically well-understood and thoroughly sampled clades. According to them, the promise of barcoding will be realized only if based on solid taxonomic foundations. If COI is lack of high resolving power other molecular markers such as cytb, 16S, and 18S could be used for identification[20].

The applications of the DNA barcoding would also improve the quality of exports as only genuine species would be allowed through DNA barcoding screening in the processed form by employing the DNA barcode scanning for the export sea food carried out by specified laboratories. The government shall insist on the exporters to get the product certified by these labs so as to authenticate the export. It is not to be refused honestly that the identifying the samples with fast pace is only possible through the modern techniques. If we say about the accuracy of the DNA barcoding we also should think about the accuracy of traditional taxonomy in the cases where specimens are old and damaged. In many cases, fish and their diverse developmental stages are difficult to identify by using morphological characteristics alone due to high diversity and morphological plasticity[17]. There are some species which are almost similar morphologically with minor differences. If such species in the preserved forms are identified by taxonomic based identifications could possibly give different inference on their identity. In such cases, DNA barcoding would be the additional tool for confirmation of identity. Thus to conclude in short, the DNA barcoding and traditional taxonomy if come together and support each other can surely go far ahead in cataloging the life on earth.

CONCLUSION

The fisheries research irrespective of the area it covers requires the proper and accurate species identification. Fisheries experiments in the areas like culture, nutrition, diseases etc. needs accurate species use as a prime base of the study. However in such experiments the fish identification is carried out using morphological keys and is the pragmatic way of identifying fish. DNA barcoding becomes less practical in this kind of research involving live and fresh animals which could be speedily identified with taxonomic keys. Hence the knowledge of taxonomy becomes important for biologists as a prime tool of identification. However a study which involves the identification of species in degraded forms requires the help of special tools. At this stage if we need the accuracy in the identification of specimen, DNA barcoding is the best option. So both taxonomic as well as molecular tools are very important in identification of animals in fisheries research.

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