SPECIES IDENTIFICATION IN FISH FILLET PRODUCTS USING DNA BARCODING

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ABSTRACT. Considering that seafood mislabeling has been widely reported throughout the world and that the authentication of food components is one of the key issues in food safety, quality and sustainability, the aim of this study was to use DNA barcoding to investigate the prevalence of mislabeling among fish fillet products from markets and supermarkets located in Apulia (SE Italy). The study reveals a high occurrence of species mislabeling in the fish fillet products. In particular, this study shows that the labels of only 32/200 fish fillet samples provided comprehensive information relating to the commercial designation, scientific name, geographical area, production method and whether previously frozen. The labeling of other samples was not compliant with European legislation. Indeed, the scientific name, which must be also indicated from the 1st January 2012, according to the Art. 68 of the EU Commission Implementing Regulation No. 404/2011, was missed in 157/168 samples, the geographical area was missed in 152/168, the commercial designation and the production method were reported in all samples. Furthermore, the results of the molecular investigations reveal a high occurrence of incorrect species declaration in fish fillet products. The commercial and/or scientific name declared failed to match the species identified in 164/200 (82 %) samples. This study is therefore a further evidence of the need for increased traceability and assessment of the authenticity of food products. Additionally, traceability may improve the management of hazards related to fish safety, as well as guaranteeing product authenticity, providing reliable information to customers, enhancing supply-side management and improving product quality and sustainability.

Keywords: species identification, mislabeling, fish fillet products, DNA barcoding, COI

1. INTRODUCTION

Both the food industry and consumers are focusing heavily on food safety, quality and sustainability. One of the key issues in food quality is the authentication of food components. Food authentication is a major concern not only for the prevention of commercial fraud, but also for the assessment of safety risks deriving from the undeclared introduction of any food ingredient that might be harmful to human health, such as potentially allergenic or toxic compounds (Rasmussen and Morrissey, 2008).

What's the reason that they can eat one but not another? If you know, it will be great you can add a note after this sentence. Therefore, food authentication associated with proper labeling statements could increase awareness among consumers regarding the composition of foods and reduce exposure to allergenic or toxic compounds.

The increasing demand for fishery products in general may lead to deliberate adulteration along the food chain, due to the substitution of high-quality species by lower quality counterparts. Although seafood labeling has to include the commercial designation, scientific name, geographical area, production method and state whether the product has been previously frozen, the commercial fish species available on the market cannot always be easily identified in processed and prepared fishery products, especially when morphological features have been removed.

Therefore, fish authentication may not only be significant from a sanitary point of view because of potentially dangerous toxic or allergenic substances, but also environmentally crucial where endangered species are threatened by Illegal, Unreported and Unregulated (IUU) fishing and retail around the world (Ward et al. 2008; Wong and Hanner, 2008; Holmes et al. 2009). In fact, fish authentication is important Illegal, Unreported and Unregulated (IUU) fishing, estimated to be worth between €10bn and €20bn per year worldwide (Agnew et al. 2009), poses a threat to the sustainable management of fisheries, not only through direct depletion of stocks, but also by undermining the competitiveness of legal fishing efforts. While a great deal of progress has been made in regulating commercial fisheries through Monitoring, Control and Surveillance (MCS) measures, and a range of technologies are utilized to identify infringements relating to individual vessels, gaps prevail in areas of catch identification (IUU fishing) and fraud throughout the food supply chain (product mislabeling).

Considering that seafood mislabeling has been widely reported throughout the world (Jacquet et al. 2008; Asensio et al. 2009; Cawthorn et al. 2011; Garcia-Vazquez et al. 2011; Hanner et al. 2011; Miller et al. 2012; Di Pinto et al. 2013) and that the authentication of food components is one of the key issues in food quality, the aim of this study was to investigate the prevalence of mislabeling among fish fillet products from markets and supermarkets located in Apulia (SE Italy) using DNA barcoding, the sequencing of the mitochondrial reference marker gene cytochrome oxidase subunit 1 (CO1) which been commonly used to identify fish species from fish fillet products (Using latest publications as citations to replace the old one such as Food Control 46 (2014) 441e445 and PLoS One. 2013 Nov 18;8(11):e79373).

MATERIAL AND METHODS

2.1 Sampling. A total of 200 samples of fillet fish products, from fish retail premises, fish markets, supermarkets and hypermarkets located in Apulia (SE Italy) were collected and stored at -20°C until processing.

2.2 Analysis of fish labeling. The samples were evaluated concerning the labeling requirements indicated by Council Regulation (EC) No. 1379/2013 (art. 35), such as commercial designation, scientific name, geographical area and production method and whether previously frozen.

2.3 DNA extraction and purification. Aliquots of each sample (10 mg) were subjected to DNA extraction and purification using the DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany) as reported by Handy et al. (2011). Positive extraction controls were obtained from each specimen of authentic species. A negative extraction control (no added tissue) was included to verify the purity of the extraction reagents. The DNA concentration and purity were established by evaluating the ratio A260nm/A280nm using a Beckman DU-640B Spectrophotometer.

2.4 Oligonucleotide primers. The oligonucleotide primers, FISHCO1LBC: 5'-TCAACYAAT CAYAAAGATATYGGCAC-3' and FISHCO1HBC: 5'-ACTTCYGGGTGRCCR AARAATCA-3' reported by Handy et al. (2011)and synthesized by PRIMM Srl (Milan, Italy), were used.

