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PRAGMATIC APPLICATIONS OF DNA BARCODING MARKERS IN IDENTIFICATION OF FISH SPECIES – A REVIEW

Saima Naz[1], Ahmad Manan Mustafa Chatha[2], Rifat Ullah Khan[3]♦

[1]Department of Zoology, Government Sadiq College Women University, Bahawalpur, Pakistan
[2]Department of Entomology, Faculty of Agriculture and Environmental, The Islamic University of Bahawalpur, Punjab, Pakistan
[3]College of Veterinary Sciences, Faculty of Animal Husbandry Veterinary Sciences, The University of Agriculture, Peshawar, Pakistan
♦Corresponding author: rukhan@aup.edu.pk

Abstract

DNA barcoding and mini-barcoding involve Cytochrome Oxidase Subunit I (*COI*) gene in mitochondrial genome and are used for accurate identification of species and biodiversity. The basic goal of the current study is to develop a complete reference database of fishes. It also evaluates the applicability of *COI* gene to identify fish at the species level with other aspects i.e., as Kimura 2 parameter (K2P) distance. The mean observed length of the sequence was ranging from 500 to 700 base pairs for fish species in DNA barcoding and from 80 to 650 base pairs for DNA mini-barcoding. This method describes the status of known to unknown samples but it also facilitates the detection of previously un-sampled species at distinct level. So, mini-barcoding is a method which focuses on the analysis of short-length DNA markers and has been demonstrated to be effective for species identification of processed food containing degraded DNA. While DNA metabarcoding refers to the automated identification of multiple species from a single bulk sample. They may contain entire organisms or a single environmental sample containing degraded DNA. Despite DNA barcoding, mini-barcoding and metabarcoding are efficient methods for species identification which are helpful in conservation and proper management of biodiversity. It aids researchers to take an account of genetic as well as evolutionary relationships by collecting their morphological, distributional and molecular data. Overall, this paper discusses DNA barcoding technology and how it has been used to various fish species, as well as its universality, adaptability, and novel approach to DNA-based species identification.

Key words: DNA barcoding, DNA mini-barcoding, metabarcoding, cytochrome c oxidase I, authentication

Fish and seafood (including all captured and farmed finfish, crustaceans, mollusks, and aquatic plants) offer a much healthier food source than terrestrial meat products (including processed meats, red meat, poultry) in the global fight against malnutrition and obesity (Tacon et al., 2020). Aquatic animal foods (whether captured or cultured) have a higher protein content on an edible weight basis than most terrestrial meats, compared with terrestrial meat products. Aquatic animal foods also have a lower caloric density and generally are much leaner than red and processed meats. They have the highest content of long chain omega-3 polyunsaturated fatty acids than any other animal foodstuff, and generally have a higher mineral and vitamin content than most terrestrial meats and processed meat products (including vitamins such as: vitamin A, vitamin D, vitamin E, vitamin B_{12}, folic acid, choline, coenzyme Q10, and minerals – calcium, magnesium, iron, copper, zinc, iodine, selenium, and trivalent chromium) (Tacon and Metian, 2018). Market demands for fish and fishery products are rapidly expanding in all continents because of the increased public awareness and consumers' positive attitudes toward fish products that are healthier than other animals and acceptable in all religions and cultures. While land animals' fats and oils cause or aggravate obesity, diabetes and cardiovascular complications, fish fats and fish oils are known to boost up immunity against these and also many other diseases (Baik et al., 2010). However, the blooming trades of fish products are greatly disputed over their frequent fraud labeling and substitution incidences in finished products (Rasmussen et al., 2009). In addition to financial harm, such intentional deceiving of consumers often results in serious consequences to their health. Authentication studies and market monitoring reflect fish products are especially vulnerable to mislabeling compared to other consumers' goods. Mislabeling was detected in 50% fish products in Germany (Kappel and Schröder, 2016), 24% seafood in South Brazil (Carvalho et al., 2015), 22% seafood in India (Nagalakshmi et al., 2016), 82% commercial fish fillets in Italy (Di Pinto et al., 2015). Morphological characteristics represent the first and obvious line for fish identification (Bottero and Dalmasso, 2011). When diagnostic traits are absent, such as in the case of fish fillets and other processed food, consumers are unable to identify the fish species. In these cases, alternative tools are needed for food surveillance.

Methods for fish identification

Fish species identification is traditionally based on external morphological features, including body shape, pattern of colors, scale size and count, number and relative position of fins, number and type of fin rays, or various relative measurements of body parts (Strauss and Bond, 1990). Besides, once most morphological features have been removed during either digestion (e.g., in stomach contents) or various processing (e.g., canning, filleting), the identification becomes difficult or even impossible. Furthermore, the identification of early life stages (egg and larvae) is even more complicated than adult identification (Callejas and Ochando, 2001). There are following methods for identification:

Protein based

Traditional and official methods used in species identification, including fish, are based chiefly on the separation and characterization of specific proteins using electrophoretic techniques, such as isoelectric focusing: IEF (Rehbein, 1990) and capillary electrophoresis: CE (Kvasnička, 2005), high performance liquid chromatography: HPLC (Hubalkova et al., 2007), or immunoassay systems, such as Enzyme-Linked ImmunoSorbent Assay: ELISA (Asensio et al., 2008). Although most of these methods are of considerable value in certain instances, they are not suitable for routine sample analysis because proteins lose their biological activity after animal death, and their presence and characteristic depend on the cell types (Asensio, 2007). Besides, because most proteins are heat labile, they become irreversibly denatured when the flesh is cooked (e.g., in food products) and thus can no longer be examined by techniques suitable for their native states. It is, however, possible to solubilize some heat-denatured proteins in denaturing solvents such as 2% sodium dodecylsulphate or 8 M urea to obtain species-specific profiles on electrophoresis. Antibodies used in immunoassay systems are also normally raised against undenatured proteins and thus useful for fresh samples only; even though some antibodies were developed against thermostable proteins. This results in that attention has now turned to DNA as a source of information.

As an alternative to protein analysis, DNA-based identification methods have been developed, chiefly in the past decade (Teletchea et al., 2005). DNA presents several advantages over protein analysis.

DNA based

Nowadays, DNA-based methods of fish identification have gained popularity over those based on proteins. DNA is more stable at high temperatures, allowing origin identification of heat-treated products. Since DNA is present in all tissues, it can be used for identification of all products, regardless of which part of the fish they are obtained from. Due to its greater variety, it allows for differentiation between specific species and subspecies (Teletchea et al., 2005). DNA barcoding has been very successfully employed in many applied fields, ranging from routine species identification (Hassold et al., 2016). DNA barcoding has been employed as a species identification tool for food authentication and safety concerns, including incorrect product labelling (Hubert et al., 2008), ingredient substitutions (Marko et al., 2004) or food contamination (Nicolè et al., 2012) as well as for regulatory use (Handy et al., 2011). DNA-based methods can also be used to monitor illegal trading involving protected or endangered species (Wallace et al., 2012) or to identify the species origin of commercially processed food (Tanabe et al., 2007). However, some of the processing and preservation methods used with seafood products are not conducive to DNA barcoding with the full-length target gene region (Chapelaet al., 2007). DNA degradation has been recognized as a considerable limitation in DNA-based analyses of these samples, and PCR amplification of full-length (i.e. ~650 bp) barcodes from moderately or highly processed samples is significantly challenging. In addition, processed seafood products often contain multiple additives, preservatives, and flavors that may affect the quantity and quality of DNA extracted from these products (Rasmussen Hellberg et al., 2011). Alternatively, a mini-barcoding approach, which focuses the analysis on shorter DNA fragments (e.g., 100–200 bp) within the full-length barcode, has been shown to be effective in obtaining DNA sequence information from specimens containing degraded DNA (Meusnier et al., 2008). The mitochondrial cytochrome c oxidase subunit I gene (*COI*) has a good amount of variation and is easy to amplify using PCR-based approaches in most animal groups (Čandek and Kuntner, 2015). The sequencing information generated from a small (≥100 bp) mini-barcode fragment of *COI* within the full-length DNA barcode region can provide the information required for identification of individual species with more than 90% species resolution (Shokralla et al., 2011). *COI*, however, is one of the most conserved mitochondrial protein-coding genes in animals and thus displays a better phylogenetic signal. COI as a barcoding marker has been used very successfully in many animal taxa. The suitability for other organisms and protists, in particular, has yet to be determined. (Mueller et al., 2006). However, extensive mini-barcode primer development and testing specifically for use with commercially processed fish species has not been carried out. The purpose of this review is to provide a critical overview of DNA barcoding and DNA mini-barcoding and also at what extent these techniques can realistically contribute to a practical identification of global biodiversity, especially of fish. Our main focus in this review is on DNA based, barcoding techniques for fish identification.

Methodology

A total of 120 articles were studied for this review article. Out of 120 articles, 102 articles were found relevant. Some of these relevant articles were used only for abstract reading and rest were used for data collection.

The criterion for the selection of research papers was to gather data regarding DNA barcoding and mini-barcoding. The key words used for these articles were DNA barcoding, DNA mini-barcoding, fish authentication by barcoding/mini-barcoding. Websites from where these papers were taken include Google Scholar, ScienceDirect, Sci-Hub, PubMed.

Overview of DNA barcoding

Hebert et al. (2003 a) proposed a technique using a primer set to amplify a 648-base pair (bp) region of the mitochondrial cytochrome c oxidase subunit 1 (*COI*) gene to ensure rapid and accurate identification of a broad range of biological specimens (Figure 1). They named this technique "DNA barcoding". Then, the Barcode of Life project was proposed to promote DNA barcoding as a global standard for sequence-based identification of eukaryotes. In 2004, this project was formally initiated by the establishment of the Consortium for the Barcode of Life (CBOL), which aims to develop standard protocol for DNA barcoding and to construct a comprehensive DNA barcode library. Few years back, the Barcode of Life project entered a new phase with the launch of the International Barcode of Life project. Tautz et al. (2002) made the case for a DNA-based taxonomic system. Empirical support for the barcoding concept ranges from studies of invertebrates (e.g. springtails and butterflies) to birds (Hogg and Hebert, 2004). However, the approach is not without controversy. For a barcoding approach to species identification to succeed, within-species DNA sequences need to be more similar to one another than to sequences in different species. Studies show that this is generally the case, but there are exceptions. Hybridization among species would create taxonomic uncertainty: mitochondrial DNA is maternally inherited and any hybrid or subsequent generation would have the maternal species DNA only (Lipscomb et al., 2003). Accurate and unambiguous identification of fish and fish products, from eggs to adults, is important in many areas. It would enable retail substitutions of species to be detected, assist in managing fisheries for long-term sustainability, and improve ecosystem research and conservation. Hitherto, a wide variety of protein- and DNA-based methods have been used for the genetic identification of fish species (Pérez-Martin and Sotelo, 2003). Fish Barcode of Life Initiative (*FISH-BOL*), as a campaign of the International Barcode of Life Project (iBOL), is to build up a standardized database of reference sequences for all fishes. With regard to fishes, the target DNA segment of 652 base-pairs near 5′ end of the mitochondrial cytochrome oxidase subunit I gene is strongly proposed (Hebert et al., 2003 b). These sequences are derived from voucher specimens preserved in the museums all around the world. Storage information (taxonomic identification, catalogue number and institution storing) and collection data (collector, collection date and location with longitude-latitude coordinates) are required to inject specimen records when creating projects for them. In addition, DNA barcodes (sequences of at least 500 bps and trace files) need to be uploaded to projects in *FISH-BOL* after finishing PCR amplification and sequencing. Thus, any problem concerning morphological identification can be solved by searching the relative data in BOLD or sending enquiries for checking voucher specimens preserved in natural history collections. Assignment of specimens to known species can be achieved through the comparison of genetic similarity with the sequence database. *FISH-BOL* provides a platform for ichthyologists to collaborate more closely, but also greatly accelerate species identification and discovery (Swartz et al., 2008). The purpose of the DNA barcoding project is to rapidly assemble a precise and representative reference library. Thus it is based on conventional and inexpensive protocols for DNA extraction, amplification and sequencing. With time, the reference library will become increasingly useful, enabling the rapid identification of low taxonomic level taxa with specific short-DNA sequences (i.e. mini-barcode) 100 bp (Hajibabaei et al., 2006).

