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DNA Authentication of Fish Products: A Tool to Reveal the Risk of Mislabeling/ Fraudulence Associated with Seafood Trade

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Abstract

Mislabeling poses a threat to the sustainability of seafood supply chains, food integrity, the economy, public health, and consumers' ethics and, can significantly affect conservation efforts. It is essential that these fraudulent and mislabeled fishes are to be revealed by PCR-based techniques. DNA Authentication is one of the effective tools and the mitochondrial Cytochrome c Oxidase subunit I gene is used to identify the mislabeling of commercially important fishes at a DNA level. Because this analysis provides strong support to detect fishes that are sold under the wrong name and fraudulent seafood products sold as deliberately substituted by low-value fishes instead of high-value fishes. It helps to prohibit the conserved fish species from the sold. This article highlighted the suitable method used to overcome mislabeling and fraudulence issues that occur in the seafood trade to create awareness to poor consumers and seafood traders.

Introduction

Of all international food commodities, seafood is the most traded one with highest total value than the pork and poultry. Since, fish provides significant amount of protein, polyunsaturated fatty acids and essential micronutrients, it has long been recognized as a valuable dietary source. At present, world population growth in combination with increase in average income and urbanization has boosted consumers to care about their diets cautiously with healthy and nutritious food. Hence, marine fish is one of their prime targets. This results in the surge of seafood production and trade worldwide.

However, it is reported that seafood mislabeling is common in markets. The mislabeling/ fraudulence can be caused by human error due to difficulties in species identification, multiple names and complicated products transformed from raw materials. Also, there is intentional fraud by sellers to gain the advantage. The economic incentive from mislabeling of seafood species is obvious with the global level of trade and consumption. In this case, sellers may deliberately substitute higher price species with lower price species. This may cause finance loss for consumers since they pay money for high quality species but get substituted species with lower quality. Moreover, it may result in health problem if substituted species contain toxins or allergic factors. In addition, endangered species can be illegally exploited for commercial purpose. There is no doubt that illegal fisheries of endangered species significantly damage biodiversity. Therefore, accurate identification of seafood species is of great importance to food management, consumer's welfare as well as biodiversity conservation.

Like other procedure of species identification, fish species are conventionally identified based on their morphology. However, morphological identification requires skilled taxonomist and consumes time. Furthermore, with the processed or cooked products, morphology of fish is altered and this approach is impossible. Recently, the applications of DNA identification in seafood authentication have become popular. Among DNA based methods, DNA barcoding has proved as a powerful tool to discriminate seafood species. Numerous studies have conducted on DNA barcoding to authenticate seafood species and evaluate their mislabeling.

Some Better Documented Cases of Fraudulent/Mislabeling of Seafood for Purposes of Deceiving Customers

Many researchers have found that about one in five fish purchased in retail and restaurants is mislabeled, and in many cases the lie was intentional. In all, it found 19% of the 25,700 global samples gleaned from the literature had been mislabeled. As early as the 1930's, canned mackerel was being labeled and sold as 'salmon'. In Maine, lobster fishers, who started an 'Imposter Lobster' campaign, are working to restrict the use of the 'Maine lobster' label by processors in New Hampshire and Nova Scotia.

In South Africa, most problematic was kob, *Argyrosomus* spp., for which some 84% of fillets provided belonged to other species, including mackerel, croaker, and warehou. Phylogenetic analyses provided strong support that the fillets sold as barracuda and wahoo were probably king mackerel (*Scomberomorus cavalla*) and that red snapper fillets included fillets of river snapper, *Lutjanus argentimaculatus*, which is a species prohibited for sale in South Africa.