2.5 PCR assay. The PCR reactions were performed in a final volume of 25 µL, using 12.5 µL of HotStarTaq Master Mix 2X (QIAGEN, Hilden, Germany), containing 2.5 units of HotStarTaq DNA Polymerase, 1.5 mM of $MgCl_2$ and 200 µL of each dNTP. Then, 1 µM of each oligonucleotide primer and 1 µL of DNA were added. The amplification profile involved an initial denaturation step at 95 °C for 15 min, followed by 30 cycles at 94 °C for 30 s, 50 °C for 40 s and 72 °C for 60 s. The positive and negative controls for the extraction and PCR were included. The PCR reactions were processed in a Mastercycler Personal (Eppendorf, Milan, Italy). All reactions were performed in duplicate.

2.6 Detection of amplified products. PCR amplified products were analyzed by electrophoresis on 1.5% (w/v) agarose NA (Pharmacia, Uppsala, Sweden) gel in 1X TBE buffer containing 0.089 M Tris, 0.089 M boric acid, 0.002 M EDTA, pH 8.0 (USB, Cleveland, OH, USA), and stained with Green Gel Safe 10000X Nucleic Acid Stain (5 µl/100 ml) (Fisher Molecular Biology, USA). A Gene Ruler™ 100 bp DNA Ladder Plus (MBI Fermentas, Vilnius, Lithuania) was used as the molecular weight marker. Image acquisition was performed using UVITEC (Eppendorf).

2.7 PCR cleanup. In order to produce an amplicon free of extra dNTPs and excess primers that might interfere with the sequencing reaction, the PCR products were purified with the QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany).

2.8 Cycle sequencing reaction. Sequencing reactions using forward and reverse COI primers were performed by PRIMM Srl (Milan, Italy).

2.9 Sequence analysis. All amplified sequences were compared with sequences available in the Barcode of Life Data System (BOLD) and GenBank databases using Geneious Pro v5.4 (Drummond et al., 2011). The bidirectional sequences with 98% HQ (98% high-quality bases) were compared with sequences from the BOLD and GenBank databases. The genetic distances *(p-distance)* (Nei& Kumar, 2000) between the sequences obtained in this study and those in the BOLD and GenBank databases were generated by Geneious Pro v5.4 (Drummond et al. 2011).

2. RESULTS

The labels of only 32/200 fish fillet samples provided comprehensive information relating to the commercial designation, scientific name, geographical area, production method and whether previously frozen. The labeling of other samples was not compliant with European legislation. In particular, the scientific name, which must be also indicated from the 1st January 2012, according to the Art. 68 of the EU Commission Implementing Regulation No. 404/2011, was missed in 157/168 samples, the geographical area was missed in 152/168, the commercial designation and the production method were reported in all samples.

Considering the Italian Ministry of Agricultural, Food and Forestry Policies (MiPAAF) Decree dated 31 January 2008, which reports the Italian name for fish species of commercial interest, the results of our molecular investigations reveal a high occurrence of incorrect species declaration, in no less than 164/200 (82 %) fish fillet products.

Specifically, DNA of sufficient yield and quality was isolated and purified from all 200 samples. All of these extractions resulted in PCR products clearly visible as single bands of expected size on agarose gel. The positive and negative controls for the extraction and PCR gave expected results. Next, the sequences obtained from the samples and compared against the BOLD and GenBank databases gave successful matches, varying from 98% to 100% pairwise sequence identity. Post-sequencing data analysis revealed that 164/200 (82 %) fillet samples were not correctly labeled. In particular, 48/56 (85.7 %) fillets of grouper (*Epinephelus marginatus*) were mislabeled, with 4/48 being identified as belonging to *Epinephelus diacanthus*, 40/48 as *Lates niloticus* and 4/48 as *Pangasius hypophthalmus*. All European perch (*Perca fluviatilis*) (100 %) samples were mislabeled; specifically, 24/64 were identified as *Lates niloticus, 36*/64 as *Pangasius hypophthalmus* and 4/64 as *Pangasius sanitwongsei*. In addition, post-sequencing data analysis found 52/80 (65 %) purported swordfish (*Xiphias gladius*) to be incorrectly labeled, with 24/52 samples being from *Prionace glauca,* 20/52 samples from *Thunnus obesus* and 8/52 as *Isurus oxyrinchus*.

3. DISCUSSION

The current importance of the fish trade requires technological developments in food production, handling, processing and distribution by a global network of operators in order to guarantee the authenticity and the origin of fish and seafood products (Di Pinto et al., 2013; Gil, 2007; Rasmussen and Morrissey, 2008).

Given the increasing demand for fish and fish products, this study also highlights the need for the sustainable management of aquatic resources. In particular, the study found extensive use of species on the IUCN red list. Specifically, among the grouper fillets, in order to replace the grouper (*Epinephelus marginatus*), which is an overfished species (IUCN, 2014) and listed as Endangered (EN) due to a suspected population size reduction of well over 50% over the last three generations and considered to be facing a very high risk of extinction in the wild (IUCN, 2014), the study highlighted the use of *Epinephelus diacanthus*, a Near Threatened (NT) species according to IUCN (2014). Moreover, the results point out important environmental issues regarding the use of threatened species, such as *Isurus oxyrinchus*, *Pangasius sanitwongsei* and *Thunnus obesus*, to replace species of high commercial importance, such as European perch (*Perca fluviatilis*) and swordfish (*Xiphias gladius*). In fact, *Isurus oxyrinchus*, *Thunnus obesus* and *Pangasius sanitwongsei* are species listed as Vulnerable (VU), considered to be facing a high risk of extinction, and Critically Endangered (CR), due to a strongly estimated population decline (IUCN, 2014) respectively. Given that the use of Endangered (EN), Vulnerable (VU) or Near Threatened (NT) species may accelerate the extinction of such species, these results may be of great value for the conservation of fish species and imposes great challenges on animal conservation. A major threat for the sustainable management of these valuable resources is Illegal, Unreported and Unregulated (IUU) fishing. Current estimates suggest that globally up to 25% of fisheries catches fall within IUU practices, identifying it as the single largest threat to achieving sustainability. Both the FAO and the European Union have placed increasing emphasis on the use of trade measures to prevent IUU-sourced fish and fish products from entering international trade. One component of this increased regulation has required the inclusion of binomial species nomenclature on catch labels throughout the distribution chain (Helyar et al. 2014).

