

RESEARCH ARTICLE

(Open Access)

DNA barcoding for species Identification in prepared fishery productsANNA MOTTOLA^{1*}, PATRIZIA MARCHETTI¹, MARILISA BOTTARO¹, ANGELA DI PINTO¹¹Department of Veterinary Medicine – University of Bari Aldo Moro – Prov. le Casamassima, km 3 - 70010 Valenzano (Bari) – ITALY

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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 quality, the aim of this study was to use DNA barcoding to investigate the prevalence of mislabeling among fresh prepared fishery products from markets and supermarkets located in Apulia (SE Italy). The study reveals a high occurrence of species mislabeling (42%) in the prepared fillet products, further evidence of the need for increased traceability and assessment of the authenticity of food products. 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. There is therefore a great need to develop fast and reliable methods to identify meat species and to quantify their levels in seafood products, in order to ensure product quality and thus to protect consumers. The study provides further evidence that molecular investigations based on DNA barcoding may be one of the most powerful tools for the assessment of species identity, food traceability, safety and fraud.

Key words: prepared fishery products, species identification, DNA barcoding**1. Introduction**

Seafood is a global commodity and is one of the most commonly traded food items in the world, but it is also object several illegal activities that misrepresents the fish you purchase, including mislabeling or substituting one species for another [8]. For this reason, both the legislators and consumers associations are focusing on food safety and quality.

Species identification is a major concern due to the increased awareness among consumers regarding the composition of foods and the need to verify labeling statements. 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. Prepared fishery products, i.e. unprocessed fishery products that have undergone an operation affecting their anatomical wholeness, are vulnerable to fraudulent labeling due to the economic profits arising from selling cheaper species as high-value ones [6].

Although the European Union law EC No. 2065/2001, art 8, requests appropriate species traceability and accurate labeling. The law says that 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 transformed fishery products. Then, the identification of species is often difficult and many scientist are looking for innovative, rapid and economical methods to identifying prepared fishery products. Innovations in the field of molecular biology have pointed out that the genetic profile of a species allows the recognition of processed products. On the basis of this discovery, the DNA barcoding was used to identify specific groups of fish species, such as tuna, flatfish anchovy and sharks [8]. Considering the importance of fish trade in the globalization era, technological developments in food production, handling, processing and distribution by a global network of operators make it necessary to ensure the authenticity and the origin of fish and seafood products [10].

Thus, the aims of the study was to use DNA barcoding, based on the universal primer region of cytochrome oxidase I (COI), in identifying “seafood substitution” in the marketplace located in Apulia region (Italy), where lower value species are mislabeled and substituted with more expensive species or with species potentially hazardous to human health.

2. Material and Methods*2.1. Sampling*

A total of 90 samples of prepared fillet fish products, including 25 grouper (*Epinephelus*

marginatus), 25 European perch (*Perca fluviatilis*) and 40 swordfish (*Xiphias gladius*) from various fish markets located in Apulia region (Italy) were collected and stored at -20°C until processing. According to Reg.(EC) N. 1224/2009, consumer labeling requirements (commercial designation, scientific name, geographical area and production method, whether previously frozen) were considered to evaluate the correct information for consumers.

2.2. 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). Briefly, aliquots (10 mg) of each fish sample were added to 50 µL ATL lysis buffer and 5.56 µL of Proteinase K (20 mg/mL) and incubated at 56 °C for 2 h. After adding 55.6 µL AL Buffer and 55.6 µL ethanol, the resulting mixture was applied to the DNeasy Mini spin column. The DNA, adsorbed onto the QIAamp silica-gel membrane during subsequent centrifugation steps at 6000 g for 1 min, was washed using 140 µL AW1 and 140 µL AW2 washing buffers. Finally, the DNA was eluted with 50 µL AE Elution Buffer (QIAGEN, Hilden, Germany). 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.

2.3. Oligonucleotide primers

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

2.4. 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 MgCl₂ 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.

2.5. 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 ethidium bromide. 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).

2.6. 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).

2.7. Cycle sequencing reaction

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

2.8. 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 [7]. The bidirectional sequences with 98% HQ (98% high-quality bases) were compared with sequences from the BOLD and GenBank databases.