In Italy, puffer fish were found being marketed as squid. In the US, the most commonly faked fish were snapper, grouper, and salmon. Asian catfish, or Pangasius, was found to be the most commonly substituted fish in the world used as a substitute for 18 varieties of common fish. An Indian survey of the authenticity of fresh and processed fish from the domestic market also used DNA barcoding. Its results showed that 22 percent of samples were mislabelled. A study of surimi products manufactured in China, India and Singapore using DNA barcoding showed that low-value species such as sardines and farmed catfish were used. Some mislabeled/fraudulent fish and shellfishes used for the production of fishery products in the seafood trade are listed below (Table 1).

Table 1: Fraudulent/ Mislabeled fish and shellfishes of Fishery Products in Seafood Trade (Source: J.L. Jacquet and D. Pauly., 2008)

Country	You Purchase (common name)	Which is supposed to be (scientific name)	But you get (scientific name)	Also called (Common Name)
US	Red snapper	*Lutjanus campechanus*	*Sebastes* spp. *Oreochromis* spp. *Coryphaena hippurus* *Ictalurus punctatus*	Rockfish Tilapia Mahi Mahi Channel catfish
US	Grouper	*Epinephelus* spp., *Mycteroperca* spp.	*Ictalurus punctatus* *Merluccius* spp. *Oreochromis* spp. *Theragra chalcogramma*	Channel catfish Hake Tilapia Alaska pollock
US	'Wild' salmon	*Oncorhynchus* spp.	*Salmo salar*	Farmed salmon
US	Mahi Mahi	Coryphaena hippurus	*Seriola lalandi*	Yellowtail
US	Halibut	*Hippoglossus* spp.	*Lates* spp.	Sea bass
US	Orange roughy	*Hoplostethus atlanticus*	*Pseudocyttus maculatus* *Zeus faber*	Oreo dory John dory
US	Swordfish	*Xiphias gladius*	*Isurus oxyrinchus*	Mako shark
US	Cod	*Gadus morhua*	*Theragra chalcogramma*	Alaska pollock
US	Dover sole	*Microstomus pacificus*	*Atheresthes stomias*	Arrow tooth flounder
US	Red drum	*Sciaenops ocellatus*	*Pogonias cromis*	Black drum
US	Rock cod	*Scorpaenidae*	*Squalus acanthias*	Spiny dogfish
US	Monkfish	*Lophius* spp.	*Tetrodon* spp.	Pufferfish
US	Scallops	Pectinidae	Various	Skate wings

Country	You Purchase (common name)	Which is supposed to be (scientific name)	But you get (scientific name)	Also called (Common Name)
US	Shrimp, crabs, scallops, lobster	*Crustaceans, Decapods*	Protein fibers extracted from offal	Surimi
US	Beluga caviar	Eggs of *Huso huso*	Eggs of *Cyclopterus lumpus*	Lumpfish roe
			Eggs of *Polyodon spatula*	Paddlefish roe
US	Patagonian toothfish	*Dissostichus eleginoides*	*Dissostichus mawsoni*	Antarctic toothfish
US	Lobster	*Homarus* spp., *Panilurus* spp., *Panulirus* spp.	*Nephrops norvegicus*	Langoustine or scampi
US	White perch	*Morone americana*	*Perca flavescens*	Yellow perch
US	Zander	*Sander lucioperca*	*Perca flavescens*	Yellow perch
US	Sauger	*Sander canadensis*	*Sander vitreus*	Walleye
US	Pink salmon	*Oncorhynchus gorbuscha*	*Oncorhynchus keta*	Chum salmon
Hong Kong	Atlantic cod	*Gadus morhua*	*Ruvettus pretiosus*	Oilfish
France	Thon blanc	*Thunninae*	*Lamna nasus*	Porbeagle
			Alopias vulpinus	Thintail thresher shark
Ecuador	Tilapia	*Oreochromis* spp.	*Merluccius gayi*	South Pacific hake
	Weakfish	Scioenidae		
	Flounder	Pleuronectidae		
Ecuador	Tuna	Thunninae	Selachians	Shark
	Flounder	Pleuronectidae		
Colombia	Pargo rojo	*Lutjanus* spp.	*Oreochromis* spp.	Tilapia
Australia	Barramundi	*Lates calcarifer*	*Lates niloticus*	Nile perch
			Polydactylus macrochir	King threadfin
Australia	King George whiting	*Sillaginodes punctatus*	*Micromesistius Australis* *Merlangius merlangus* *Sillago* spp. *Merluccius* spp.	Southern blue whiting North sea whiting Silver whiting Hake
Australia	Red emperor	*Lutjanus sebae*	*Lethrinus Choerorhynchus*	Spangled emperor
			Lethrinus miniatus	Redthroat emperor
Australia	Dhufish	*Glaucosoma hebraicum*	*Glaucosoma buergeri*	Northern pearl perch
			Glaucosoma scapulare	Pearl perch