Fish product mislabeling is used either to launder IUU fish into the legitimate marketplace or simply to defraud the industry and consumer in order to obtain a higher sale price. Mislabeling is also of growing concern to certification schemes (‘eco-labeling’) that rely on credible species and origin identification to support consumer demand for ‘sustainable’ products. False labeling, even of legally-caught fish, destroys confidence in systems designed to reduce IUU fishing. It is important to highlight that, under the European Union legislative framework, two important and related regulations addressing IUU fishing and fisheries control have recently been implemented: (i) Reg. EC 1005/2008 establishing a Community system to prevent, deter and eliminate illegal, unreported and unregulated fishing, the so-called ‘IUU regulation’, introducing a catch certification scheme, and (ii) Reg. EC 1224/2009 which places emphasis on traceability in support of fisheries control and control along the supply chain.

This study represents a further evidence of the need for increased traceability, and assessment of the authenticity of seafood products. Fish mislabeling is widespread throughout the world. In fact, cases of fraudulent mislabeling of lesser-valued species (masquerading as more valuable species) are becoming more common as commercial quotas on certain high-value species become more restrictive in the world (Cawthorn et al. 2011).

Traceability is an essential component of any risk management strategy, and a key requirement for post-marketing surveillance. The fishing industry requires a full traceability system as part of a broader commercial agenda, using the developing standards as a means of promoting greater seafood quality and safety. Traceability provides the ability to identify and track a product or a component to its point of origin, which may be a particular lot or batch, production line and time frame, field, or supplier. It also provides a means of identifying the units for recall. This concept aims to create a link between the various steps in the supply chain from catching or harvesting to the retail stage, registering the names of the fishing vessel or aquaculture production unit, of the fish processors, distributors, and retailers until the moment the food is placed on the consumer's table.

The need to improve transparency and traceability in the fishing industry by implementing full traceability—every step in the supply chain having the correct procedures and protocols in place to trace receipt, processing, and shipping of seafood—is a crucial step in eliminating illegal fishing, seafood mislabeling and fraud (Thompson et al. 2005; Maralit et al. 2013). This study underlines that there is a great need for a comprehensive tracing system, which must address a wide range of problems – from illegal fishing practices on the high seas, to legal and common practices that are environmentally degrading in fish-farming, to areas both of conflict and consensus between consumers and suppliers of seafood. Adoption and implementation of an effective tracing system may control and reduce IUU fishing and fraud throughout the food supply chain (product mislabeling) (Ogden et al. 2008). Moreover, efficient monitoring may control fishing capacity and fishing effort at levels commensurate with the sustainability of fish stocks, protect the marine environment from destructive fishing practices, as well as from the marine debris and pollution associated with fishing activities, promote trade regimes that contribute to sustainable fisheries and promote responsible and sustainable aquaculture (Huxley-Jones et al. 2012).

A tracing system must also satisfy a wide array of needs, from small, local fisheries to national requirements for an industry that spans international markets. Additionally, tracing seafood must be economically feasible in order to gain acceptance. Traceability may improve the management of hazards related to fish safety, as well as guaranteeing product authenticity, providing reliable information to customers, enhancing supply-side management and improving product quality (Thompson et al. 2005; Maralit et al. 2013)..

Then, labels may provide information on the sustainability characteristics of a particular fish, both as an important service to consumers to support informed choices, and a means of motivating the fishing industry to adopt more environmentally-sound practices.

4. CONCLUSIONS

Given the increasing demand for transparency in the food industry and the enforcement of proper labeling have provided a driving force for the development of suitable analytical methodologies for species identification. Methods for identifying fish and fish products are needed to support legislation and routine audits within the industry, so as to act as enforcement tools for prosecuting illegal activities. Here, we propose DNA barcoding as a powerful technique for monitoring the trade of animal species, which helps in restricting the excessive use and illegal trade of such species in order to guarantee the conservation of threatened and vulnerable species. Indeed, the seafood industry currently lacks a simple, standardized, widespread method for tracing seafood products purchased along the supply chain. Specifically, DNA traceability could offer a more precise form of traceability for fish and byproducts. Therefore, Council Regulation (EC) No 1224/2009, establishing a Community control system for ensuring compliance with the rules of the common fisheries policy, provides the use of new technologies such as genetic analysis (Article 13), in order to improve compliance with the rules of the common fisheries policy in a cost-effective way.

A tracing system that combines genetic analysis with conventional methods of traceability may give companies and consumers the information they need to make sustainable seafood choices. A great effort should therefore be made to create a strong standardized monitoring program or strategy and to evoke consumer awareness on several aspects of accurate labeling information.

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Appendix A. Grouper fillet results.