DNA barcoding is a basic tool to identify various fish species. In a case study, genetic variations were analyzed by amplifying a gene fragment of 650 base pairs in different populations of *Tor putitora* cytochrome oxidase c subunit 1 (CO1) gene sequence confirms not only species but also separate species populations (Khaliq et al., 2015). *Cyprinus carpio*, *Cirrihinus mrigala* and *Ctenopharyngodon idella* were identified through DNA barcoding and three *COI* barcodes recovered from three genera of family Cyprinidae through sequencing with F1 and R1 primers indicated polymorphic sites (116), singleton variable sites (109), and no parsimony informative sites. All the amplified sequences contain functional mitochondrial *COI* sequences that were used for species identification (Karim et al., 2016). Mitochondrial sequence differences are linked with morphological analysis, speciation and DNA barcoding**.** As the genetic distance between groups was greater than within groups, so the *COI* gene barcode of *S. indica* is more effective to identify *Sillago* species (Xiao et al., 2016). Genetic identification of two freshwater fish species was done by employing a short sequence of *COI* gene which also specifies the nucleotide divergence among species (Fatima, 2018).

The mitochondrial DNA (mtDNA) *COI* gene was used as a DNA barcode to explain the systematics of *Sillago*. mtDNA analysis resolves queries regarding taxonomy of fish species, their identification and population genetics (Nalugwa et al., 2010). Species identified by morphological features and DNA barcoding plays a vital role to study biodiversity and sustainable development of fish resources (Gao et al., 2011). DNA barcodes of *COI* gene were characterized using fish species and distinct genetic boundaries were observed due to small intraspecific and large interspecific differences. An average 652 bp region of *COI* gene was sequenced for marine fish species in China and the average genetic distance was 50-fold more between the species compared to within species (Zhang and Hanner, 2012). The interspecific ge-

netic distance was observed more than the intraspecific genetic distance. A study indicated that interspecific genetic distance was >0.02 in more than 98% of closely related species but intraspecific distance was <0.01 (Hebert et al., 2003 b). An average intraspecific distance of 0.002 and interspecific distance of 0.137 was observed for six *Thryssa* species. Increase in interspecific genetic distances indicated *COI* gene sequence as a suitable marker for identification of species (Ma et al., 2015). According to reviewed research articles specific mitochondrial *COI* barcode sequences were acquired from various fish species. The observed length of all barcode sequences ranged between 283 and 707 bp for DNA barcoding with no stop codons, insertions or deletions. All the searched sequences were greater than 600 bp. Specific nucleotide pair frequency or base composition analysis were used for *COI* sequences. This study is subjected to find out the possible known barcodes for fish species through DNA barcoding. mtDNA gene specific fragment is commonly used as a species barcode and its greater efficacy of species identification has been described in fish species of various countries which are mentioned as Pakistan, China, India, Indonesia, Bangladesh, Japan, Turkey, Iran, Philippines, Thailand, Myanmar, Iraq, Saudi Arabia, Taiwan, Kazakhstan and Vietnam (Table 1). In this review, a brief description is given for amplified *COI* barcode sequences in different fish species as well as mini-barcode sequences. Specific primer is used for amplification of target region without deletion or insertion. The summarized data indicates maximum number of fish species barcoded in India and minimum number of species barcoded in Iraq. Mostly the species of family Cyprinidae, Carangidae, Sillaginidae, Gerreidae, Labridae, Lutjanidae and Mullidae are frequently found to be barcoded. Pakistan has large natural inland resources which include many lakes, canals and their tributaries.

Benefits of DNA barcoding

The task of identifying species is underpinned by the discipline of evolutionary biology, particularly the fields of taxonomy (the classification of organisms into groups according to their morphological similarity), phylogeography (the evolutionary relatedness of organisms in space and time), and population genetics (distribution of genetic variation within and among populations) (Avise, 2000). The most commonly applied methods of analysis for species involve the use of mitochondrial DNA, particularly cytochrome b and cytochrome oxidase I. The purpose of the DNA barcoding project is to rapidly assemble a precise and representative reference library. Thus, it is based on conventional and inexpensive protocols for DNA extraction, amplification and sequencing. With time, the reference library will become increasingly useful, enabling the rapid identification of low taxonomic level taxa with specific short-DNA sequences (i.e. mini-barcode) of 100 bp (Hajibabaei et al., 2006).

It has a wide range of applications including identification of species that have similar morphological characters (Bingpeng et al., 2018), individuals at different developmental stages, damaged and incomplete specimens (Wang et al., 2016), processed products i.e., food forensics (Marko et al., 2004), cryptic species, and new species discovery. It can, for example, identify fish specimens based on samples of whole fish, fillets, fins, eggs or tissue fragments as only a tiny fragment of material is required to generate a DNA barcode. Moreover, samples can be viable even if they are modified, subject to processing or heat damage (Hanner et al., 2011). DNA barcoding might enhance biodiversity inventories by being faster and cheaper, and by overcoming the taxonomic impediment (Gaston O'Neill, 2004).