2.9. Analysis of genetic distances.

The genetic distances (*p*-distance) between the sequences obtained in this study and those in the BOLD and GenBank databases were generated by Geneious Pro v5.4 [7].

3. Results and Discussion

Only 22/90 fish fillet samples provided total information required in according to the art. 58 of the Council Regulation (EC) n. 1224/2009. The labeling of the other samples was not compliant with European legislation. In particular, the geographical area was missed in 32/68 samples. Relatively the name printed on the label, the results of the molecular investigations

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reveals a high occurrence of mislabeling in prepared fillet products, in fact the commercial and/or scientific name declared failed to match the species identified in 38/90 (42%) (Table 1, Table 2 and Table 3.)

Table 1. Grouper fillet results

Sample number	Commercial designation	Scientific* name	FAO code	Sequence from COI Identification	Sequence from BOLD Database	Similarity	**p-distance	E-value	Mislabelling
1	Grouper	<i>Epinephelus marginatus</i>	FAO 37	<i>Epinephelus marginatus</i>	<i>E. marginatus</i> -DQ108016	98	0,005	0.0	NO
2	Grouper	<i>Epinephelus marginatus</i>	FAO 37	<i>Epinephelus marginatus</i>	<i>E. marginatus</i> -DQ108016	99	0,003	0.0	NO
3	Grouper	n.d.	n.d.	<i>Epinephelus marginatus</i>	<i>E. marginatus</i> -DQ108016	99	0,003	0.0	NO
4	Grouper	<i>Epinephelus marginatus</i>	FAO 37	<i>Epinephelus diacanthus</i>	<i>E. diacanthus</i> -DQ108019	99	0,006	0.0	YES
5	Grouper	<i>Epinephelus marginatus</i>	FAO 37	<i>Epinephelus marginatus</i>	<i>E. marginatus</i> -DQ108016	98	0,005	0.0	NO
6	Grouper	n.d.	n.d.	<i>Epinephelus diacanthus</i>	<i>E. diacanthus</i> -DQ108019	99	0,005	0.0	YES
7	Grouper	n.d.	n.d.	<i>Epinephelus marginatus</i>	<i>E. marginatus</i> -DQ108016	98	0,007	0.0	NO
8	Grouper	n.d.	n.d.	<i>Lates niloticus</i>	<i>L. niloticus</i> -DQ108018	99	0,003	0.0	YES
9	Grouper	n.d.	n.d.	<i>Epinephelus marginatus</i>	<i>E. marginatus</i> -DQ108016	99	0,007	0.0	NO
10	Grouper	<i>Epinephelus marginatus</i>	n.d.	<i>Epinephelus diacanthus</i>	<i>E. diacanthus</i> –DQ108019	98	0,005	0.0	YES
11	Grouper	<i>Epinephelus marginatus</i>	FAO 37	<i>Epinephelus marginatus</i>	<i>E. marginatus</i> -DQ108016	100	0,005	0.0	NO
12	Grouper	<i>Epinephelus marginatus</i>	n.d.	<i>Lates niloticus</i>	<i>L. niloticus</i> -DQ108018	99	0,006	0.0	YES
13	Grouper	n.d.	n.d.	<i>Epinephelus marginatus</i>	<i>E. marginatus</i> -DQ108016	99	0,007	0.0	NO
14	Grouper	n.d.	FAO 37	<i>Epinephelus marginatus</i>	<i>E. marginatus</i> -DQ108016	99	0,005	0.0	NO
15	Grouper	n.d.	n.d.	<i>Epinephelus marginatus</i>	<i>E. marginatus</i> -DQ108016	99	0,003	0.0	NO
16	Grouper	<i>Epinephelus marginatus</i>	FAO 37	<i>Epinephelus marginatus</i>	<i>E. marginatus</i> -DQ108016	99	0,002	0.0	NO
17	Grouper	<i>Epinephelus marginatus</i>	n.d.	<i>Epinephelus marginatus</i>	<i>E. marginatus</i> -DQ108016	98	0,005	0.0	NO
18	Grouper	<i>Epinephelus marginatus</i>	FAO 37	<i>Epinephelus marginatus</i>	<i>E. marginatus</i> -DQ108016	98	0,002	0.0	NO
19	Grouper	n.d.	FAO 37	<i>Epinephelus marginatus</i>	<i>E. marginatus</i> -DQ108016	98	0,007	0.0	NO
20	Grouper	<i>Epinephelus marginatus</i>	FAO 37	<i>Epinephelus marginatus</i>	<i>E. marginatus</i> -DQ108016	99	0,006	0.0	NO
21	Grouper	n.d.	n.d.	<i>Epinephelus marginatus</i>	<i>E. marginatus</i> -DQ108016	99	0,006	0.0	NO
22	Grouper	<i>Epinephelus marginatus</i>	FAO 37	<i>Pangasius hypophthalmus</i>	<i>P. hypophthalmus</i> -DQ108017	99	0,006	0.0	YES
23	Grouper	<i>Epinephelus marginatus</i>	FAO 37	<i>Epinephelus marginatus</i>	<i>E. marginatus</i> -DQ108016	99	0,002	0.0	NO
24	Grouper	<i>Epinephelus marginatus</i>	FAO 37	<i>Epinephelus marginatus</i>	<i>E. marginatus</i> -DQ108016	99	0,003	0.0	NO
25	Grouper	<i>Epinephelus marginatus</i>	FAO 37	<i>Epinephelus marginatus</i>	<i>E. marginatus</i> -DQ108016	100	0,003	0.0	NO