Sample number	Commercial designation	Scientific name[a]	FAO code	Sequence from COI Identification	Sequence from BOLD Database	Similarity (%)	p-distance[b]	E-value	IUCN status[c]
1	Grouper	*Epinephelus marginatus*	FAO 37	*Epinephelus marginatus*	*E. marginatus* - DQ108016	98	0,005	0.0	**EN**
2	Grouper	*Epinephelus marginatus*	n.d	*Epinephelus diacanthus*	*E. diacanthus* - DQ108019	99	0,005	0.0	**NT**
3	Grouper	*Epinephelus marginatus*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,003	0.0	**LC**
4	Grouper	*Epinephelus marginatus*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,003	0.0	**LC**
5	Grouper	*Epinephelus marginatus*	FAO 37	*Epinephelus marginatus*	*E. marginatus* - DQ108016	98	0,006	0.0	**EN**
6	Grouper	*Epinephelus marginatus*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,009	0.0	**LC**
7	Grouper	*Epinephelus marginatus*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,007	0.0	**LC**
8	Grouper	*Epinephelus marginatus*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	98	0,008	0.0	**LC**
9	Grouper	*Epinephelus marginatus*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,007	0.0	**LC**
10	Grouper	*Epinephelus marginatus*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,005	0.0	**LC**
11	Grouper	*Epinephelus marginatus*	n.d	*Pangasius hypophtalmus*	*P. hypophtalmus*-DQ108017	99	0,008	0.0	**EN**
12	Grouper	*Epinephelus marginatus*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,004	0.0	**LC**
13	Grouper	*Epinephelus marginatus*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,004	0.0	**LC**
14	Grouper	*Epinephelus marginatus*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,002	0.0	**LC**
15	Grouper	*Epinephelus marginatus*	FAO 37	*Epinephelus marginatus*	*E. marginatus* - DQ108016	99	0,007	0.0	**EN**
16	Grouper	*Epinephelus marginatus*	n.d	*Epinephelus diacanthus*	*E. diacanthus* - DQ108019	99	0,005	0.0	**NT**
17	Grouper	*Epinephelus marginatus*	n.d	*Pangasius hypophtalmus*	*P. hypophtalmus*-DQ108017	99	0,008	0.0	**EN**
18	Grouper	*Epinephelus marginatus*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,004	0.0	**LC**
19	Grouper	*Epinephelus marginatus*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,004	0.0	**LC**
20	Grouper	*Epinephelus marginatus*	FAO 37	*Epinephelus marginatus*	*E. marginatus* - DQ108016	99	0,002	0.0	**EN**
21	Grouper	*Epinephelus marginatus*	n.d	*Epinephelus diacanthus*	*E. diacanthus* - DQ108019	99	0,005	0.0	**NT**
22	Grouper	*Epinephelus marginatus*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	98	0,009	0.0	**LC**
23	Grouper	*Epinephelus marginatus*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,009	0.0	**LC**
24	Grouper	*Epinephelus marginatus*	n.d	*Pangasius hypophtalmus*	*P. hypophtalmus*-DQ108017	99	0,009	0.0	**EN**
25	Grouper	*Epinephelus marginatus*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,007	0.0	**LC**
26	Grouper	*Epinephelus marginatus*	n.d	*Pangasius hypophtalmus*	*P. hypophtalmus*-DQ108017	99	0,008	0.0	**EN**
27	Grouper	*Epinephelus marginatus*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,008	0.0	**LC**
28	Grouper	*Epinephelus marginatus*	FAO 37	*Epinephelus marginatus*	*E. marginatus* - DQ108016	99	0,004	0.0	**EN**
29	Grouper	*Epinephelus marginatus*	FAO 37	*Epinephelus marginatus*	*E. marginatus* - DQ108016	99	0,004	0.0	**EN**
30	Grouper	*Epinephelus marginatus*	FAO 37	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,009	0.0	**LC**
31	Grouper	*Epinephelus marginatus*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,005	0.0	**LC**
32	Grouper	*Epinephelus marginatus*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	98	0,008	0.0	**LC**
33	Grouper	*Epinephelus marginatus*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	98	0,005	0.0	**LC**
34	Grouper	*Epinephelus marginatus*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,003	0.0	**LC**
35	Grouper	*Epinephelus marginatus*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	100	0,007	0.0	**LC**
36	Grouper	*Epinephelus marginatus*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,005	0.0	**LC**
37	Grouper	*Epinephelus marginatus*	n.d	*Epinephelus diacanthus*	*E. diacanthus* - DQ108019	99	0,009	0.0	**NT**
38	Grouper	*Epinephelus marginatus*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,005	0.0	**LC**
39	Grouper	*Epinephelus marginatus*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,009	0.0	**LC**
40	Grouper	*Epinephelus marginatus*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,007	0.0	**LC**
41	Grouper	*Epinephelus marginatus*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,006	0.0	**LC**
42	Grouper	*Epinephelus marginatus*	FAO 37	*Epinephelus marginatus*	*E. marginatus* - DQ108016	99	0,004	0.0	**EN**
43	Grouper	*Epinephelus marginatus*	FAO 37	*Epinephelus marginatus*	*E. marginatus* - DQ108016	99	0,004	0.0	**EN**
44	Grouper	*Epinephelus marginatus*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,009	0.0	**LC**
45	Grouper	*Epinephelus marginatus*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,003	0.0	**LC**
46	Grouper	*Epinephelus marginatus*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,009	0.0	**LC**
47	Grouper	*Epinephelus marginatus*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,005	0.0	**LC**
48	Grouper	*Epinephelus marginatus*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,002	0.0	**LC**
49	Grouper	*Epinephelus marginatus*	FAO 37	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,007	0.0	**LC**
50	Grouper	*Epinephelus marginatus*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,005	0.0	**LC**
51	Grouper	*Epinephelus marginatus*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	98	0,005	0.0	**LC**
52	Grouper	*Epinephelus marginatus*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,005	0.0	**LC**
53	Grouper	*Epinephelus marginatus*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,002	0.0	**LC**
54	Grouper	*Epinephelus marginatus*	FAO 37	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,004	0.0	**LC**
55	Grouper	*Epinephelus marginatus*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,007	0.0	**LC**
56	Grouper	*Epinephelus marginatus*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,002	0.0	**LC**

Appendix B. Perch fillet results.

