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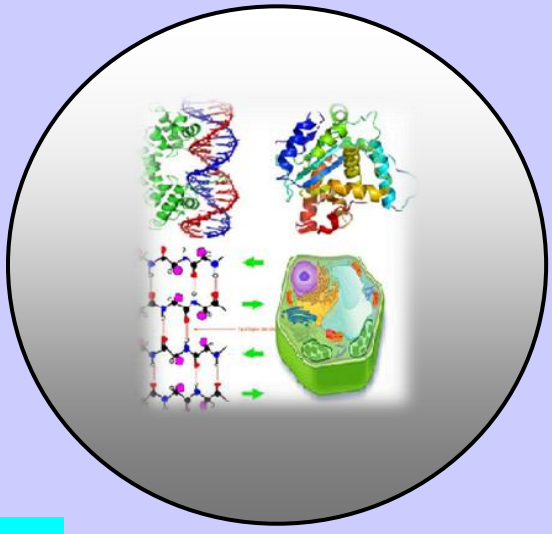

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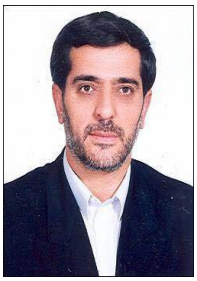

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# Differentiation of *Charah sorrah* and *Alopias pelagicus* using DNA Barcoding based on 16s rDNA

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**ABSTRACT**

***Fraud in the marine food products got importance in most countries for more than three quarters of a century. Nowadays, with the increasing demand for consumption of sea foods so that demand for knowledge of its contents and compounds increased. Present study was done on two shark species that are mainly offered to the Iranian market and they are known as shark (Charcharhinus sorrah, Alopias pelagicus), these two types are under fraud because of good capability to be a fillet, low cost and prohibition against distribution and supply in the Shiites' markets so that it is more possible to be distributed in the markets as another fishes' fillet. In this study we aimed to detect these two species using 16s rDNA. This study was conducted on 60 samples obtained from 5 species of fish including sharks as control positive, and Pomadasyskakkan, Scomberomorus Commerson and Acipenser stellatus species as control negative. After PCR done for Charah sorrah and Alopias pelagicus according to the protocol of electrophoresis in gel agarose 2% it was seen that the amplified fragment size was 179 bp and 1100, respectively, so, based on sharp band and size of marker showed safe and specific replication therefore can be used for enzymatic digestion and comparing with other samples.***
***Keywords: Charah sorrah, Alopias pelagicus, 16s rDNA, Barcoding and PCR.***

## INTRODUCTION

Fraud in the marine food products got importance in most countries for more than three quarters of a century. Nowadays, with the increasing demand for consumption of sea foods so that demand for knowledge of its contents and compounds increased (l amendin et al., 2014). In addition to the importance of food safety, consumers need clear and accurate

information about foods that they buy, because this information is necessary in choosing which products they want to buy. It depends on factors such as Economic, eating habits, socio-religious as well as medical considerations in terms of health risks (Asensio et al., 2009). In this regard, fish and seafood are more at risk of fraud and mislabeling than other raw animal products. High diversity in fish species as well as misdiagnosis of fish meats after deboning and making fillet in processed form make mistake in detection of products each other for example, the most of the fish species are the same in terms of taste, texture and appearance; so, it is hard to detect after the deboning and making the fillet (Farrell, Clarke and Mariani 2009). Therefore, it is most preferable by some sellers to replace the one expensive fish meat with another which is cheap and same characteristics by doing mislabeling to receive high profits (Aida et al., 2004).