*Scientific name according to Decree of the Italian Ministry of Agricultural, Food and Forestry Policies (MiPAAF) dated 31 January 2008.

** The genetic distance (p-distance) between the sequences obtained in this study and those in BOLD database

Table 2. Perch fillet results.

Sample number	Commercial designation	Scientific* name	FAO code	Sequence from COI Identification	Sequence from BOLD Database	Similarity	**p-distance	E-value	Mislabelling
1	Perch	n.d.	FAO 51	<i>Pangasius hypophthalmus</i>	<i>P. hypophthalmus</i> - DQ108017	98	0,003	0.0	YES
2	Perch	n.d.	n.d.	<i>Lates niloticus</i>	<i>L. niloticus</i> - DQ108018	98	0,005	0.0	YES
3	Perch	n.d.	FAO 51	<i>Lates niloticus</i>	<i>L. niloticus</i> - DQ108018	99	0,005	0.0	YES
4	Perch	<i>Perca fluviatilis</i>	FAO 51	<i>Pangasius hypophthalmus</i>	<i>P. hypophthalmus</i> - DQ108017	98	0,003	0.0	YES
5	Perch	n.d.	n.d.	<i>Pangasius hypophthalmus</i>	<i>P. hypophthalmus</i> - DQ108017	99	0,009	0.0	YES
6	Perch	n.d.	n.d.	<i>Pangasius hypophthalmus</i>	<i>P. hypophthalmus</i> - DQ108017	99	0,007	0.0	YES
7	Perch	<i>Perca fluviatilis</i>	FAO 51	<i>Perca fluviatilis</i>	<i>P. fluviatilis</i> - DHQ961088.1	99	0,002	0.0	No
8	Perch	<i>Perca fluviatilis</i>	FAO 51	<i>Perca fluviatilis</i>	<i>P. fluviatilis</i> - DHQ961088.1	98	0,005	0.0	No
9	Perch	<i>Perca fluviatilis</i>	FAO 51	<i>Perca fluviatilis</i>	<i>P. fluviatilis</i> - DHQ961088.1	98	0,005	0.0	No
10	Perch	n.d.	FAO 51	<i>Lates niloticus</i>	<i>L. niloticus</i> - DQ108018	99	0,002	0.0	YES
11	Perch	<i>Perca fluviatilis</i>	FAO 51	<i>Pangasius hypophthalmus</i>	<i>P. hypophthalmus</i> - DQ108017	99	0,007	0.0	YES
12	Perch	n.d.	n.d.	<i>Pangasius hypophthalmus</i>	<i>P. hypophthalmus</i> - DQ108017	98	0,004	0.0	YES
13	Perch	<i>Perca fluviatilis</i>	FAO 51	<i>Perca fluviatilis</i>	<i>P. fluviatilis</i> - DHQ961088.1	98	0,008	0.0	No
14	Perch	n.d.	n.d.	<i>Perca fluviatilis</i>	<i>P. fluviatilis</i> - DHQ961088.1	99	0,004	0.0	No
15	Perch	<i>Perca fluviatilis</i>	FAO 51	<i>Perca fluviatilis</i>	<i>P. fluviatilis</i> - DHQ961088.1	99	0,005	0.0	No
16	Perch	<i>Perca fluviatilis</i>	FAO 51	<i>Perca fluviatilis</i>	<i>P. fluviatilis</i> - DHQ961088.1	99	0,007	0.0	No
17	Perch	n.d.	n.d.	<i>Pangasius sanitwongsei</i>	<i>P. sanitwongsei</i> - DQ108015	100	0,005	0.0	YES
18	Perch	<i>Perca fluviatilis</i>	FAO 51	<i>Perca fluviatilis</i>	<i>P. fluviatilis</i> - DHQ961088.1	98	0,002	0.0	No
19	Perch	n.d.	n.d.	<i>Perca fluviatilis</i>	<i>P. fluviatilis</i> - DHQ961088.1	98	0,008	0.0	No
20	Perch	<i>Perca fluviatilis</i>	FAO 51	<i>Perca fluviatilis</i>	<i>P. fluviatilis</i> - DHQ961088.1	99	0,004	0.0	No
21	Perch	<i>Perca fluviatilis</i>	FAO 51	<i>Perca fluviatilis</i>	<i>P. fluviatilis</i> - DHQ961088.1	98	0,006	0.0	No
22	Perch	n.d.	n.d.	<i>Pangasius hypophthalmus</i>	<i>P. hypophthalmus</i> - DQ108017	99	0,005	0.0	YES
23	Perch	<i>Perca fluviatilis</i>	FAO 51	<i>Pangasius sanitwongsei</i>	<i>P. sanitwongsei</i> - DQ108015	99	0,009	0.0	YES
24	Perch	<i>Perca fluviatilis</i>	FAO 51	<i>Perca fluviatilis</i>	<i>P. fluviatilis</i> - DHQ961088.1	99	0,007	0.0	No
25	Perch	n.d.	FAO 51	<i>Pangasius hypophthalmus</i>	<i>P. hypophthalmus</i> - DQ108017	99	0,005	0.0	YES