Sample number	Commercial designation	Scientific name[a]	FAO code	Sequence from COI Identification	Sequence from BOLD Database	Similarity (%)	p-distance[b]	E-value	IUCN status[c]
1	Perch	*Perca fluviatilis*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	98	0,005	0.0	**LC**
2	Perch	*Perca fluviatilis*	n.d	*Pangasius hypophtalmus*	*P. hypophtalmus* - DQ108017	99	0,007	0.0	**EN**
3	Perch	*Perca fluviatilis*	n.d	*Pangasius hypophtalmus*	*P. hypophtalmus* - DQ108017	99	0,009	0.0	**EN**
4	Perch	*Perca fluviatilis*	n.d	*Pangasius hypophtalmus*	*P. hypophtalmus* - DQ108017	99	0,009	0.0	**EN**
5	Perch	*Perca fluviatilis*	FAO 51	*Pangasius hypophtalmus*	*P. hypophtalmus* - DQ108017	98	0,009	0.0	**EN**
6	Perch	*Perca fluviatilis*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,008	0.0	**LC**
7	Perch	*Perca fluviatilis*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,008	0.0	**LC**
8	Perch	*Perca fluviatilis*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	96	0,004	0.0	**LC**
9	Perch	*Perca fluviatilis*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,004	0.0	**LC**
10	Perch	*Perca fluviatilis*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,005	0.0	**LC**
11	Perch	*Perca fluviatilis*	FAO 51	*Pangasius hypophtalmus*	*P. hypophtalmus* - DQ108017	99	0,005	0.0	**EN**
12	Perch	*Perca fluviatilis*	FAO 51	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,008	0.0	**LC**
13	Perch	*Perca fluviatilis*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,005	0.0	**LC**
14	Perch	*Perca fluviatilis*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,003	0.0	**LC**
15	Perch	*Perca fluviatilis*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,007	0.0	**LC**
16	Perch	*Perca fluviatilis*	n.d	*Pangasius hypophtalmus*	*P. hypophtalmus* - DQ108017	99	0,005	0.0	**EN**
17	Perch	*Perca fluviatilis*	n.d	*Pangasius hypophtalmus*	*P. hypophtalmus* - DQ108017	99	0,009	0.0	**EN**
18	Perch	*Perca fluviatilis*	FAO 51	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,005	0.0	**LC**
19	Perch	*Perca fluviatilis*	FAO 51	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,009	0.0	**LC**
20	Perch	*Perca fluviatilis*	FAO 51	*Pangasius hypophtalmus*	*P. hypophtalmus* - DQ108017	99	0,007	0.0	**EN**
21	Perch	*Perca fluviatilis*	n.d	*Pangasius hypophtalmus*	*P. hypophtalmus* - DQ108017	99	0,005	0.0	**EN**
22	Perch	*Perca fluviatilis*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	98	0,004	0.0	**LC**
23	Perch	*Perca fluviatilis*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,004	0.0	**LC**
24	Perch	*Perca fluviatilis*	n.d	*Pangasius hypophtalmus*	*P. hypophtalmus* - DQ108017	99	0,007	0.0	**EN**
25	Perch	*Perca fluviatilis*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,003	0.0	**LC**
26	Perch	*Perca fluviatilis*	n.d	*Pangasius hypophtalmus*	*P. hypophtalmus* - DQ108017	99	0,009	0.0	**EN**
27	Perch	*Perca fluviatilis*	n.d	*Pangasius sanitwongsei*	*P. sanitwongsei* - DQ108015	99	0,005	0.0	**CR**
28	Perch	*Perca fluviatilis*	n.d	*Pangasius hypophtalmus*	*P. hypophtalmus* - DQ108017	99	0,002	0.0	**EN**
29	Perch	*Perca fluviatilis*	n.d	*Pangasius hypophtalmus*	*P. hypophtalmus* - DQ108017	99	0,009	0.0	**EN**
30	Perch	*Perca fluviatilis*	n.d	*Pangasius hypophtalmus*	*P. hypophtalmus* - DQ108017	99	0,009	0.0	**EN**
31	Perch	*Perca fluviatilis*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,005	0.0	**LC**
32	Perch	*Perca fluviatilis*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	98	0,008	0.0	**LC**