DNA barcoding provides an additional advantage over traditional tissue-sampling methods, which were only able to provide a positive or negative match indication. Because DNA among similar species bears a similar resemblance, researchers can usually narrow down a mystery fish to a specific species type even if they aren't able to match its DNA with a sequence in the library. There are many genes are used in barcoding of fishes' meat in field of food fraud that mitochondrial and nuclear genomes are the most popular. Using mitochondrial DNA is common. Mitochondrial 16s rDNA is a gene that is evolved later and the rate of mutation in this gene is low; so, it is selected for assessment of taxonomy in term of family and genus. 16s rDNA gene has protected and variable areas in which protected areas large enough for joining to the primers and is used for study of phylogenetic relationships of the species. Present study was done on two shark species that are mainly offered to the Iranian market and they are known as shark (charcharhinus sorrah, Alopias pelagicus), these two types are under fraud because of good capability to be a fillet, low cost and prohibition against distribution and supply in the Shiites' markets so that it is more possible to be distributed in the markets as another fishes' fillet. In this study we aimed to detect these two species using 16s rDNA.

## MATERIAL AND METHODS

### Sampling

This study was conducted on 60 samples obtained from 5 species of fish including sharks as control positive, and *Pomadasys kakkan, Scomberomorus commerson* and *Acipenser stellatus* species as control negative. These fish were randomly obtained from certain reliable sources after morphological investigation and thorough compatibility and final confirmation and were used for setting the primers and setting up the experiments. The samples were transferred in dry ice within falcons to the laboratory and maintained in freezer -20˚C till the time of study. The type and name of species is shown in table 1.

**Table 1. Scientific and Common names of some fishes.**

| | **Scientific Name** | **Common Name** |
|---|---|---|
| 1 | *Charcharhinus sorrah* | Shark |
| 2 | *Alopias pelagicus* | Shark |
| 3 | *Pomadasys kakkan* | Javelin grunter |
| 4 | *Scomberomorus commerson* | Narrow-barred-spanish mackerel |
| 5 | *Acipenser stellatus* | South caspian stelute sturgeon star storgeon stellate sturgeon |

**DNA extraction**

An amount of 200mg meat of fishes were grounded in liquid nitrogen. Then, 500 µL lysis buffer was added, transferred into tubes, and incubated at 65°C for 15 min. After cooling in room temperature for 5 min, 700 µL chloroform-isoamyl alcohol was added, and then centrifuged at 10000 g for 5 min. The supernatant was transferred into new eppendr of tubes. An equal volume of cold isopropanol was added into tubes, and mixed gently by inversion. The tubes were then centrifuged at 10000rpm for 5 min. The supernatant was discarded, and the pellet of DNA was dried at room temperature. Then, the dried DNA was dissolved in 100 µL distilled water. The extracted DNA was quantified by a spectrophotometer, NanoDrop1000 (Nano Drop, Wilmington, USA).

**Specific multiplex PCR**

One set of primers were designed with oligo7 software for specific regions of each sequence of mitochondrial 16s ribosomal gene of fishe (Table 2). An initial denaturation at 94°C for 5 min was followed by 32 cycles of amplification (denaturation at 94°C for 2 min, annealing at 50°C for 1 min, and extension at 72°C for 1 min), ending with a final extension at 72°C for 10 min.

**Table 2. Primer sets for multiplex PCR for detection of fishes in market.**

| Species | Sequence of primer | Size of amplicon | Ta (annealing temperature) |
|---|---|---|---|
| *Carcharhinus sorrah* | CASF: 5'- TCTAATTTACATTTCA ACC-3'<br>CASR:5'-zGATGCAAAAGGTACGAGGG-3' | 179 | 57°C |
| *Alopias pelagicus* | ALPF: 5'- TTATTAATTACCTTATACAC-3'<br>ALPR: 5'-TTAAAGTTCTTTTCTTAGGC -3' | 1100 | 57°C |

## RESULTS

**Results of PCR for Charah sorrah**

After PCR done for Charah sorrah according to the protocol of electrophoresis in gel agarose 2% it was seen that the amplified fragment size was 179 bp, so, based on sharp band and size of marker showed safe and specific replication therefore can be used for enzymatic digestion and comparing with other samples.