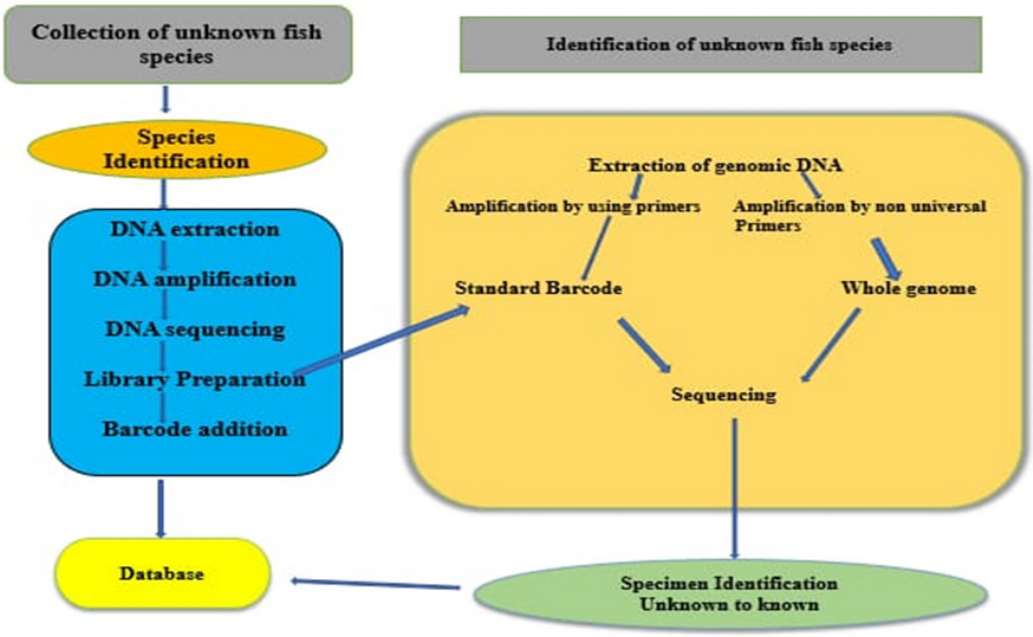


Figure 1. An overview of DNA barcoding

Table 1. Mitochondrial *COI* barcode sequence for fish samples

Countries	Number of order, genera, family, sub family, class	Location	Specific primers	Barcoded species	Specific barcode	K2P (Intraspecific distance) (%)	Reference
1	2	3	4	5	6	7	8
Pakistan	1 family, 1 genus	Swat, Indus, Poonchand Hingol River.	FishF1 5'-TCAACCAACCACAAAGACATTGGCAC-3'and FishR1 5'-TAGACTTCTGGGTGGCCAAAGAATCA-3'	1	650bp	0.5	Khaliq et al., 2015
	1 family, 3 sub families, 3 genus	Manawan, Lahore	FishF15'-TCAACCAACCACAAAGACATTGGCAC-3' and FishR1 5'-TAGACTTCTGGGTGGCCAAAGAATCA-3'	3	650–680bp	0.28	Karim et al., 2016
	1 order, 1 family, 1 genus	Coast of Pakistan	L5956-*COI*: 5'-CACAAAGACATTGGCACCCT-3'; H6558-*COI*: 5'-CCTCCTGCAGGGTCAAAGAA-3'	72	601bp	0.171–0.222	Xiao et al., 2016
	2 genera, 1 family, 1 sub family, 1 order	River Ravi at Head Balloki region	Fish F1: 5'TCAACCAACCACAAAGACATTGGCAC3' Fish R2: 5'ACTTCAGGGTGACCGAAGAATCAGAA3'	2	658bp	0.45 and 0.012	Fatima, 2018
	11 families, 6 orders	Faisalabad and Karachi	Forward 5′-AACCACCGTTGTTATTCAACTACAA-3′ Reverse 5′-CCGACTTCCGGATTACAAGACCG-3′	17	Cyt b barcode (1,141 bp)	0.311	Ghouri et al., 2020
China	85 genera, 55 families, 15 orders, 2 classes	Along the coast of China	M13F 5′-TGTAAAACGACGGCCAGT-3′ M13R 5′-CAGGAAACAGCTATGAC-3′	121	652bp	0.319	Zhang and Hanner, 2011
	1 order, 1 family	Yellow Sea and Bohai Sea	L5956-*COI* 5'-CACAAAGACATTGGCACCCT-3' H6558-*COI* 5'-CCTCCTGCAGGGTCAAAGAA-3'	6	609bp	0.163–0.212	Gao et al., 2011
	159 genera, 80 families, 22 orders, 2 classes	South China Sea	M13F 5′-TGTAAAACGACGGCCAGT-3′ M13R 5′-CAGGAAACAGCTATGAC-3′	242	652bp	0.18	Zhang and Hanner, 2012
	1 genus	Seas off Fujian and Zhejiang provinces of China	CFF (5'-TCRACYAAYCAYAAAGAYATYGGCAC-3') CFR (5'-ACTTCWGGGTGRCCRAAGAATCA-3')	6	543bp	0.002	Ma et al., 2015
	1 order, 1 family	Nansha, port of Guangzhou	Forward primer 5′-GGCTACAATCCACCGCTTAA-3′ Reverse primer 5′-AGGCGACAATGTGGGAAAT-3′	12	651bp	0–1.1	Yan et al., 2016
India	79 genera, 37 families, 7 orders	East and West Coast of India	FishF1-5′TCAACCAACCACAAAGACATTGGCAC-3′and FishR1-5′TAGACTTCTGGGTGGCCAAAGAATCA-3′	115	655bp	0.30	Lakra et al., 2011
	1 family	River Brahmaputra	FishF1-5′TCAACCAACCACAAAGACATTGGCAC-3′ and FishR1-5′TAGACTTCTGGGTGGCCAAAGAATCA3′	4	˜655bp	0.8	Laskar et al., 2013
	63 genera, 34 families, 10 orders	Narmada River	Not mentioned	85	625bp	0.36	Khedka et al., 2014
	50 genera, 19 families, 5 orders	Ganges and Brahmaputra river	FishF1-5′-TCAACCAACCACAAAGACATTGGCAC-3′ and FishR1-5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′	72	655bp	0.40	Lakra et al., 2016
	30 genera, 4 orders, 9 families	South Indian waters, Cauvery and Bhavani river	Fish F1-5'-TCAACCAACCACAAAGACATTGGCAC-3' and Fish R1-5'-TAGACTTCTGGGTGGCCAAAGAATCA-3'	46	635bp	0.32	Raja and Perumal, 2017
	60 genera, 34 families, 10 orders, 2 sub-classes	East and West Coasts of India	FishF1 5′-TCAACCAACCACAAAGACATTGGCAC-3′ and FishR1 5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′	111	655bp	0.32	Bineesh et al., 2017

Table 1 – contd.

1	2	3	4	5	6	7	8
Indonesia	14 species, 11 genera, 6 families, 5 orders	Lake Laut	FishF1 5′ TCAACCAACCACAAAGACATTGGCAC3′ and FishR1 5′TAGACTTCTGGGTGGCCAAAGAATCA3′	10	655bp	22.9	Muchlisin et al., 2013
	Not mentioned	Aceh, Jakarta, West Java, Central Java, East Java, Bali, West Kalimantan, South Sulawesi, North Sulawesi, Maluku, and West Papua	FISH-BCL (5′-TCAACYAATCAYAAAGATATYGGCAC-3′) and FISH-BCH (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′)	40	600-654bp	Nil	Sembiring et al., 2015
	8 families, 5 genera	Jakarta Bay and Banda Sea	Fish-*COI*-F (5'-ACT TCAAACTTCCAYAAAGAYatyGG-3) and *COI*-Fish-R(5'-TAGACTTCTGGG TGG CCR AAR Aay CA-3 ‘)	33	471bp	0.0–1.30	Wibowow et al., 2018
	73 genera, 24 families	Ambon harbor	*COI*-2C_VF1LFt1–C_VR1LRt1 LepF1_t1TGTAAAACGACGGCCAGTATTCAACCAATCATAAAGA-TATTGG VF1_t1 1 TGTAAAACGACGGCCAGTTCTCAACCAACCACAAAGA-CATTGG VF1d_t1 1 TGTAAAACGACGGCCAGTTCTCAACCAACCACAAR-GAYATYGG VF1i_t1 3 TGTAAAACGACGGCCAGTTCTCAACCAACCAIAAI-GAIATIGG LepRI_t1 1 CAGGAAACAGCTATGACTAAACTTCTGGATGTC-CAAAAAATCA VR1d_t1 1 CAGGAAACAGCTATGACTAGACTTCTGGGTGGC-CRAARAAYCA VR1_t1 1 CAGGAAACAGCTATGACTAGACTTCTGGGTGGCCAAA-GAATCA VR1i_t1 3 CAGGAAACAGCTATGACTAGACTTCTGGGTGICCIAAIAAI-CA *COI*-3 C_FishF1t1–C_FishR1t1 VF2_t1 TGTAAAACGACGGCCAGTCAACCAACCACAAAGACATTG-GCAC FishF2_t1 TGTAAAACGACGGCCAGTCGACTAATCATAAAGA-TATCGGCAC FishR2_t1 CAGGAAACAGCTATGACACTTCAGGGTGACCGAA-GAATCAGAA FR1d_t1 1 CAGGAAACAGCTATGACACCTCAGGGT-GTCCGAARAAYCARAA M13F (−21) TGTAAAACGACGGCCAGT M13R (−27) CAGGAAACAGCTATGAC	760	652bp	0.32	Limmon et al., 2020
	32 genera and 17 families	Indonesia	FishF1 5′-TCAACCAACCACAAAGACATTGGCAC-3′ FishR1 5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′	72 species	639bp	0–1.55	Fadli et al., 2020
	57 genera, 22 families and 7 orders	Indo- Myanmar	FishF1 5′-TCAACCAACCACAAAGACATTGGCAC-3′ FishR1 5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′	109 species	>600bp	0.42	Barman et al., 2018
	11 genera, 6 families, and 5 orders	Indonesia	FishF1 5′-TCAACCAACCACAAAGACATTGGCAC-3′ FishR1 5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′	14 species	655bp	0.15	Muchlisinet al., 2013

Table 1 – contd.

1	2	3	4	5	6	7	8
Bangla-desh	9 genera	Dhaka metropolitan city	FishF1 5′-TCAACCAACCACAAAGACATTGGCAC-3′ Fish F2 5′-TCGACTAATCATAAAGATATCGGCAC-3′ Fish R15′-TAGACTTCTGGGTGGCCAAAGAATCA-3′ FishR2 5′-ACTTCAGGGTGACCGAAGAATCAGAA-3′	10	653bp	71.42	Smriti et al., 2017
	1 family, 1 genus	TanguarHaor, Sunamganj	FishF1 (5′-TCAACCAACCACAAAGACATTGGCAC-3′) and FishR1 (5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′)	4	658bp	1.2	Ahmed et al., 2018
	7 orders, 24 families, 55 genera	TanguarHaor and the HakalukiHaor	forward primer-5′TCAACCAACCACAAAGACATTGGCAC-3′and reverse primer-5′TAG ACTTCTGGGTGGCCAAAGAATCA-3′	81	652bp	30.34	Ahmed et al., 2019
	114 genera, 49 families, 16 orders	Local fish markets of Bang-ladesh	FishF1 5′TCAACCAACCACAAAGACATTGGCAC3′ and FishR1 5′ TAGACTTCTGGGTGGCCAAAGAATCA3′	153	652bp	0.38	Ahmed et al., 2020
Japan	144 genera, 93 families, 21 orders, 2 classes	South Japan	FishF2 t1:5′-TGTAAAACGACGGCCAGTCGACTAATCATAAAGATATC GGCAC-3′ VF2_t1: 5′-TGTAAAACGACGGCCAGTCAACCAACCACAAAGACATT GGCAC-3′ FishR2_t1: 5′-CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGAA TCAGAA-3′ FR1d_t1: 5′-CAGGAAACAGCTATGACACCTCAGGGTGTCCGAARAAY CARAA-3′	158	500bp	0.3	Zhang and Hanner, 2011
Turkey	4 genera	Western part	FishF1 5′-TCAACCAACCACAAAGACATTGGCAC-3′ FishR1 5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′ FishF2 5′-TCGACTAATCATAAAGATATCGGCAC-3′ FishR2 5′-ACTTCAGGGTGACCGAAGAATCAGAA-3′	5	654bp	0.009	Keskin et al., 2013
	70 genera, 40 families, 19 orders, 1 class	Turkish ichthyofauna	Fish-F2 5′-TCGACTAATCATAAAGATATCGGGAC-3′ and Fish-R2 5′-ACTTCAGGGTGACCGAAGAATCAGAA-3′	89	654bp	0.32	Keskİn and Atar, 2013
Iraq	1 genera	Little Zab River drainage	LCO1490-JJ: 5′CHACWAAYCATAAAGATATYGG-3′ and HCO2198- JJ: 5′-AWACTTCVGGRTGVCCAAARAATCA-3′	2	Not mentioned	>8	Freyhof et al., 2016
Iran	5 genera	Tehran	FishF1-5′-TCAACCAACCACAAAGACATTGGCAC-3′ FishR1-5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′	5	650bp	Nil	Changizi et al., 2013
	8 genera	Iran	FishF1-5′-TCAACCAACCACAAAGACATTGGCAC-3′ FishR1-5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′	9	650bp	Nil	Changizi et al., 2013
Philippines	18 genera, 16 families, 7 orders	Laguna de Bay	FishF1-5′-TCAACCAACCACAAAGACATTGGCAC-3′ FishF2-5′-TCGACTAATCATAAAGATATCGGCAC-3′ FishR1-5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′ FishR2-5′-ACTTCAGGGTGACCGAAGAATCAGAA-3′	18	650bp	1.33	Aquino et al., 2011
	6 genera, 1 family	Variable geographical regions	FishF1-5′-TCA-ACC-AAC-CAC-AAA-GAC-ATT-GGG-AC-3′ FishR1-5′-TAG-ACT-TCT-GGG-TGG-CCA-AAG-AAT-CA-3′ FishF2-5′-TCG-ACT-AAT-CAT-AAA-GAT-ATC-GGC-AC-3′ FishR2-5′-ACT-TCA-GGG-TGA-CCG-AAG-AAT-CAG-AA-3′ FF2d -5′-TTC-TCC-ACC-AAC-ACA-ARG-AYA-TYG-G-3′ FR1d -5′-CAC-CTC-AGG-GTG-TCC-GAA-RAA-YCA-RAA-3′	27	500–608bp	0.68	Alcantara and Yam-bot, 2016

Table 1 – contd.