Authentication of Fishery Products

Hence, authentication of fish species is most important now a days and it is done by DNA barcoding that consists of Sample Collection and Preservation, DNA Extraction, Polymerase Chain Reaction Analysis (PCR) and Gene Sequencing Analysis (Figure 1). The mislabeled and substituted fishes can be revealed by product samples compared with references (Figure 2).

1. Sample Collection and Preservation

- The collected samples may be consisted of two groups such as either market samples (such as raw, sushi, fillet, dry) or cooked fish that are collected from supermarkets, online markets, fish markets and restaurants while reference samples will be caught from the sea.
- All samples should be labeled and deposited by a part of muscle or/and fin clip should be cut and stored in absolute

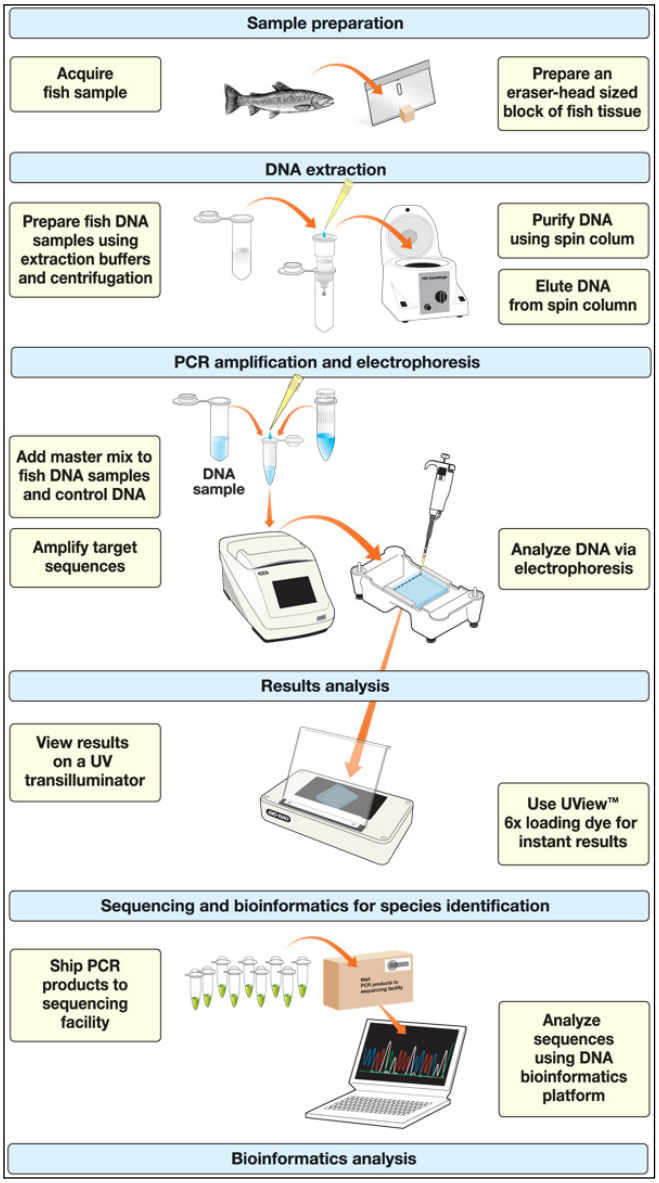

Figure 1: Protocol for DNA authentication of Fishes (Source: https://www.bio-rad.com)