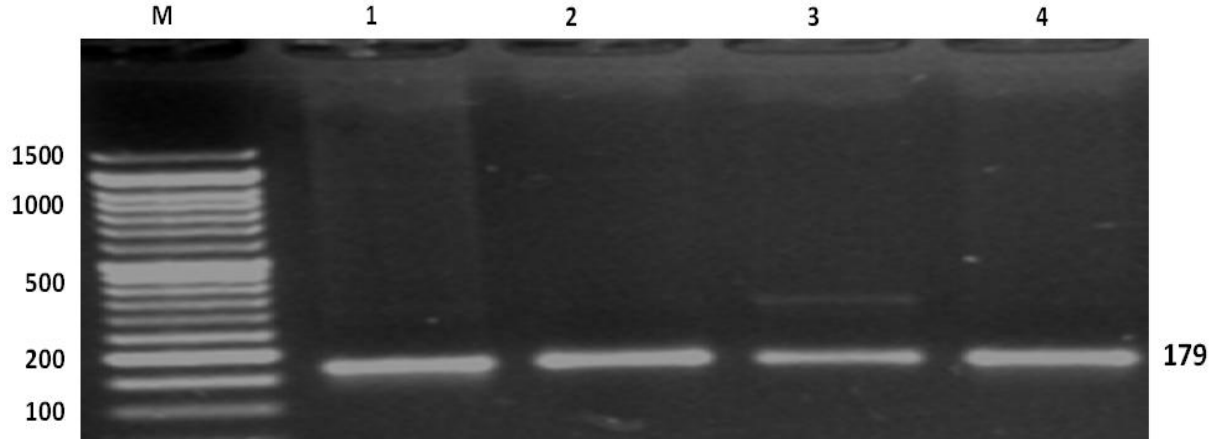


**Fig 1. Protocol of electrophoresis in gel agarose 2% which shows the amplified fragment size is 179 bp.**

**Results of PCR for *Alopias pelagicus***

Extracting of DNA by the method of phenol chloroform and according to the protocol of electrophoresis in gel agarose 2% showed the amplified fragment size was 1100 bp, so, based on sharp band and size of marker showed safe and specific replication therefore can be used for enzymatic digestion and comparing with other samples.