*Scientific name according to Decree of the Italian Ministry of Agricultural, Food and Forestry Policies (MiPAAF) dated 31 January 2008.

** The genetic distance (p-distance) between the sequences obtained in this study and those in BOLD database

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Table 3. Swardfish fillet results.

Sample number	Commercial designation	Scientific* name	FAO code	Sequence from COI Identification	Sequence from BOLD Database	Similarity	**p-distance	E-value	Mislabelling
1	Swordfish	<i>Xiphias gladius</i>	FAO 57	<i>Prionace glauca</i>	<i>P. glauca</i> - HM007788	99	0,002	0.0	YES
2	Swordfish	n.d	n.d	<i>Isurus oxyrinchus</i>	<i>I. oxyrinchus</i> - DQ885060	99	0,005	0.0	YES
3	Swordfish	n.d.	n.d	<i>Prionace glauca</i>	<i>P. glauca</i> - HM007788	98	0,002	0.0	YES
4	Swordfish	<i>Xiphias gladius</i>	FAO 57	<i>Xiphias gladius</i>	<i>X. Gladius</i> - FJ605764	99	0,007	0.0	NO
5	Swordfish	<i>Xiphias gladius</i>	FAO 57	<i>Prionace glauca</i>	<i>P. glauca</i> - HM007788	99	0,003	0.0	YES
6	Swordfish	n.d.	n.d	<i>Xiphias gladius</i>	<i>X. Gladius</i> - FJ605764	99	0,006	0.0	NO
7	Swordfish	n.d.	FAO 57	<i>Prionace glauca</i>	<i>P. glauca</i> - HM007788	99	0,008	0.0	YES
8	Swordfish	n.d.	n.d	<i>Xiphias gladius</i>	<i>X. Gladius</i> - FJ605766	99	0,005	0.0	NO
9	Swordfish	<i>Xiphias gladius</i>	FAO 57	<i>Xiphias gladius</i>	<i>X. Gladius</i> - FJ605767	98	0,005	0.0	NO
10	Swordfish	n.d	n.d	<i>Prionace. Glauca</i>	<i>P. glauca</i> - HM007788	99	0,005	0.0	YES
11	Swordfish	n.d.	n.d	<i>Thunnus obesus</i>	<i>T. obesus</i> - HQ24921	99	0,005	0.0	YES
12	Swordfish	<i>Xiphias gladius</i>	FAO57	<i>Xiphias gladius</i>	<i>X. Gladius</i> - FJ605768	100	0,008	0.0	NO
13	Swordfish	<i>Xiphias gladius</i>	FAO57	<i>Xiphias gladius</i>	<i>X. Gladius</i> - FJ605769	98	0,002	0.0	NO
14	Swordfish	<i>Xiphias gladius</i>	FAO57	<i>Xiphias gladius</i>	<i>X. Gladius</i> - FJ605770	98	0,005	0.0	NO
15	Swordfish	n.d	n.d	<i>Prionace. Glauca</i>	<i>P. glauca</i> - HM007788	99	0,004	0.0	YES