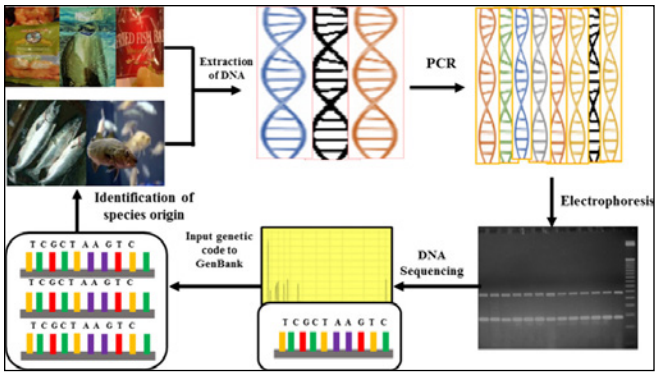

Figure 2: Schematic diagram of DNA authentication of seafood products (Source: Sultana *et al*., 2018)

alcohol (99.9%) at -20 °C for molecular study. Fish samples should be preserved in 10% formalin for Morphometric and meristic characterization.

2. DNA Extraction

• DNA can be extracted from muscle or fin clip of fish samples using phenol chloroform technique or using a Genomic DNA Extraction Kit/ CTAB (Cetyl Trimethyl Ammonium Bromide) Method. Among these, we can see the widely used and basic method *i.e*., Phenol-chloroform method by following the method of Kumar *et al*. (2007) with little modification.

• First, approximately 50 mg of muscle tissues can be taken from each individual of alcohol preserved specimen and dried on a tissue paper.

• The tissues should cut into small pieces and placed in a micro centrifuge tube (2 ml) containing 940 µl lysis buffer. Cell lysis buffer is used to lyse cell membrane, then intact nuclei are released into lysis buffer and it will be pelleted (EDTA is designed to lyse outer cell membrane of cells).

• Then 30 µl of 20% SDS and 20 µl of Proteinase K (10 mg/ml) should be added to lyse nuclear membrane and to digest protein. It will be incubated at 48 °C for 45-50 min in a water bath.

• After incubation, an equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) can be separately added to the tube containing lysed cells. DNA released into the solution may be extracted with phenol-chloroform to remove proteinaceous material. The contents may be mixed properly by gently inverting the eppendorf tube for 10 min to precipitate the proteins and other part of the nucleic acids.

• The tube will be centrifuged at 9,200 rpm for 10 min. Two layers such as top aqueous layer and bottom layer can be formed.

• The top aqueous layer should be transferred to a new 2 ml micro centrifuge tube. The DNA will be precipitated from the aqueous layer by adding equal volume of ice cold isopropanol and 0.2 volumes of 10 M Ammonium acetate and inverting gently for 10 min. The precipitated DNA must be pelleted by centrifugation at 13,200 rpm for 10 min.

• The supernatant can be removed by pouring out carefully without loss of DNA pellet. Then the pellet will be washed with 500 µl chilled 70% ethanol, air dried and resuspended in 200 µl Tris EDTA buffer and stored at -20 °C.

• The purity of isolated DNA can be checked with UV Spectrophotometer at 260 nm and 280 nm and the isolated DNA may be confirmed with 1% agarose gel electrophoresis.

3. Polymerase Chain Reaction Analysis (PCR)

• The extracted DNA can be subjected into PCR for amplification. During the PCR process, A 652 bp segment can be amplified from the mitochondrial Cytochrome C Oxidase subunit I (COI) gene using primer (Rajeshkannan *et al*., 2019).