33	Perch	*Perca fluviatilis*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	98	0,002	0.0	**LC**
34	Perch	*Perca fluviatilis*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,004	0.0	**LC**
35	Perch	*Perca fluviatilis*	FAO 51	*Lates niloticus*	*L. niloticus* - DQ108018	100	0,007	0.0	**LC**
36	Perch	*Perca fluviatilis*	n.d	*Pangasius sanitwongsei*	*P. sanitwongsei* - DQ108015	99	0,002	0.0	**CR**
37	Perch	*Perca fluviatilis*	n.d	*Pangasius hypophtalmus*	*P. hypophtalmus* - DQ108017	99	0,005	0.0	**EN**
38	Perch	*Perca fluviatilis*	n.d	*Pangasius hypophtalmus*	*P. hypophtalmus* - DQ108017	99	0,005	0.0	**EN**
39	Perch	*Perca fluviatilis*	n.d	*Pangasius hypophtalmus*	*P. hypophtalmus* - DQ108017	99	0,003	0.0	**EN**
40	Perch	*Perca fluviatilis*	n.d	*Pangasius sanitwongsei*	*P. sanitwongsei* - DQ108015	99	0,003	0.0	**CR**
41	Perch	*Perca fluviatilis*	n.d	*Pangasius hypophtalmus*	*P. hypophtalmus* - DQ108017	99	0,006	0.0	**EN**
42	Perch	*Perca fluviatilis*	n.d	*Pangasius hypophtalmus*	*P. hypophtalmus* - DQ108017	99	0,004	0.0	**EN**
43	Perch	*Perca fluviatilis*	FAO 51	*Pangasius hypophtalmus*	*P. hypophtalmus* - DQ108017	99	0,007	0.0	**EN**
44	Perch	*Perca fluviatilis*	FAO 51	*Pangasius hypophtalmus*	*P. hypophtalmus* - DQ108017	99	0,005	0.0	**EN**
45	Perch	*Perca fluviatilis*	n.d	*Pangasius hypophtalmus*	*P. hypophtalmus* - DQ108017	99	0,005	0.0	**EN**
46	Perch	*Perca fluviatilis*	n.d	*Pangasius hypophtalmus*	*P. hypophtalmus* - DQ108017	99	0,005	0.0	**EN**
47	Perch	*Perca fluviatilis*	n.d	*Pangasius hypophtalmus*	*P. hypophtalmus* - DQ108017	99	0,008	0.0	**EN**
48	Perch	*Perca fluviatilis*	n.d	*Pangasius hypophtalmus*	*P. hypophtalmus* - DQ108017	99	0,004	0.0	**EN**
49	Perch	*Perca fluviatilis*	n.d	*Pangasius hypophtalmus*	*P. hypophtalmus* - DQ108017	99	0,004	0.0	**EN**
50	Perch	*Perca fluviatilis*	n.d	*Pangasius hypophtalmus*	*P. hypophtalmus* - DQ108017	98	0,002	0.0	**EN**
51	Perch	*Perca fluviatilis*	n.d	*Pangasius hypophtalmus*	*P. hypophtalmus* - DQ108017	99	0,005	0.0	**EN**
52	Perch	*Perca fluviatilis*	FAO 51	*Pangasius hypophtalmus*	*P. hypophtalmus* - DQ108017	99	0,007	0.0	**EN**
53	Perch	*Perca fluviatilis*	n.d	*Pangasius hypophtalmus*	*P. hypophtalmus* - DQ108017	99	0,007	0.0	**EN**
54	Perch	*Perca fluviatilis*	n.d	*Pangasius hypophtalmus*	*P. hypophtalmus* - DQ108017	99	0,009	0.0	**EN**
55	Perch	*Perca fluviatilis*	n.d	*Pangasius hypophtalmus*	*P. hypophtalmus* - DQ108017	99	0,009	0.0	**EN**
56	Perch	*Perca fluviatilis*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	100	0,008	0.0	**LC**
57	Perch	*Perca fluviatilis*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	98	0,008	0.0	**LC**
58	Perch	*Perca fluviatilis*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,004	0.0	**LC**
59	Perch	*Perca fluviatilis*	n.d	*Lates niloticus*	*L. niloticus* - DQ108018	99	0,009	0.0	**LC**
60	Perch	*Perca fluviatilis*	FAO 51	*Pangasius sanitwongsei*	*P. sanitwongsei* - DQ108015	99	0,009	0.0	**CR**
61	Perch	*Perca fluviatilis*	FAO 51	*Pangasius hypophtalmus*	*P. hypophtalmus* - DQ108017	99	0,005	0.0	**EN**
62	Perch	*Perca fluviatilis*	n.d	*Pangasius hypophtalmus*	*P. hypophtalmus* - DQ108017	99	0,009	0.0	**EN**
63	Perch	*Perca fluviatilis*	n.d	*Pangasius hypophtalmus*	*P. hypophtalmus* - DQ108017	99	0,005	0.0	**EN**
64	Perch	*Perca fluviatilis*	n.d	*Pangasius hypophtalmus*	*P. hypophtalmus* - DQ108017	99	0,005	0.0	**EN**