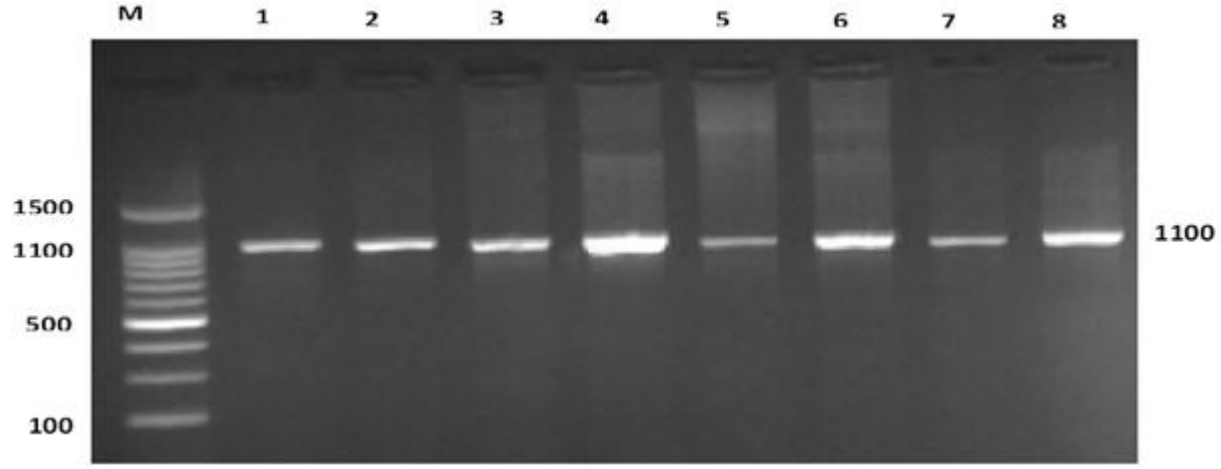


**Fig 2. protocol of electrophoresis in gel agarose 2% which shows the amplified fragment size is 1100 bp.**

## DISCUSSION AND CONCLUSION

Identifying species in marine products using the specific genes and application of polymerase chain reaction (PCR) has received much attention in recent years (Barbuto et al., 2010). Nowadays, standard methods such as molecular techniques related to the DNA which is a modern technique, strong, fast and efficient, attractive than other methods and is recommended for detection species in processed products (Wang et al., 2008). Mitochondrial DNA due to its advantages is preferable than genomic DNA for studies of diagnosis and detection the fraud in processed products. Since mitochondrial DNA has a large number of copies per cell, as well as, due to lack of genetic recombination is preferable for diagnosing interspecies and interspecies genetic differences (Holmes et al., 2009; Ward et al.,2008). Among the mitochondrial genes, there are areas that give useful phylogenic information to the researchers such as D-loop, cytochrome B, NADH dehydrogenase (ND1 to ND6) and 16s rDNA (Hebert et al., 2004).

Cytochrome genes are originates from copper and iron saturated chromoproteins, so far, a variety of which are known in mitochondria. Studies on gene cytochrome B show that this gene is used to identify the species of vertebrates; of course some areas which are highly protected can also be used to identify species of invertebrate (Hebert etal., 2003). The gene cytochrome B has high intergroup variations which is used to sequencing (Gharibkhani and pourkazemi, 1390).

The rate of nucleotide diversity and gene mutations in gene cytochrome b is more than 16s rDNA, as a result, the number of polymorphic sites in gene cytochrome b is high, but 16s rDNA which later evolves so the rate of mutation in this gene is low. Gene's cytochrome b and 16srDNA are widely used as genetic markers to identify species of fish because of

having strong power in detection species by DNA barcoding and its application in designing DNA oligonucleotide probes with low density in microarray technique (Hajibabaei et al., 2002).

In Taiwan, of cytochrome b and 16s rDNA used to identify the endangered animal remains. Although till now there is no report indicating detection of shark using ribosomal genes, but in the case of cheating aquatic food and meats using these genes have been many reports and all of these methods declare reliability of ribosomal gens method's.

Landi et al., 2014 emphasize the discriminatory power of COI barcodes and their application to cases requiring species level resolution starting from query sequences. Their results highlight the value of public reference libraries of reliability grade-annotated DNA barcodes, to identify species from different geographical origins. They concluded that the ability to assign species with high precision from DNA samples of disparate quality and origin has major utility in several fields, from fisheries and conservation programs to control of fish products authenticity.

Tanaka et al., 2013 also used mitochondrial cytochrome B, 16s rDNA and 12s rDNA for differentiation and relationship of the species in phylogeny.

Marc Kochzius et al., 2010 evaluated the applicability of the three mitochondrial genes 16S rRNA (16S), cytochrome *b* (cyt *b*), and cytochrome oxidase subunit I (COI) for the identification of 50 European marine fish species by combining techniques of "DNA barcoding" and microarrays. Based on their data, a DNA microarray containing 64 functional oligonucleotide probes for the identification of 30 out of the 50 fish species investigated was developed. It represents the next step towards an automated and easy-to-handle method to identify fish, ichthyoplankton, and fish products.

Luis Fernando et al., 2009 identified those shark species being exploited off northern Brazil, by using the 12S-16S molecular marker. The study emphasizes the value of molecular techniques for the identification of cryptic shark species, and the potential of the 12S-16S marker as a tool for phylogenetic inferences in a study of elasmobranchs.

Ghiasvand et al., 2011 also used DNA barcoding method to detect of two fish species using cytochrome oxidase area.

**Suggestions**

According to the results of the study and confirming 16srDNA mitochondrial genes in determining species and fraud Shark meat and considering the extended fraud in the markup of fish and the extent of fraud in the labeling of meat and shark fillets, therefore, using DNA barcoding method will facilitates the detecting fraud in foods and consumer confidence will provide about the labels on products. DNA barcoding can serve as a valuable tool and act as low in monitoring organizations for authentication species, protect consumer health, food safety control and management of the environmental protection (Costa and Carvalho, 2007).

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