16	Swordfish	<i>Xiphias gladius</i>	FAO 57	<i>Prionace. Glauca</i>	<i>P. glauca</i> - HM007788	99	0,004	0.0	YES
17	Swordfish	n.d.	n.d	<i>Prionace. Glauca</i>	<i>P. glauca</i> - HM007788	99	0,006	0.0	YES
18	Swordfish	<i>Xiphias gladius</i>	FAO 57	<i>Xiphias gladius</i>	<i>X. Gladius</i> - FJ605774	99	0,007	0.0	NO
19	Swordfish	<i>Xiphias gladius</i>	FAO 57	<i>Xiphias gladius</i>	<i>X. Gladius</i> - FJ605775	99	0,008	0.0	NO
20	Swordfish	<i>Xiphias gladius</i>	FAO 57	<i>Xiphias gladius</i>	<i>X. Gladius</i> - FJ605776	98	0,008	0.0	NO
21	Swordfish	<i>Xiphias gladius</i>	FAO 57	<i>Xiphias gladius</i>	<i>X. Gladius</i> - FJ605777	98	0,009	0.0	NO
22	Swordfish	n.d.	FAO 57	<i>Xiphias gladius</i>	<i>X. Gladius</i> - FJ605778	99	0,006	0.0	NO
23	Swordfish	n.d.	n.d	<i>Xiphias gladius</i>	<i>X. Gladius</i> - FJ605779	98	0,007	0.0	NO
24	Swordfish	<i>Xiphias gladius</i>	FAO 57	<i>Xiphias gladius</i>	<i>X. Gladius</i> - FJ605780	99	0,004	0.0	NO
25	Swordfish	<i>Xiphias gladius</i>	FAO 57	<i>Isurus oxyrinchus</i>	<i>I. oxyrinchus</i> - DQ885060	99	0,004	0.0	YES
26	Swordfish	n.d.	n.d	<i>Xiphias gladius</i>	<i>X. Gladius</i> - FJ605780	98	0,005	0.0	NO
27	Swordfish	<i>Xiphias gladius</i>	FAO 57	<i>Xiphias gladius</i>	<i>X. Gladius</i> - FJ605781	98	0,002	0.0	NO
28	Swordfish	<i>Xiphias gladius</i>	FAO 57	<i>Xiphias gladius</i>	<i>X. Gladius</i> - FJ605782	98	0,003	0.0	NO
29	Swordfish	n.d.	n.d	<i>Prionace. glauca</i>	<i>P. glauca</i> - HM007788	99	0,002	0.0	YES
30	Swordfish	<i>Xiphias gladius</i>	FAO 57	<i>Prionace. glauca</i>	<i>P. glauca</i> - HM007788	99	0,002	0.0	YES
31	Swordfish	<i>Xiphias gladius</i>	FAO 57	<i>Prionace. glauca</i>	<i>P. glauca</i> - HM007788	99	0,002	0.0	YES
32	Swordfish	<i>Xiphias gladius</i>	FAO 57	<i>Prionace. glauca</i>	<i>P. glauca</i> - HM007788	99	0,005	0.0	YES
33	Swordfish	<i>Xiphias gladius</i>	FAO 57	<i>Xiphias gladius</i>	<i>X. Gladius</i> - FJ605787	100	0,006	0.0	NO