Appendix C. Swordfish fillet results.

Sample number	Commercial designation	Scientific name*	FAO code	Sequence from COI Identification	Sequence from BOLD Database	Similarity (%)	p-distance*	E-value	IUCN status*
1	Swordfish	*Xiphias glaudius*	n.d	*Prionace glauca*	*P. glauca* - HM007788	98	0,009	0.0	**NT**
2	Swordfish	*Xiphias glaudius*	FAO 57	*Xiphias gladius*	*X. gladius* - FJ605763	99	0,005	0.0	**LC**
3	Swordfish	*Xiphias glaudius*	FAO 57	*Xiphias gladius*	*X. gladius* - FJ605763	99	0,009	0.0	**LC**
4	Swordfish	*Xiphias glaudius*	n.d	*Prionace glauca*	*P. glauca* - HM007788	99	0,007	0.0	**NT**
5	Swordfish	*Xiphias glaudius*	n.d	*Prionace glauca*	*P. glauca* - HM007788	98	0,006	0.0	**NT**
6	Swordfish	*Xiphias glaudius*	n.d	*Thunnus obesus*	*T. obesus* - HQ024921	99	0,004	0.0	**VU**
7	Swordfish	*Xiphias glaudius*	n.d	*Thunnus obesus*	*T. obesus* - HQ024921	99	0,004	0.0	**VU**
8	Swordfish	*Xiphias glaudius*	n.d	*Xiphias gladius*	*X. gladius* - FJ605763	98	0,009	0.0	**LC**
9	Swordfish	*Xiphias glaudius*	n.d	*Thunnus obesus*	*T. obesus* - HQ024921	99	0,003	0.0	**VU**
10	Swordfish	*Xiphias glaudius*	n.d	*Thunnus obesus*	*T. obesus* - HQ024921	99	0,009	0.0	**VU**
11	Swordfish	*Xiphias glaudius*	n.d	*Prionace glauca*	*P. glauca* - HM007788	99	0,005	0.0	**NT**
12	Swordfish	*Xiphias glaudius*	FAO 57	*Xiphias gladius*	*X. gladius* - FJ605763	99	0,002	0.0	**LC**
13	Swordfish	*Xiphias glaudius*	FAO 57	*Xiphias gladius*	*X. gladius* - FJ605763	99	0,009	0.0	**LC**
14	Swordfish	*Xiphias glaudius*	FAO 57	*Xiphias gladius*	*X. gladius* - FJ605763	99	0,009	0.0	**LC**
15	Swordfish	*Xiphias glaudius*	n.d	*Prionace glauca*	*P. glauca* - HM007788	99	0,004	0.0	**NT**
16	Swordfish	*Xiphias glaudius*	n.d	*Prionace glauca*	*P. glauca* - HM007788	99	0,009	0.0	**NT**
17	Swordfish	*Xiphias glaudius*	n.d	*Prionace glauca*	*P. glauca* - HM007788	99	0,002	0.0	**NT**
18	Swordfish	*Xiphias glaudius*	n.d	*Thunnus obesus*	*T. obesus* - HQ024921	99	0,004	0.0	**VU**
19	Swordfish	*Xiphias glaudius*	n.d	*Thunnus obesus*	*T. obesus* - HQ024921	99	0,009	0.0	**VU**
20	Swordfish	*Xiphias glaudius*	FAO 57	*Xiphias gladius*	*X. gladius* - FJ605763	99	0,002	0.0	**LC**
21	Swordfish	*Xiphias glaudius*	FAO 57	*Xiphias gladius*	*X. gladius* - FJ605763	99	0,005	0.0	**LC**
22	Swordfish	*Xiphias glaudius*	FAO 57	*Xiphias gladius*	*X. gladius* - FJ605763	98	0,005	0.0	**LC**
23	Swordfish	*Xiphias glaudius*	n.d	*Isurus oxyrinchus*	*I. oxyrinchus* - DQ885060	99	0,003	0.0	**VU**
24	Swordfish	*Xiphias glaudius*	n.d	*Thunnus obesus*	*T. obesus* - HQ024921	99	0,003	0.0	**VU**
25	Swordfish	*Xiphias glaudius*	n.d	*Prionace glauca*	*P. glauca* - HM007788	99	0,006	0.0	**NT**
26	Swordfish	*Xiphias glaudius*	n.d	*Prionace glauca*	*P. glauca* - HM007788	99	0,008	0.0	**NT**
27	Swordfish	*Xiphias glaudius*	n.d	*Xiphias gladius*	*X. gladius* - FJ605763	99	0,007	0.0	**LC**
28	Swordfish	*Xiphias glaudius*	n.d	*Prionace glauca*	*P. glauca* - HM007788	99	0,009	0.0	**NT**
29	Swordfish	*Xiphias glaudius*	n.d	*Prionace glauca*	*P. glauca* - HM007788	99	0,007	0.0	**NT**
30	Swordfish	*Xiphias glaudius*	n.d	*Thunnus obesus*	*T. obesus* - HQ024921	99	0,005	0.0	**VU**
31	Swordfish	*Xiphias glaudius*	FAO 57	*Xiphias gladius*	*X. gladius* - FJ605763	99	0,008	0.0	**LC**
32	Swordfish	*Xiphias glaudius*	n.d	*Thunnus obesus*	*T. obesus* - HQ024921	98	0,004	0.0	**VU**
33	Swordfish	*Xiphias glaudius*	FAO 27	*Xiphias gladius*	*X. gladius* - FJ605763	98	0,004	0.0	**LC**
34	Swordfish	*Xiphias glaudius*	FAO 27	*Xiphias gladius*	*X. gladius* - FJ605763	99	0,002	0.0	**LC**
35	Swordfish	*Xiphias glaudius*	FAO 27	*Xiphias gladius*	*X. gladius* - FJ605763	100	0,005	0.0	**LC**
36	Swordfish	*Xiphias glaudius*	n.d	*Prionace glauca*	*P. glauca* - HM007788	99	0,008	0.0	**NT**
37	Swordfish	*Xiphias glaudius*	FAO 57	*Xiphias gladius*	*X. gladius* - FJ605763	99	0,009	0.0	**LC**
38	Swordfish	*Xiphias glaudius*	FAO 57	*Xiphias gladius*	*X. gladius* - FJ605763	99	0,009	0.0	**LC**
39	Swordfish	*Xiphias glaudius*	n.d	*Xiphias gladius*	*X. gladius* - FJ605763	99	0,009	0.0	**LC**