1	2	3	4	5	6	7	8
Vietnam	8 genera	Mekong River, Hau and Tien rivers	16Sar 5'-CGCCTGTTTATCAAAAACAT-3' and 16Sbr 5'-CCGGTCTGAACTCAGATCACGT- 3' HCO 5' – TAAACTTCGGGTGACCAAA – 3' LCO 5' – GGTCAACAAATCATAAAGATA – 3'	11	600bp	Nil	Quyen et al., 2015
		Big C Long Bien and Aeon Mall Long Bien in Hanoi	MAB F:5′-TCAACCAACCACAAAGACATTGGCAC-3′ R: 5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′ Fish F:5′-CGACTAATCATAAAGATATCGGCAC-3′ R:5′-TTCAGGGTGACCGAAGAATCAGAA-3′ Cytb 5′-AAAAACCACCGTTGTTATTCAACTA-3′ 5′-GCICCTCARAATGAYATTTGTCCT CA-3′		500–700bp	Nil	Ha et al., 2018
	273 genera, 113 families, 43 orders	Do Son and Ha Long of HaiPhong–Quang-Ninh Provinces,Phan Rang of NinhThuan Province and TacCau and Ha Tien of KienGiang Province	FishF1 (5'-TCAACCAACCACAAAGACATTGGCAC-3') FishF2 (5'-TCGACTAATCATAAAGATATCGGCAC-3') and FishR1 (5'- TAGACTTCTGGGTGGCCAAAGAATCA-3') FishR2 (5'-ACTTCAGGGTGACCGAAGAATCAGAA-3')	458	313–683bp	0.34	Thu et al., 2019
	Nil	Mekong Delta	FishR2_t1: 5′-CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGAA TCAGAA-3′ FR1d_t1: 5′-CAGGAAACAGCTATGACACCTCAGGGTGTCCGAARAAY CARAA-3′	Not mentioned	Nil	12.4–18.7	Duong et al., 2020
	4 genera	18 locations belonging to 11 provinces	FishF1-5′-TCA-ACC-AAC-CAC-AAA-GAC-ATT-GGG-AC-3′ FishR1-5′-TAG-ACT-TCT-GGG-TGG-CCA-AAG-AAT-CA-3′ FishF2-5′-TCG-ACT-AAT-CAT-AAA-GAT-ATC-GGC-AC-3′	40	655bp	Nil	Durand et al., 2020
Thailand	1 genus	Khorat Plateau basin	VF2_t1 1 5′-TGTAAAACGACGGCCAGTCAACCAACCACAAAGACAT TGGCAC-3′ FishF2_t15′-TGTAAAACGACGGCCAGTCGACTAATCATAAAGATATC GGCAC -3′ FishR2_t1 15′- CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGA ATCAGAA-3′ FR1d_t1 1 5′-CAGGAAACAGCTATGACACCTCAGGGTGTCCGAARAA YCARAA-3′ M13F5′- TGTAAAACGACGGCCAGT-3′ M13R 5′-CAGGAAACAGCTATGAC-3′	11	652bp	0.5	Sriwattanarothai et al., 2010
	5 orders, 10 families, 31 genera	Bingzhongluo, Puladi, Maji, County Areas of Fugong, Pihe, Chenggan, Liuku, Hongqi Dam, Mangkuan, Xiaopingtian, Sanjiangkou, Longzhen Bridge, Kongguang Village, ManglongRiver, Mangkuan River, Mangliu River, and Longwang Pond.	FishF1 (5'-TCAACCAACCACAAAGACATTGGCAC-3') FishR1 (5'- TAGACTTCTGGGTGGCCAAAGAATCA-3')	46	648bp	0.41	Chen et al., 2015

Table 1 – contd.

1	2	3	4	5	6	7	8
Vietnam	8 genera	Mekong River, Hau and Tien rivers	16Sar 5'-CGCCTGTTTATCAAAAACAT-3' and 16Sbr 5'-CCGGTCTGAACTCAGATCACGT- 3' HCO 5' – TAAACTTCGGGTGACCAAA – 3' LCO 5' – GGTCAACAAATCATAAAGATA – 3'	11	600bp	Nil	Quyen et al., 2015
		Big C Long Bien and Aeon Mall Long Bien in Hanoi	MAB F:5′-TCAACCAACCACAAAGACATTGGCAC-3′ R: 5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′ Fish F:5′-CGACTAATCATAAAGATATCGGCAC-3′ R:5′-TTCAGGGTGACCGAAGAATCAGAA-3′ Cytb 5′-AAAAACCACCGTTGTTATTCAACTA-3′ 5′-GCICCTCARAATGAYATTTGTCCT CA-3′		500–700bp	Nil	Ha et al., 2018
	273 genera, 113 families, 43 orders	Do Son and Ha Long of HaiPhong–Quang-Ninh Provinces,Phan Rang of NinhThuan Province and TacCau and Ha Tien of KienGiang Province	FishF1 (5'-TCAACCAACCACAAAGACATTGGCAC-3') FishF2 (5'-TCGACTAATCATAAAGATATCGGCAC-3') and FishR1 (5'- TAGACTTCTGGGTGGCCAAAGAATCA-3') FishR2 (5'-ACTTCAGGGTGACCGAAGAATCAGAA-3')	458	313–683bp	0.34	Thu et al., 2019
	Nil	Mekong Delta	FishR2_t1: 5′-CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGAA TCAGAA-3′ FR1d_t1: 5′-CAGGAAACAGCTATGACACCTCAGGGTGTCCGAARAAY CARAA-3′	Not mentioned	Nil	12.4 to 18.7	Duong et al., 2020
	4 genera	18 locations belonging to 11 provinces	FishF1-5′-TCA-ACC-AAC-CAC-AAA-GAC-ATT-GGG-AC-3′ FishR1-5′-TAG-ACT-TCT-GGG-TGG-CCA-AAG-AAT-CA-3′ FishF2-5′-TCG-ACT-AAT-CAT-AAA-GAT-ATC-GGC-AC-3′	40	655bp	Nil	Durand et al., 2020
Thailand	1 genus	Khorat Plateau basin	VF2_t1 1 5′-TGTAAAACGACGGCCAGTCAACCAACCACAAAGACAT TGGCAC-3′ FishF2_t15′-TGTAAAACGACGGCCAGTCGACTAATCATAAAGATATC GGCAC -3′ FishR2_t1 15′- CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGA ATCAGAA-3′ FR1d_t1 1 5′-CAGGAAACAGCTATGACACCTCAGGGTGTCCGAARAA YCARAA-3′ M13F5′- TGTAAAACGACGGCCAGT-3′ M13R 5′-CAGGAAACAGCTATGAC-3′	11	652bp	0.5	Sriwattanarothai et al., 2010
	5 orders, 10 families, 31 genera	Bingzhongluo, Puladi, Maji, County Areas of Fugong, Pihe, Chenggan, Liuku, Hongqi Dam, Mangkuan, Xiaopingtian, Sanjiangkou, Longzhen Bridge, Kongguang Village, ManglongRiver, Mangkuan River, Mangliu River, and Longwang Pond.	FishF1 (5'-TCAACCAACCACAAAGACATTGGCAC-3') FishR1 (5'- TAGACTTCTGGGTGGCCAAAGAATCA-3')	46	648bp	0.41	Chen et al., 2015

Table 1 – contd.

1	2	3	4	5	6	7	8
	1 genus	Bangakong Basin, Nakhornnayok Province	FishF1 (50-TCAACCAACCACAAAGACATTGGCAC-30) and FishR1(50-TAGACTTCTGGGTGGCCAAAGAATCA-30)	3	707bp	0.000–0.005	Panprommin et al., 2017
	18 families, 8 orders	Kwan Phayao	FishF1-5´TCAACCAACCACAAAGACATTGGCAC3´, FishF2-5´TCGACTAATCATAAAGATATCGGCAC3´, FishR1-5´TAGACTTCTGGGTGGCCAAAGAATCA3´, FishR2-5´ACTTCAGGGTGACCGAAGAATCAGAA3´	52	639bp	Nil	Panprommin et al., 2019
	31 genera, 19 families and 9 orders	Tributaries of the lower Ing River in Chang Rai Province, northern Thailand	FishF1 (5'-TCAACCAACCACAAAGACATTGGCAC-3') FishF2 (5'-TCGACTAATCATAAAGATATCGGCAC-3') and FishR1 (5'- TAGACTTCTGGGTGGCCAAAGAATCA-3') FishR2 (5'-ACTTCAGGGTGACCGAAGAATCAGAA-3')	35	640bp	0.000–0.004	Panprommin et al., 2020
Saudi Arabia	6 genera	Red Sea coastal waters of Al-Khuraybah	FishF1 5′-TCAACCAACCACAAAGACATTGGCAC-3′ and FishR1 5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′	6	621–643bp	Nil	Trivedi et al., 2014
	13 orders, 54 families	Offshore and inshore Saudi waters of The Gulf	FishF1-5′-TCAACCAACCACAAAGACATTGGCAC-3′ , FishF2-5′-TCGACTAATCATAAAGATATCGGCAC-3′ , FishR1-5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′, FishR2-5′-ACTTCAGGGTGACCGAAGAATCAGAA-3′	117	678bp	0.20–0.28	Rabaoui et al., 2019
	1 family, 2 genus	Red Sea, Farasan Island	FishF1 (5′-TCAACCAACCACAAAGACATTG- GCAC-3 ′), and reverse FishR1 (5′-TAGACTTCTGGGT-GGCCAAAGAATCA-3′)	30	655bp	48.89	Hassan et al., 2020
Taiwan	87 families, 79 genera	Northern, southern and northwestern waters of Taiwan	FishF1-5′-TCAACCAACCACAAAGACATTGGCAC-3′ FishR1-5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′	69	650bp	0.39	Ko et al., 2013
		Harbor loadings, fish markets, supermarkets, street venders, and restaurants in six cities and eight counties of Taiwan	LCO1490:59-GGTCAACAAATCATAAAGATATTGG-39 and HCO2198:59-TAAACTTCAGGGTGACCAAAAAATCA-39	548	391bp	0.042–0.109	Vanson Liu et al., 2013
	21 families	TungKang, XinGang, NanFangAo, Taipei, Taichung, Changhua, and Kaohsiung	FishF1-5′-TCAACCAACCACAAAGACATTGGCAC-3′ FishF2-5′-TCGACTAATCATAAAGATATCGGCAC-3′ FishR1-5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′ FishR2-5′-ACTTCAGGGTGACCGAAGAATCAGAA-3′	61	523 ± 69bp	Nil	Chuang et al., 2016
	Nil	Three different custom offices	FishF1þ2 (50-TCRACYAAYCAYAAAGAYATYGGCAC-30) reverse: FishR1 (50- TAGACTTCTGGGTGGCCAAAGAATCA-30) and FishR2 (50-ACTTCAGGGTGACCGAAGAATCAGAA-30).	34	650bp	Nil	Chang et al., 2016
	Nil	Northern Taiwan	L2510 (5_-GCCTGTTTAACAAAAACAT-3_) and H3059 (5_-CGGTCTGAACTCAGATCACGT-3_) FishF2 (5_-TCGACTAATCATAAAGATATCGGCAC-3_) and FishR2 (5_-ACTTCAGGGTGACCGAAGAATCAGAA-3_)	1277	590 and 680bp	Nil	Wang et al., 2017
	85 genera, 39 families, 11 orders	Taiwan Strait	FishF1-5′-TCAACCAACCACAAAGACATTGGCAC-3′ FishF2-5′-TCGACTAATCATAAAGATATCGGCAC-3′ FishR1-5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′ FishR2-5′-ACTTCAGGGTGACCGAAGAATCAGAA-3′	85	655bp	0.21	Bingpeng et al., 2018

Table 1 – contd.