FP 5' – TCA ACC AAC CAC AAA GAC ATT GGC AC – 3'

RP 5' – TAG ACT TCT GGG TGG CCA AAG AAT CA – 3'

• It can be performed in 25 µl or 50 µl reaction tube containing *Taq* 2X PCR master mix red (1.5 U *Taq* DNA polymerase) with 1.5 mM $MgCl_2$ and 20 ng of template DNA.

• The components should be mixed thoroughly and the PCR amplification can be performed in thermal cycler with following steps consists of Initial denaturation of 94 °C for 2 min, 35 cycles of 94 °C for 30 sec (Denaturation), 52 °C for 40 sec (Annealing) and 72 °C for 1 min (Extension) with final extension 72 °C for 10 min.

• The final PCR products may be confirmed with 2% agarose gel electrophoresis at 80-100 V for 30-35 min using 100 bp DNA ladder and the molecular weight of the PCR products (652 bp) may be determined with DNA ladder.

• The quality of DNA content in the PCR product can be analyzed by using biophotometer for further analysis.

4. Gene Sequencing Analysis Reveals Mislabeled Fishes

• The sequences of PCR products of COI gene can be analyzed and sequenced by using next generation sequencing through the sequencer machines. Then obtained sequences range must be at least 200-300 bp nucleotide length is effective in identifying specimens (Hajibabaei *et al*., 2006). Then, the sequences of all samples are analyzed with Basic Local Alignment Search Tool (BLAST) individually for the comparison of global database.

• The sequence of samples can be aligned in FASTA format, analyzed by using softwares like FASTQC, CLC Genomics Workbench, OrthoANI Tool, MolQuest, OmicsBox, ClustalW and MEGA 6.0 for analyzing nucleotide sequence characteristics and genetic divergence.

• The COI sequences of the samples will be used to estimate genetic divergence values and for constructing phylogenetic tree. The COI sequence of each sample can be aligned to yield a final alignment.

• Based on COI sequence data analysis, Transition/ transversion bias (R) and the average nucleotide frequency for all the samples to be observed. The mean GC content and genetic distance will be obtained from MEGA 6.0 values.

• The phylogenic relationship among the samples can be clearly established with Neighbour Joining (NJ) tree model and closely related species must be clustered under the same node while dissimilar species are clustered under separate nodes.

• DNA barcode sequences of all the samples should be submitted to GenBank and the GenBank accession numbers can be obtained which is an approved genetic barcode of respective samples.

• Based on the accession numbers, the obtained seafood market or cooked fish sample gene sequences will be compared with reference sequences to check the originality of fishes which had used to prepare the ready to eat and ready to cook seafood product and if it has, the mislabeled and substituted fishes can be revealed by this technique.

Conclusion

This article reveals that seafood mislabeling appears to be motivated primarily by economic gain through intentionally misleading buyers at every level of the seafood supply chain, across the world and concludes clearly that the use of DNA authentication process against the mislabeled and substituted fishes in seafood products is the only right way to identify and avoid illegal activities in seafood trading. This is also reinforced the usefulness of COI barcodes to overcome the difficulties in species identification, multiple names, conservation of endangered fishes and complicated products transformed from raw materials.

References

Hajibabaei, M., Singer, G.A.C., Hickey, D.A., 2006. Benchmarking DNA barcodes: an assessment using available primate sequences. *Genome*. 49, 851 -854.

Kumar, R., Singh, P.J., Nagapune, N.S., Kushwaha, B., Srivastava, S.K., Lakra, W.S., 2007. A non-invasive technique for rapid extraction of DNA from fish scales. *Ind.J. Exp.Biol*. 45, 992-997.

Rajeshkannan, R., J. Jaculine Pereira, K. Karal Marx, P. Jawahar, D. Kiruthiga Lakshmi and Devivaraprasad Reddy., 2019. DNA Barcoding on Cardinalfishes (Apogonidae) of Thoothukudi Coast. *Int.J.Curr.Microbiol.App.Sci.* 8(08): 1293-1306.