40	Swordfish	*Xiphias glaudius*	n.d	*Prionace glauca*	*P. glauca* - HM007788	99	0,008	0.0	**NT**
41	Swordfish	*Xiphias glaudius*	n.d	*Thunnus obesus*	*T. obesus* - HQ024921	99	0,007	0.0	**VU**
42	Swordfish	*Xiphias glaudius*	FAO 27	*Thunnus obesus*	*T. obesus* - HQ024921	99	0,004	0.0	**VU**
43	Swordfish	*Xiphias glaudius*	n.d	*Isurus oxyrinchus*	*I. oxyrinchus* - DQ885060	99	0,009	0.0	**VU**
44	Swordfish	*Xiphias glaudius*	n.d	*Prionace glauca*	*P. glauca* - HM007788	99	0,007	0.0	**NT**
45	Swordfish	*Xiphias glaudius*	n.d	*Prionace glauca*	*P. glauca* - HM007788	99	0,005	0.0	**NT**
46	Swordfish	*Xiphias glaudius*	n.d	*Prionace glauca*	*P. glauca* - HM007788	99	0,009	0.0	**NT**
47	Swordfish	*Xiphias glaudius*	n.d	*Isurus oxyrinchus*	*I. oxyrinchus* - DQ885060	99	0,005	0.0	**VU**
48	Swordfish	*Xiphias glaudius*	n.d	*Thunnus obesus*	*T. obesus* - HQ024921	99	0,005	0.0	**VU**
49	Swordfish	*Xiphias glaudius*	n.d	*Prionace glauca*	*P. glauca* - HM007788	99	0,007	0.0	**NT**
50	Swordfish	*Xiphias glaudius*	n.d	*Thunnus obesus*	*T. obesus* - HQ024921	99	0,005	0.0	**VU**
51	Swordfish	*Xiphias glaudius*	FAO 57	*Xiphias gladius*	*X. gladius* - FJ605763	98	0,009	0.0	**LC**
52	Swordfish	*Xiphias glaudius*	FAO 57	*Xiphias gladius*	*X. gladius* - FJ605763	99	0,005	0.0	**LC**
53	Swordfish	*Xiphias glaudius*	n.d	*Isurus oxyrinchus*	*I. oxyrinchus* - DQ885060	99	0,009	0.0	**VU**
54	Swordfish	*Xiphias glaudius*	n.d	*Thunnus obesus*	*T. obesus* - HQ024921	99	0,007	0.0	**VU**
55	Swordfish	*Xiphias glaudius*	n.d	*Thunnus obesus*	*T. obesus* - HQ024921	99	0,005	0.0	**VU**
56	Swordfish	*Xiphias glaudius*	n.d	*Thunnus obesus*	*T. obesus* - HQ024921	99	0,004	0.0	**VU**
57	Swordfish	*Xiphias glaudius*	n.d	*Prionace glauca*	*P. glauca* - HM007788	99	0,004	0.0	**NT**
58	Swordfish	*Xiphias glaudius*	FAO 27	*Xiphias gladius*	*X. gladius* - FJ605763	99	0,007	0.0	**LC**
59	Swordfish	*Xiphias glaudius*	n.d	*Xiphias gladius*	*X. gladius* - FJ605763	99	0,003	0.0	**LC**
60	Swordfish	*Xiphias glaudius*	n.d	*Prionace glauca*	*P. glauca* - HM007788	99	0,009	0.0	**NT**
61	Swordfish	*Xiphias glaudius*	n.d	*Prionace glauca*	*P. glauca* - HM007788	98	0,005	0.0	**NT**
62	Swordfish	*Xiphias glaudius*	n.d	*Prionace glauca*	*P. glauca* - HM007788	99	0,002	0.0	**NT**
63	Swordfish	*Xiphias glaudius*	n.d	*Isurus oxyrinchus*	*I. oxyrinchus* - DQ885060	99	0,009	0.0	**VU**
64	Swordfish	*Xiphias glaudius*	n.d	*Isurus oxyrinchus*	*I. oxyrinchus* - DQ885060	99	0,009	0.0	**VU**
65	Swordfish	*Xiphias glaudius*	FAO 57	*Xiphias gladius*	*X. gladius* - FJ605763	99	0,003	0.0	**LC**
66	Swordfish	*Xiphias glaudius*	FAO 57	*Xiphias gladius*	*X. gladius* - FJ605763	99	0,008	0.0	**LC**
67	Swordfish	*Xiphias glaudius*	n.d	*Prionace glauca*	*P. glauca* - HM007788	99	0,002	0.0	**NT**
68	Swordfish	*Xiphias glaudius*	FAO 57	*Xiphias gladius*	*X. gladius* - FJ605763	99	0,004	0.0	**LC**
69	Swordfish	*Xiphias glaudius*	n.d	*Prionace glauca*	*P. glauca* - HM007788	99	0,006	0.0	**NT**
70	Swordfish	*Xiphias glaudius*	FAO 57	*Xiphias gladius*	*X. gladius* - FJ605763	99	0,002	0.0	**LC**
71	Swordfish	*Xiphias glaudius*	n.d	*Isurus oxyrinchus*	*I. oxyrinchus* - DQ885060	99	0,005	0.0	**VU**
72	Swordfish	*Xiphias glaudius*	n.d	*Thunnus obesus*	*T. obesus* - HQ024921	100	0,009	0.0	**VU**
73	Swordfish	*Xiphias glaudius*	n.d	*Thunnus obesus*	*T. obesus* - HQ024921	99	0,005	0.0	**VU**
74	Swordfish	*Xiphias glaudius*	n.d	*Thunnus obesus*	*T. obesus* - HQ024921	99	0,009	0.0	**VU**
75	Swordfish	*Xiphias glaudius*	n.d	*Thunnus obesus*	*T. obesus* - HQ024921	99	0,007	0.0	**VU**
76	Swordfish	*Xiphias glaudius*	FAO 27	*Xiphias gladius*	*X. gladius* - FJ605763	99	0,003	0.0	**LC**
77	Swordfish	*Xiphias glaudius*	FAO 27	*Xiphias gladius*	*X. gladius* - FJ605763	99	0,004	0.0	**LC**
78	Swordfish	*Xiphias glaudius*	n.d	*Prionace glauca*	*P. glauca* - HM007788	99	0,004	0.0	**NT**
79	Swordfish	*Xiphias glaudius*	n.d	*Isurus oxyrinchus*	*I. oxyrinchus* - DQ885060	98	0,009	0.0	**VU**
80	Swordfish	*Xiphias glaudius*	FAO 57	*Xiphias gladius*	*X. gladius* - FJ605763	99	0,003	0.0	**LC**