1	2	3	4	5	6	7	8
Kazakh-stan	2 genera	Chara River and Mikhailovs-ki Lake in the Irtish River system	L15923 5′-TTA AAG CAT CGG TCT TGT AA-3′ and H16500 5′-GCC CTG AAA TAG GAA CCA GA-3′	3	283bp	Nil	Sakai et al., 2009
Australia	Three subgroups, 122 genera	Australia	TAGACTTCTGGGTGGCCAAAGAA-5 0FishF1 5′-TCAACCAACCACAAAGACATTGGCAC-3′ FishR1 5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′ FishF2 5′TCGACTAATCATAAAGATATCGGCAC3′ FishR2 5′ACTTCAGGGTGACCGAAGAATCAGAA3′	207 spe-cies	655bp	0.39	Ward et al., 2005
Brazil	26 orders, 62 families, and 123 genera, 2 sub-classes	Brazil	FishF1 5′-TCAACCAACCACAAAGACATTGGCAC-3′ FishR1 5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′	182 fish species	650bp	0.10–3.40	Guimarães-Costa et al., 2020
	98 genera, 57 families, 17 orders and 2 classes	Brazil	FishF1 5′-TCAACCAACCACAAAGACATTGGCAC-3′ FishR1 5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′	128 spe-cies	626bp	0.33	Guimarães-Costa et al., 2019
	62 families, 110 genera	Brazil	FishF1 5′-TCAACCAACCACAAAGACATTGGCAC-3′ FishR1 5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′ FishF2 5′TCGACTAATCATAAAGATATCGGCAC3′ FishR2 5′ACTTCAGGGTGACCGAAGAATCAGAA3′	135 spe-cies	652bp	0.31	Ribeiro et al., 2012
	75 genera and 22 families	Brazil	FishF1 5′-TCAACCAACCACAAAGACATTGGCAC-3′ FishR1 5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′	101 spe-cies	600bp	0.5	Carvalho et al., 2015
America	71 genera, 35 families and 10 orders	South Americas	FishF1 5′-TCAACCAACCACAAAGACATTGGCAC-3′ FishR1 5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′	79 species	648bp	0.53	Díaz et al., 2016
	Nil	North Americas	FishF1 5′-TCAACCAACCACAAAGACATTGGCAC-3′ FishR1 5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′	391 spe-cies	650bp	0.21	Steinke et al., 2009
	98 genera, and 63 families	South Americas	FishF1 5′-TCAACCAACCACAAAGACATTGGCAC-3′ FishR1 5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′ FishF2 5′TCGACTAATCATAAAGATATCGGCAC3′ FishR2 5′ACTTCAGGGTGACCGAAGAATCAGAA3′	125 spe-cies	>600bp	0.23	Mabragaña et al., 2011
Canada	136 genera, 81 families and 28 orders	Atlantic Canada	FishF1 5′-TCAACCAACCACAAAGACATTGGCAC-3′ FishR1 5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′	177 spe-cies	650bp	0.32	Mccusker et al., 2013
	85 genera and 28 families	Canada	FishF1-5′TCAACCAACCACAAAGACATTGGCAC3′ FishR1-5′TAGACTTCTGGGTGGCCAAAGAATCA3	190 spe-cies	652bp	0.3	Hubert et al., 2008
	Nil	Canada	FishF1 5′-TCAACCAACCACAAAGACATTGGCAC-3′ FishR1 5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′	201 species	490–652bp	0.25	Steinke et al., 2009
Cuba	17 genera and 10 families	Cuba	FishF1 5′-TCAACCAACCACAAAGACATTGGCAC-3′ FishR1 5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′	27 species	652bp	0.6	Lara et al., 2010

Cytochrome Oxidase Subunit I=*COI*

DNA barcoding provides a rapid, accurate, and cost-effective method for accurate species identification using a short, recognized standard DNA fragment and has been widely used to distinguish animal, plant, fungal, and bacterial species (Hebert et al., 2003 b).

Limitations of DNA barcoding

DNA barcoding has gained considerable attention in research as a tool for species identification in food authentication and safety assessment, including ingredient substitutions, incorrect product labeling food contamination as well as for regulatory practice. For the identification of animal species in food products, the conventional full-length DNA barcode is a ~650-base pair (bp) region in the mitochondrial gene for cytochrome c subunit I (*COI*). However, for some moderately or highly processed food, the quality of DNA could be severely affected, thus PCR amplification of full-length barcodes from these samples may be challenging (Hajibabaei et al., 2006). The main limitation of the barcoding approach comes from its single-locus identification system. Even if several regions from these organelle DNAs are sequenced, this is still a single-locus approach because the different genes of mitochondrial or chloroplast DNA are linked. It is well known that identical mitochondrial or chloroplast DNA sequences can be present in different related species due to introgression, or due to incomplete lineage sorting since the time of speciation (Ballard Whitlock, 2004). Another statistical difficulty is caused by the low number of individuals analyzed per species, which does not allow a precise estimation of the intraspecific variation compared with interspecific variation (Matz Nielsen, 2005).

DNA-based species identification depends on distinguishing intra-specific from inter-specific genetic variation. The ranges of these types of variation are unknown and may differ between taxa. It seems difficult to resolve recently diverged species or new species that have arisen through hybridization. There is no universal gene for DNA barcoding, no single gene that is conserved in all domains of life and exhibits enough sequence divergence for species discrimination. The validity of DNA barcoding therefore depends on establishing reference sequences from taxonomically confirmed specimens. This is likely to be a complex process that will involve cooperation among a diverse group of scientists and institutions. Barcode sequences are, in general, short (approx. 500–1000 bp) and this fundamentally limits their utility in resolving deep branches in phylogenies. Some controversy exists over the value of DNA barcoding which could result in incorrect species recognition (Schindel and Miller, 2005). Conventional barcoding methodology is often limited by its failure to amplify and sequence degraded DNA, which is often found in museum specimens and in preserved and processed biological material (food products, decayed tissues) (Meusnier et al., 2008).

Approaches to repair DNA *in vitro* are inefficient and not cost-effective as the DNA damage and degradation in museum samples is complex and difficult to characterize (Patel et al., 2010). In comparison, short sequences (i.e., 100 bp) are usually stable in museum specimens (Zimmermann et al., 2008; Hajibabaei et al., 2008). The use of a short or minimalist barcode (100–300 bp, referred herein as "mini-barcode") greatly expands the applications of DNA barcoding (Hajibabaei et al., 2006). In many cases, damaged DNA can be extracted from a sample but the DNA is broken into small fragments due to hydrolysis of the DNA backbone (Poinar et al., 2006). This fragmented DNA is unable to be amplified using standard barcoding primers. Models derived from preserved specimen and tissue samples by capillary electrophoresis predict a rapid initial decline in average DNA fragment size in the first 5 years followed by a more gradual change in the following time period (Zimmermann et al., 2008).

Future use of DNA barcoding

DNA barcoding represents the key foundation step in the process of coordinating the use of DNA for taxonomy at the species level. It has already accelerated the routine establishment of 'DNA ready' collections for herbaria and museums. It has triggered a formalization of links between sequence data and voucher specimens in Genbank, and the development of informatics systems linking specimens, sequences, names and associated information. It has without doubt stimulated biologists to use DNA data at the species level. It has increased attention to coordinating activities and to think beyond producing local solutions for individual studies (Hollingsworth, 2007).

Several biologists have questioned both the scientific validity of the approach, and its broader implications for the future of taxonomy. However, the coordinated use of genetics in other disciplines and the societal need for biological identifications had given the several benefits. But, still it seems to be difficult to imagine that an appropriately implemented coordinated use of genetics in species level taxonomy can be anything other than beneficial. The exact form of this approach can be expected to evolve as technologies develop, and the future will undoubtedly involve approaches that go beyond single gene sequencing. But as long as there is a demand for the conservation and utilization of species, there will be a need for their identification. A system which enables this to be automated has to be worth developing (Hogg and Hebert, 2004).

DNA mini-barcoding (a derived DNA barcoding technique)

DNA barcoding has gained considerable attention in research as a tool for species identification in food authentication and safety assessment, including ingredient substitutions, incorrect product labeling food contamination as well as for regulatory practice. For the identification of animal species in food products, the conventional full-length DNA barcode is a ~650-base pair (bp) region in the mitochondrial gene for cytochrome c subunit I (*COI*). However, for some moderately or highly

processed foods, the quality of DNA could be severely affected, thus PCR amplification of full-length barcodes from these samples may be challenging. Alternatively, a mini-barcoding method, which focuses on the analysis of short-length DNA markers (100–300 bp) has been demonstrated to be effective for species identification of processed food containing degraded DNA (Figure 2). Commonly used mini-barcodes are DNA fragments within the full-length *COI* barcode region (≥100 bp), which can provide information for individual species identification with ≥90% species resolution. Apart from *COI*, other mini-barcodes such as cytochrome b (cytb) and 16S ribosomal RNA (16S rRNA) genes have been used in phylogenetic analysis or to complement *COI* in DNA barcoding (Xing et al., 2021). DNA degradation has been recognized as a considerable limitation in DNA-based analyses of samples and PCR amplification of full-length (i.e., ~650 bp) barcodes from moderately or highly processed samples is significantly challenging. In addition, processed seafood products often contain multiple additives, preservatives and flavors that may affect the quantity and quality of DNA extracted from these products (Shokralla et al., 2011). Alternatively, a mini-barcoding approach, which focuses the analysis on shorter DNA fragments (e.g., 100–200 bp) within the full-length barcode, has been shown to be effective in obtaining DNA sequence information from specimens containing degraded DNA (Meusnier et al., 2008). The sequencing information generated from a small (≥100 bp) mini-barcode fragment of *COI* within the full-length DNA barcode region can provide the information required for identification of individual species with more than 90% species resolution (Hajibabaei et al., 2006).

Fish processing for preservation is the major cause of DNA degradation. Consequently, molecular analyses to reveal species substitution may face DNA degradation limitations and, therefore, become biased by technical questions. The possibility of analyzing short DNA fragments such as *COI* mini-barcoding seems to solve this issue (Table 2). According to the reviewed articles, specific mitochondrial *COI* mini-barcode sequences were acquired from various fish products available in markets worldwide. The observed length of all mini-barcode sequences ranged between 88 and 650 bp for DNA mini-barcoding with no stop codons, insertions, or deletions. This study is subjected to find out the possible known mini-barcodes for fish products available in markets through DNA mini-barcoding. Sometimes fish products available in markets are mislabeled due to many reasons. DNA mini-barcoding is a powerful tool to identify such products. In this study, data of different countries was observed. The reviewed articles show a success rate in range of 47%–100%. Maximum success rate was in China, Indonesia, Italy and Orange County while minimum success rate was observed in Malaysia.