Sample number	Commercial designation	Scientific* name	FAO code	Sequence from COI Identification	Sequence from BOLD Database	Similarity	**p-distance	E-value	Mislabelling
34	Swordfish	<i>Xiphias gladius</i>	FAO 57	<i>Thunnus obesus</i>	<i>T. obesus</i> - HQ24921	100	0,006	0.0	YES
35	Swordfish	<i>Xiphias gladius</i>	FAO 57	<i>Xiphias gladius</i>	<i>X. Gladius</i> - FJ605764	99	0,005	0.0	NO
36	Swordfish	n.d.	n.d.	<i>Isurus oxyrinchus</i>	<i>I. oxyrinchus</i> - DQ885060	99	0,002	0.0	YES
37	Swordfish	<i>Xiphias gladius</i>	FAO 57	<i>Prionace. glauca</i>	<i>P. glauca</i> - HM007788	99	0,002	0.0	YES
38	Swordfish	<i>Xiphias gladius</i>	FAO 57	<i>Xiphias gladius</i>	<i>X. Gladius</i> - FJ605787	100	0,004	0.0	NO
39	Swordfish	<i>Xiphias gladius</i>	FAO 57	<i>Xiphias gladius</i>	<i>X. Gladius</i> - FJ605764	100	0,002	0.0	NO
40	Swordfish	<i>Xiphias gladius</i>	n.d.	<i>Isurus oxyrinchus</i>	<i>I. oxyrinchus</i> - DQ885060	99	0,005	0.0	YES

***Scientific** name according to Decree of the Italian Ministry of Agricultural, Food and Forestry Policies (MiPAAF) dated 31 January 2008.

** The genetic distance (p-distance) between the sequences obtained in this study and those in BOLD database

In particular 6/25 fillets of grouper (*Epinephelus marginatus*) were mislabeled, with 3/6 being identified as belonging to *Epinephelus diacanthus*, 2/6 as *Lates niloticus* and 1/6 as *Pangasius hypophthalmus* (Table 1). Moreover, 13/25 European perch (*Perca fluviatilis*) were mislabeled; specifically, 3/13 were identified as *Lates niloticus*, 8/13 as *Pangasius hypophthalmus* and 2/13 as *Pangasius sanitwongsei* (Table 2). Then, post-sequencing data analysis found 19/40 purported swordfish (*Xiphias gladius*) to be incorrectly labeled (Table 3), with 13/19 samples being from *Prionace glauca*, 2/9 samples from *Thunnus obesus* and 4/9 as *Isurus oxyrinchus*. All interpretable sequences obtained in this study revealed *p*-distance values in according with taxonomic position (Table 1, Table 2 and Table 3).

Bimolecular method used for identification provided optimal yield DNA extracted and purified from all 90 samples. PCR products were 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 (Table 1, Table 2 and Table 3).

The results of this study reveal a high occurrence of incorrect species declaration in prepared fillet products, further evidence of the need for increased traceability and assessment of the authenticity of food products. In comparison with other studies [8,1,6], the present study reports and confirms the strong trends in

seafood mislabeling levels among retail types, prevalently using species of lower commercial value to replace overfished, imperiled or vulnerable species, such as *Isurus oxyrinchus* and *Thunnus obesus*. Fish mislabeling is widespread throughout the world. Species substitution in fish occurs frequently, particularly in imported products which are not recognizable visually and are indistinguishable on a morphological basis after processing and freezing [8].

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. The need to improve transparency and traceability in the fishing industry by implementing full traceability is a crucial step in eliminating illegal fishing, seafood mislabeling and fraud. In addition, voluntary certification and the creation of a global label could not only effectively raise the quality standards of production procedures but also increase the chances that a company's products will be chosen by specific importers, retailers or consumers. Thus, every prepared fillet fishery/aquaculture product would be traceable at all stages of production, processing and distribution, from catching or harvesting to the retail stage and adequately labeled according with art. 58 of Reg.(EC) N. 1224/2009. Adoption of emerging packaging technologies for fresh and prepared fish fillet commercialization associated with a detailed global label that identifies seafood by a unique code may allow consumers to track seafood uniquely. 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. The study provides further evidence that DNA barcoding may be one of the most powerful tools for the assessment of species identity, food traceability, safety and fraud as suggested by the art. 13 of the Reg.(EC) N. 1224/2009. 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.

4. References

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