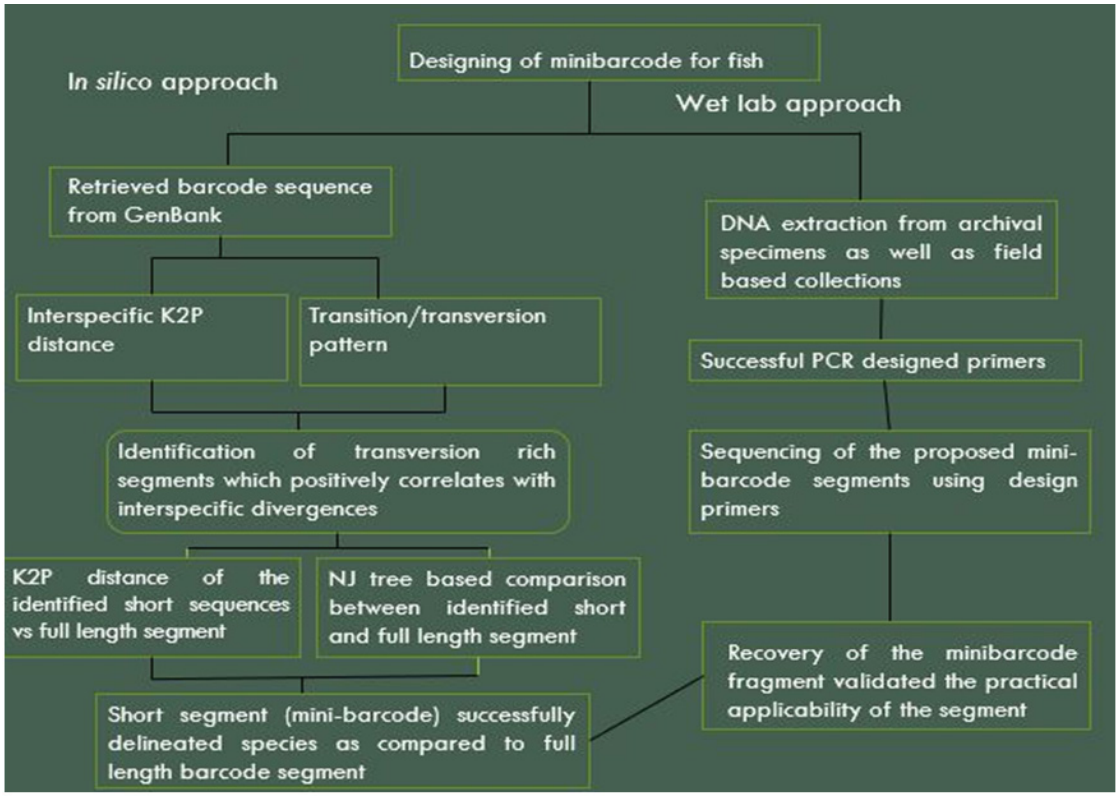


Figure 2. DNA mini-barcoding (derived technique of DNA barcoding)

Table 2. A DNA mini-barcoding system for authentication of processed fish/fish products

Location	No. of samples	Type of sample	Length (bp)	Success rate (%)	Primer sets	Sequence of primer	DNA extraction methods	Reference
USA	50	Processed shark	150	N/A	3	FishF2_t1 5'-TGTAAAACGACG-GCCAGTCGA. CTAATCATAAA-GATATCGG CAC-3', VF2_t1 5'-TG-TAAAACGACGGCCAGTCAACC AACCA CAAAGACATTG, *COI*-MINIR 5' AAGATTACAAAA-GCGTGGGC-3'	DNeasy commercial kit	Fields et al., 2015
USA	126	Fresh/frozen fish (whole/ fillets)	208–226	67–90	1	Mini_SH-D Mini_SH-E	DNeasy Blood and Tissue Kit, Spin-Column protocol	Pollack et al., 2018
Emilia Romagna	71	Fresh and frozen commercial fish	295	87.3, 97.2	2	ATCACAAAGACATTGGCACCCT AATGAAGGGGGGAGGAGTCAGAA CACGACGTTGTAAAACGACACYAAI-CAYAAAGAYATIGGCAC-30 GGATAACAATTTCACACAGGCT-TATRTTRTTTATICGIGGRAAIGC	Wizard Genomic DNA Purifica-tion Kit	Filonzi et al., 2021
Los Angeles	35	Shark products (jerky, fin soup, cartilage pills, fresh or grilled fillets)	127	54.3	3	TGTAAAACGACGGCCAGTCAAC-CAACC ACAAAGACATTGGCAC TGTAAAACGACGGCCAGTCGAC-TAATC ATAAAGATATCGGCAC AAGATTACAAAAGCGTGGGC	DNeasy Blood and Tissue Kit, Spin-Column protocol	Hellberg et al., 2019
North East India	51	Ornamental fish	171	N/A	1	5′ - GCNTTCCCNCGAATRAANAACAT-3′ 5′ - GATNGTNGTGATGAAGTTNAT - 3′	Phenol-Chloro-form Iso-amyl alcohol method	Dhar and Ghosh, 2017
Taiwan Strait	365	Commercial fish (fresh, fil-let or frozen)	313	92.86–100	1	5′GGWACWGGWTGAACWGTWTAYC-CYCC3′ 5′TAIACYTCIGGRTGICCRAARAAY-CA3	QIAmpTM tissue DNA extraction kit, DNeasymeri-con Food Kit	Xing et al., 2020
Canada	285	Fish (raw/ frozen)	>100	75	1	TGTAAAACGACGGCCAGTTCCAC-TAATCACAARGATATTGGTAC CAGGAAACAGCTATGACGAAAAT-CATAATGAAGGCATGAGC	QiagenDNeasy Blood & Tissue kits	Hu et al., 2018

Cytochrome Oxidase Subunit I=*COI.*

Benefits of DNA mini-barcoding

The methodology refers to the analysis of short DNA fragments and it can be applied in different fields of systematics (from museum collection research to forensic applications). *COI* mini-barcoding is, therefore, useful to assess the correct taxonomy in processed products. This is particularly the case with commercial fish, for which the correct preservation under stable refrigerated conditions is a major issue during fishing, transportation and distribution. In addition, fish processing for preservation is the major cause of DNA degradation. Consequently, molecular analyses to reveal species substitution may face DNA degradation limitations and, therefore, become biased by technical questions. The possibility of analyzing short DNA fragments such as *COI* mini-barcoding seems to solve this issue. In the field of ichthyology, the application of mini-barcoding is nowadays possible thanks to the availability of specific databases accounting for millions of *COI* sequences. To give a general idea, BOLD database reports cover more than 24,000 barcoded species among *Actinopterygii* and *Elasmobranchii*. On the other hand, it is noteworthy to observe that data redundancy may generate confusion or technical biases due to contradictory attribution within a genus level. Starting from past experience of the application of *COI* and *CytB* barcoding, besides the use of additional markers for the identification of species substitution in fish products, a new investigation was carried out to verify the usefulness of *COI* mini-barcoding as an innovative methodology to substitute for classic barcoding. The research was focused on sampling and analyzing the same taxa purchased in the same department stores to assess the evolution of cryptic species mislabeling after a decade, also considering the application of new European regulations (Filonzi et al., 2021).

Limitations of DNA mini-barcoding

The DNA sequence may contain unstable mutation sites or be difficult to combine into a unified sequence. Thus, selecting the position and length of DNA mini-barcodes is critical in discriminating between multiple species (Hajibabaei et al., 2006). Nucleotide signatures tech-

nique (20–50 bp in length) containing stable SNP sites could succeed in discrimination of closely-related species. However, this approach is useless for species that cannot be distinguished using DNA barcoding. For example, no mini-barcoding markers could be developed from ITS2 sequences to distinguish three species of Ephedrae herbs (*Ephedra sinica*, *Ephedra intermedia*, and *Ephedra equisetina*) as the entire ITS2 sequence fails to identify these species (Chen et al., 2019). The development of DNA mini-barcoding should ensure reliable PCR amplification results. Although challenging, novel primer pairs based on 100–200 bp sequences must be designed that avoid dimer formation, hairpin formation, and false priming. Specially designed primer sets can drastically increase the success of amplifying degraded DNA (Hajibabaei et al., 2006). Although fish mini-barcodes have been developed, they have not yet been extensively researched for use with regulatory samples. Furthermore, there is currently a lack of information regarding the most appropriate technique to use for fish samples that have been cooked in different ways (Shokralla et al., 2015).

Overview of metabarcoding

NGS (next generation sequencing) combined with DNA barcoding is referred to as metabarcoding. DNA metabarcoding is a process in which environmental sample is used for identifying a number of organisms simultaneously as shown in Figure 3. Environmental sample or eDNA refers to any DNA collected from soil, water or air. It mostly contains degraded DNA and many of the times only short barcodes can be used from it. Therefore, highly conserved and versatile primers are required in metabarcoding studies along with other constraints imposed by this technique (Riaz et al., 2011). The eDNA detection method will become more valuable and essential if it could reveal the entire fish diversity in a given area. One approach to this end is metabarcoding combined with massively parallel sequencing (Lawson Handley, 2015).

More recently, fish-universal primers for eDNA metabarcoding have been developed, which will promote fish diversity research (Miya et al., 2015). In this regard, the set of fish-universal PCR primers, MiFish are suitable for eDNA metabarcoding. These MiFish primers amplify hyper-variable regions of the mitochondrial 12S ribosomal RNA (rRNA) gene and enable taxonomic identity to be distinguished mostly at the species level. The fact that eDNA metabarcoding using these primers detected >90% of fish species (i.e. 168 species from 14 orders) in aquarium tanks indicates that the primers can cover phylogenetically diverse species. Moreover, because the amplicon length is ~170 bp, the target region can be PCR-amplified even from degraded genetic material, and the short amplicons are suitable for massively parallel sequencing using MiSeq. Thus, eDNA metabarcoding is becoming an increasingly useful approach for revealing the composition of entire fish communities (Valentini et al., 2016).

The sample's details of metabarcoding is given (Table 3). According to reviewed articles, specific metabarcode sequences were acquired from various samples. For some samples Mi FISH primer is used.

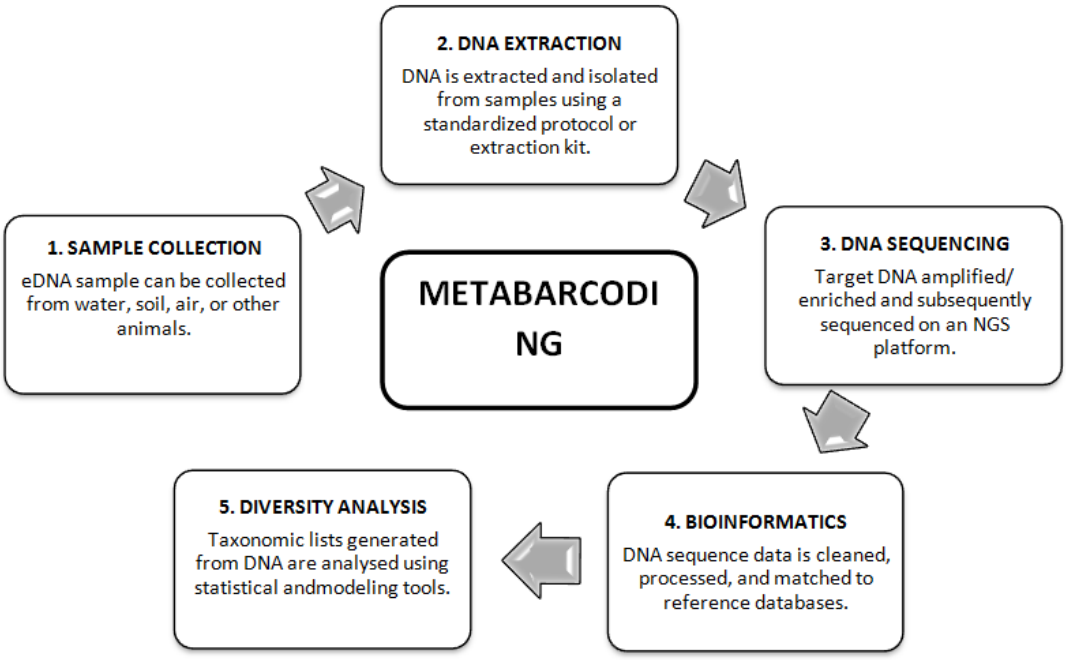


Figure 3. Overview of metabarcoding pipeline

Table 3. Detection of fish species or taxa using metabarcoding of water and sediment samples at different localities of world

Country	Primers	No. of sequences reads	Base pairs length (bp)	No. of fish species/taxa	DNA extraction protocol	Sample type	Sequencing library	No. of samples	References
1	2	3	4	5	6	7	8	9	10
Czech Republic	12S-V5-F and 12S-V5-R	22.46 million	73–110 ~ 106	31 taxa	Mu-DNA water protocol	Water samples	IlluminaMiSeq®	189	Blabolil et al., 2021
Taiwan	12S rRNA (MiFish-U/E), ND5 (MiFish-tuna-ND5), and *COI* (Fish-miniA/C/E; Fish1/2; Fish1F/2F with Shark *COI*-MINIR, Shark-MiniV1-R, and Shark-MiniV2-R)	50,534,995	150	153	QiagenDNeasy Blood and Tissue Kit	Filtered sea-water	Illumina Sequencing	1 Liter (three times sampled twice)	Lee et al., 2021
Aka, Tama, Miya, Takatsu, Sendai River-Japan	MiFish U and MiFish-E	17,600	170	182	DNeasy Blood and Tissue Kit	Water samples	MiSeq platform	30	Ahn et al., 2020
Eastern Bass Strait	COX1 Minibar, BilSSU1100_F and BilSSU1300_R, 16S Chord-cephA	107 922	260–650	8	QiagenDNeasy Blood and Tissue kit	Stomachs, intestinal tract	Mega BLAST algorithm	151	Berry et al., 2015
Greece	FishF1,FishR1 FishF2, FishR2	1002	624	55	Protocol of Hillis et al.,	Freshwater fishes	ABI 3730xl DNA Analyzers	544	Tsoupas et al., 2022
The river Mulde, Germany	MiSeq	9,906,197	129–209	25	salt precipitation protocol	Water samples	v2 250 bp PE Illumina kit	18 (1-L)	Macher et al., 2021
Churaumi Aquari-um, Okinawa, Japan	MiFish	2,157,365	170	410 species	Anacapa Toolkit	Seawater	IlluminaMiSeq		Miya et al., 2020
Okinawa Churaumi Aquarium, Japan	MiFish-U-F/R	14.86 million	163–185	168 species	DNeasy Blood and Tissue Kit	Seawater from tanks	MiSeq platform	180	Miya et al., 2015
Paraná River	MiFish F, MiFish R	17,616,032	163–185	29	Magnetic beads	River water	IlluminaMiSeq Reagent 600V3 (PE 300b)	4447	Dal Pont et al., 2021
Japan	MiFish-U and -E	8,094,567	~170	128	DNeasy Blood and Tissue Kit	Water samples	IlluminaMiSeq v2 Reagent kit	1-L(47 sites)	Yamamoto et al., 2017
Brazil	MiFishU32, Teleo17 and V05F_89835)	Nil	952	67 fish species	Salting-out protocol	Fin clips	ABI 3500 Genetic Analyzer	132	Milan et al., 2020
UK fishing venue	12s	10,751,170.	106	12 fish taxa	Mu-DNA protocol	Water samples	Meta BEAT (metaBarcod-ing and Environmental Analysis Tool) v0.97.11	98	Di Muri et al., 2020
Northwest Atlantic Ocean	Cytochrome C oxidase I (*COI*), 12S and 18S	1,250,418	163–185	15 species	DNeasy Power Water Kit	Water sample	IlluminaNovaSeq 6000	48	McClenaghan et al., 2020

Table 3 – contd.

1	2	3	4	5	6	7	8	9	10
Atlantic and Pacific waters	Cytochrome oxidase I (*COI*)	5343 (West Africa #1) and 22,649 (West Africa #4). 6294 in Rangiroa (Polynesia) to 14,794 in the Mediterranean Prévost lagoon	650	Nil	Power Water DNA Isolation Kit	Water samples	MiSeqIllumina platform	6-L	Garcia-Vazquez et al., 2021
England (The river Lugg)	12S-V5-F 12S-V5-R	1131	144	7 species	Nexxtec 1-step DNA Isolation Kit for Tissues & Cells	Water samples	IlluminaMiSeq platform Team, 2019).	55	Muha et al., 2021
Lagoon of the North shore of Moorea	ml*COI*intF/jgHCO2198 *COI*	69,663	313	3 species	QIAGEN DNeasy Blood & Tissue kit.	Digestive tract	NEBNext Quick DNA Sample Prep Reagent Set 2 chemistry	25	Leray et al., 2015
Tanzania	12S-V5 MiFish-U 16s_Teleo Cichlid_CR	11.5 million and 8.6 million paired-end reads	170 106 278 307	63 species	QiagenDNeasy® Blood & Tissue Kit	Water sample	IlluminaMiSeq	10 L	Doble et al., 2020
Tigris Basin	MiFish-U	3,862,683	~ 172	32 genera/species of fishes,	DNeasy® Blood & Tissue Kit	Water sample	IlluminaMiSeq DNA sequencer (Illumina)	27	Hashemzadeh Segherloo et al., 2021
Japan	MiFish(U/E)	Nil	172	290 fish species	DNeasy Blood and Tissue Kit	Water samples	MiSeq v2 Reagent Kit for 2 × 150 bp PE (Illumina)	150	Kumeet et al., 2021
Scorpion State Marine Reserve	MiFish Universal Teleost 12S primerMiFish Universal Teleost 12S primerMiFish Universal Teleost 12S primer	four million reads	Nil	40 unique species,	DNAeasy Tissue and Blood Kit	Seawater samples	Reagent Kit V3 with 20% PhiX	27 samples and 8 controls.	Gold et al., 2021
Scotland	s14F1 s15.3	7535877	Nil	10	DNA Elution Accessory Kit	Sediments ranging between muds and silty-sands	Illumina sequencing	45	Pawlowski et al., 2014
Volga River	Cyt b 12s 16s	12 M	330–413	23	Phenol–chloroform–isoamyl alcohol protocol	Water samples	IlluminaMiSeq (reagent kit v3),	60 eDNA samples	Lecaudey et al., 2019
Scripps Pier and Matlahuayl Marine Reserve	*COI* VF1 *COI* VR1	15,197,856	710	17 species	QiagenDNeasy Blood and Tissue Kit	Plankton samples	IlluminaMiSeq	4,500 eggs	Duke et al., 2020
Australia	16SF/D 16S2R-degenerate	740,847	Nil	73 taxa	DNeasy Blood and Tissue Kit	Seawater samples	MiSeq V2 Reagent Kit Illu-mina MiSeq platform	500 ml	Stat et al., 2019

Table 3 – contd.

1	2	3	4	5	6	7	8	9	10
The Rainbow Reef tank at Ripley's Aquarium of Canada in Toronto, Ontario,	*COI*, 12S, and 16S.	28,079,780	155–365	107 species	PowerSoil DNA Isolation Kit	Seawater	IlluminaMiSeq	5	Morey et al., 2020
Koide River watershed, which flows through Kanagawa Prefecture, Japan	MiFish-U primers	1,762,797 (aqueous) and 1,918,090 (sedimentary)	Nil	Aqueous (57) and sedimentary (88) fish species	Salivette (Sarstedt, Nümbrecht, Germany) and DNeasy Blood & Tissue Kit (PowerSoil DNA Isolation Kit;	Water and sediment samples	IlluminaMiSeq v2 Reagent kit	10–4000 mL/50 g	Sakata et al., 2020
Bassen-thwaite Lake, Derwent Water and Windermere) in the English Lake District, UK,	12S_F1 and 12S_R1 CytB_L14841 and CytB_ H15149	Nil	~106–460	Fourteen	PowerWater DNA Isolation Kit	Water samples	IlluminaMiSeq	78 (2L)	Hänfling et al., 2016
Beijing area	Tele02 primer pair	53.8 million	129–209	37	DNeasy Blood and Tissue Kit	Water samples	IlluminaHiSeq X-Ten	100	Zhang et al., 2020
St. Joseph River in northwestern Indiana, USA	L14735/H15149c, Ac12S, Am12S, and Ac16S primer sets	5.4 million	50–500	12 species	Chloroform–isoamyl alcohol and an isopropanol precipitation of DNA	Water samples	IlluminaMiSeq	8	Olds et al., 2016
Tian-e-Zhou Reserve Poyang Lake	16S_eDNAF 16S_eDNAR	286 million	133–140	75 fish species	Chloroform–isoamyl alcohol and an isopropanol precipitation of DNA	Water samples	IlluminaHiSeq 4000 platform.	15(1-L)	Qu et al., 2020
Daqing River Basin	*COI* 12 S	4,545,260 *COI* reads 6,678,447 12 S	12	15 species	DNeasyPowerWater Kit	Surface water samples			Xie et al., 2021
Lake Biwa, Japan	MiFish-U-F and MiFish-U-R primers	9,083,566	370	Nodanuma (31), Sonenuma (22), Ibanaiko(33) and Nishinoko (31) lineages for individual samples; 30, 20, 29, and 27 lineages for pooled samples	DNeasy Blood and Tissue Kit	Water samples	MiSeq platform using the MiSeq v2 Reagent Kit	43 (individual); 60 (pooled)	Sato et al., 2017

Table 3 – contd.

1	2	3	4	5	6	7	8	9	10
Okinawa Churaumi Aquarium, Okinawa, Japan	MiFish-U/E	10,073,869	Nil	232 fish species	DNeasy blood and tissue kits	Surface seawater	IlluminaMiSeq	10L	Fukaya et al., 2019
Germany	MiFish primers	1,221,036	170	27 species	First-Magnetic Forensic Kit (GENIAL), NucleoSpin Soil Kit (MACHEREY-NAGEL), and the DNeasyPowerSoil Kit (QIAGEN).	Suspended particulate matter (SPM)	IlluminaMiSeq	9	Díaz et al., 2020
Lake Geneva (Thonon les Bains, France)	MiFish-U-F and MiFish-U-R	15,478,824	163–185	126 species	Macherey-Nagel NucleoSpin Soil kit (Macherey-Nagel)	Water samples	IlluminaMiSeq sequencing	30	Rivera et al., 2021
England	L14912,H15149(Cytb) And 12S_V5_F,12S_V5_R(12S)	17.15 million (Cytb) and 15.97 million (12S)	414 (Cytb) 106 (12S)	24 species	DNeasyPowerWater Kit	Water samples	IlluminaMiSeq	252	Li et al., 2019
Upper Great Lakes Region, North America	12S forward and reverse primers	7-81,209	Nil	Nil	gMax Mini Genomic DNA Extraction Kit	Water samples	IlluminaMiSeq System	327	Euclide et al., 2021
Italy, France, Spain and Greece	Tele02 primer set	16,197,599	~167	74	DNeasy Blood & Tissue Kit	Water samples	HiSeq4000	132 samples/25 Mediterranean fish species	Aglieri et al., 2021
France and The Netherlands	batra_Fbatra_Rbatra_blk-teleo_Fteleo_Rteleo_blk	20 211 018	Nil	24 taxa	DNeasy Blood &Tissue kit	Tissue samples	IlluminaMiSeqsequencer	86 fish species	Valentini et al., 2016
Hokkaido, Japan	MiFishU-F and MiFish-U-R primers	641.056	Nil	24 species	DNeasy Blood and Tissue Kit	Water samples	MiSeq v2 Reagent Kit	31	Fujii et al., 2019
RV Celtic Voyager in the Irish and Celtic seas	12S V5	13,149,751	106	19 taxa in water, 20 taxa in larvae	e QIAGEN PowerSoil kit	Water and larvae samples	IlluminaMiseq	42 water biological replicate samples	Ratcliffe et al., 2021

Cytochrome Oxidase Subunit I=*COI*

Bioinformatics tools

Bioinformatics has played a crucial role in the advancement of metabarcoding. In recent years, many bioinformatics tools have been developed and are constantly being improved to efficiently and effectively perform various steps involved in the metabarcoding process. After obtaining NGS data, quality filtering is the first essential step, because it removes erroneous data that may otherwise potentially lead to misidentification of species. Sequencing errors introduced during NGS can be recognized because raw reads have predicted error probabilities for each base indicated by Phred quality scores. Sequence errors can be removed during quality filtering and trimming, e.g. by truncating reads at the position where their quality begins to drop. A Phred score of 20, which corresponds to a 1% error rate in base calling, is often used as a minimum threshold in quality filtering. Bokulich et al. (2013) published guidelines for quality-filtering strategies to enable efficient extraction of high-quality data from Illumina amplicon sequencing data. In their studies on TMs, Coghlan et al. (2012) used the commercially available software Geneious (Kearse et al., 2012). Other software tools for quality filtering of reads include PRINSEQ (Schmieder and Edwards, 2011) and Trimmomatic (Bolger et al., 2014).

Benefits of metabarcoding

The greatest advantage of DNA metabarcoding is its ability to identify each single species within complex multi-ingredient and processed mixtures simultaneously, where the application of DNA barcoding and conventional analytical methods is limited considerably. DNA metabarcoding enables simultaneous high-throughput multi-taxa identification by using the extracellular and/or total DNA extracted from complex samples containing DNA of different origins (Taberlet et al., 2018). eDNA metabarcoding is a powerful and cost-efficient approach for biomonitoring as it allows whole communities to be screened (Hering et al., 2018). eDNA metabarcoding from water samples is non-invasive, safe and simple, and derived taxonomic richness estimates are generally more complete than classical assessments (Hänfling et al., 2016; Pont et al., 2018; Boivin-Delisle et al., 2021).

While fish species are primary targets, eDNA monitoring data can also provide reliable information on many other taxa either living in or in the vicinity of water bodies such as mammals (Andruszkiewicz et al., 2017; Closek et al., 2019), amphibians (Bálint et al., 2018; Harper et al., 2018), and birds (Ushio et al., 2018; Day et al., 2019; Schütz et al., 2020). The collection of eDNA samples during monitoring studies thus can provide highly valuable information on a much broader scale without any additional costs or sampling effort, if the same metabarcoding primers are used. Often universal, i.e., degenerate primers (Riaz et al., 2011; Miya et al., 2015; Taberlet et al., 2018), have the potential to efficiently target fish and lamprey, and moreover also to amplify DNA of species of birds and mammals as a bycatch, without reducing the fish detection rate.

Detection of rare and endangered or invasive species is a particularly useful application of eDNA metabarcoding. Balasingham et al. (2018) used *COI* markers to detect three endangered fish species, one invasive fish species, and 78 native fish species from two tributaries of the Great Lakes in the U.S.

Limitations and comparison of metabarcoding with traditional survey methods

DNA metabarcoding data is usable for qualitative evaluation only, to determine presence of taxa, and not for quantitative assessment of relative species abundance based on sequence read numbers, as many variables considerably impact the obtained sequence read results (Staats et al., 2016).

It has been shown in most cases, that eDNA surveys provide similar or higher detection probabilities than UV, and reveal cryptic species known to occupy habitats better than visual methods (Port et al., 2016). However, eDNA surveys were still unable to cover all the species detected by UV (Mirimin et al., 2021; Valdivia-Carrillo et al., 2021). When comparing eDNA and traditional EF methods in large rivers, eDNA detection was more sensitive when the species were at low abundances (Czeglédi et al., 2021). Similarly, Pont et al. (2018) confirmed the possibility of detecting local fish assemblages using eDNA metabarcoding through a comparison with results from long-term EF surveys in a large river. When comparing eDNA and net-based methods, Shaw et al. (2016) demonstrated that more fish species were identified by eDNA metabarcoding with 12S rRNA primers than by conventional fyke net surveys in three sites along the North Para River in Australia.

Such high and reliable detection probabilities provided by eDNA methods equate to substantial time and economic savings when species are rare, or when survey scales are large, upholding the utility of eDNA methods as alternative non-invasive, time- and cost-effective tools for biological surveys. Nonetheless, the existing eDNA methods also have considerable limitations, notably in morphological measurement, age structure analysis, life or development stage identification, and direct enumeration, compared to conventional surveys for fish monitoring and conservation. Unlike traditional surveys, eDNA analysis does not provide real-time data on habitat variation and the exact locations of target species. The results of eDNA analysis can also be susceptible to environmental factors such as temperature, pH, and water chemistry, as well as difficulties associated with the production, persistence, and transport of eDNA, in addition to variability in the size and number of target species, and to challenges related to the sampling process (Sato et al., 2017). Thus, eDNA analysis should not be used as an alternative but only as a supplement to established methods in the advancement of sampling, monitoring, and conservation. A brief comparison of advantages and disadvantages of different barcoding techniques are given in Table 1.

Table 4. Comparison of benefits and drawbacks of barcoding techniques

Method	Advantages	Disadvantages
DNA barcoding	Identification of species Help in detection of incorrect labeling of products Rapid Accurate Cost effective	Single-locus identification system No precise estimation of the intraspecific variation compared with interspecific variation No universal gene Sequences are short (approx. 500–1000 bp) Cannot amplify and sequence degraded DNA
Mini-barcoding	Analyze short DNA fragments Can analyze degraded DNA fragments Used for identification of processed products	Unable to amplify unstable mutation sites Useless for species that cannot be distinguished using DNA barcoding Dimer formation, hairpin formation, and false priming occurs which hinder the amplification of DNA No extensive use of primers in research
Metabarcoding	Simultaneous high-throughput multi-taxa identification Powerful and cost-efficient approach for bio-monitoring Non-invasive, safe and simple, and derived taxonomic richness estimates are complete for water samples Provide reliable information Can detect rare and endangered or invasive species	Usable for qualitative evaluation only Unable to cover all the species More sensitive when the species were at low abundances Does not provide real-time data on habitat variation

Challenges

Due to a lack of taxonomic reference material and corresponding intraspecific variation information, many challenges remain pertaining to species-level identification of fish using eDNA metabarcoding. Although the target fragment of the international Barcode of Life (iBOL), a part of the mitochondrial *COI*, has been shown to have more reference sequences from fishes, it is less suitable for metabarcoding work for fishes (Collins et al., 2019; Zhang et al., 2020). Fragments of the mitochondrial 12S genes have been proven to be a better barcode for fishes (Evans et al., 2016), however, databases for fragments of this gene are less complete than for *COI* and heavily biased towards certain geographic regions and taxonomic groups (Belle et al., 2019). As such, there is a need to expand databases for understudied areas and taxonomic groups to improve the application of this technology (Schenekar et al., 2020). It has also been found that the sequences of many fishes living in Lake Taihu are very close and cannot be fully identified using short 12S rRNA fragments (about 150 bp amplified with MiFish primer), and it has been suggested that a longer marker for metabarcoding or specific primers for a certain genus are required to solve this identification problem (Wu et al., 2021). A multi-barcode approach (i.e. using more than one gene marker for the same community) has also been recommended to improve taxonomic resolution and to reduce false negatives (Deagle et al., 2014). However, due to primer and gene copy variation biases, one should be cautious when using this approach as barcodes may not be equally applicable for quantitative analyses. In addition, an increased cost of primers, sequencing, and labor, as well as the necessity for duplicity of reference databases are other obvious downsides of the multiple-barcode approach.

Conclusion and recommendations

DNA barcoding, mini-barcoding and metabarcoding are standard techniques used for proper identification, checking mislabeling and discovery of different species. It overcomes the taxonomy related issues. DNA sequenced based libraries make them more accurate methods. In short, DNA barcodes, mini-barcodes and metabarcodes reference library have opened various opportunities to monitor artisanal fisheries by considering their dynamics at species level. They also contribute to achieve more effective monitoring, conservation, and management of fisheries and have a major role in aquaculture industry. Specific taxonomic groups have been targeted both regionally and globally for detailed species validation projects.

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