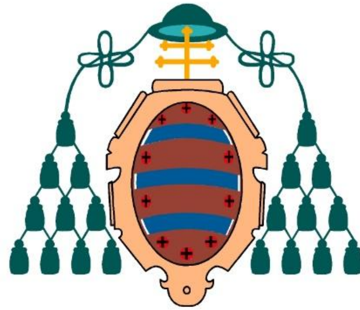

MASTER UNIVERSITARIO EN CONSERVACIÓN MARINA

Master of Science in Marine Conservation

Detection of Illegal, Unreported and Unregulated (IUU) fishing in sharks using barcoding

TRABAJO FIN DE MASTER

Master Thesis

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Detection of IUU in sharks using barcoding

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(To be submitted to <Journal Title>)

Abstract

To meet the ever-growing demands by seafood consumers as human population increases, many producers in the industry have resorted to the use of fraudulent means along chains to meet these demands. Mislabelling of fish and fish products have attained much attention over the years, especially after public awareness was raised on the high level of substitution of high-value fish with low-value fish in various fish markets, restaurants and processed fish products. The use of DNA barcoding as a genetic tool for the validation of seafood has since been applied to a variety of commercial fish species such as salmon, trout, tilapia, cod and sharks to determine their rate of mislabelling. This report followed similar methods to detect the rate of mislabelling in shark species and other commercial fisheries in Ghana. Eighty-five samples were collected from both Ghanaian and Spanish regions, and genetic information was obtained from sequencing results after polymerase chain reaction (PCR) amplification of the 650 base region of cytochrome c oxidase I (COI) gene. Identity of species obtained in GenBank showed a 94% (16 out of 17) and an 18% (3 out of 17) mislabelling rate in Ghanaian shark and Asturian shark samples, respectively. Availability of various species of sharks under threat in the fish markets is a sign of weak regulations regarding the conservation measures, especially in the Ghanaian market. The results of this study portray the value of DNA barcoding as an essential tool for enhancing traceability in the seafood business.

Keywords: DNA barcoding, mislabelling, seafood traceability, fisheries, species

1. INTRODUCTION

Fish and fishery products to date remain one of the most popular food commodities traded worldwide accounting for up to 20.5kg of per capita food fish consumption in 2018 (FAO, 2020). More than half of the world's population depends on fish as a source of protein, and the supply has thus led to the depletion of global saltwater fish stocks (Britten et al., 2016). Current quantities of fish stocks at unsustainable levels have increased from ten per cent in 1974 to 34.2 per cent in 2017 (FAO, 2020).

The overall decline in sustainability of fish and fishery resources have driven seafood consumers toward a change in lifestyle, by making informed choices about the conservation status of the wide variety of species consumed. International regulations designed to promote the sustainable use of marine resources, such as the United Nations Convention on the Law of the Sea (UNCLOS) (1982), the FAO Code of Conduct for Responsible Fisheries (the Code) (1995), the United Nations Fish Stocks Agreement (1995), as well as rules and regulations made by regional fisheries management organisations (RFMOs), have provided a degree of protection for some marine resources. Regulations by Federal Governments and the implementation of Eco-labels from both Governmental (e.g. 'the Flower' by the European Union) and Non-governmental organisations (NGOs) such as the Marine Stewardship Council (MSC) have been conceived to boost traceability in the seafood chain. Consumers are therefore given a variety of information about the methods used to catch the fish, damage caused to protected species in the process of fishing, types of gear used, place of fishing and fishing techniques used.

However, despite these efforts to ensure traceability in the seafood chain, many illegal and fraudulent means such as unreported fish catch and mislabelling of fish and fish products are carried out by the fishing industry to meet the high demand for seafood (Fox et al., 2018). The strong evidence of mislabelling detected in processed and pre-packaged seafood (Bénard-Capelle et al., 2015; Jacquet & Pauly, 2008; Minoudi et al., 2020; Muñoz-Colmenero et al., 2016; Von Der Heyden et al., 2010) has led to the use of genetic tools in authenticating seafood to detect Illegal, Unreported and Unregulated (IUU) fishing.

Advancement in genetics and molecular techniques has made it possible to authenticate and identify species, whether fresh or processed with high accuracy using different molecular markers such as Restriction Fragment Length Polymorphism (RFLP) (Teletchea, 2009), Microarrays (Kochzius et al., 2010), Single Nucleotide Polymorphisms (SNPs) (Machado-Schiaffino et al., 2008), minisatellites (Miller et al., 1996) and microsatellites (Klein et al., 2019). DNA barcoding has become a widely used method for identifying species through Polymerase Chain Reaction (PCR) amplification and sequencing (Clark, 2015). DNA barcoding has proven to be a useful tool applied in the conservation of fish species because of its ability to provide information on genetic diversity of species, detection of hybridisation, population structure, and detection of polymorphism in species (Haig, 1998; Klein et al., 2019; Van Der Merwe & Gledhill, 2015; Wringe et al., 2019).

Different short DNA sequences of the genome such as the cytochrome c oxidase subunit I (COI), 18S rRNA, cytochrome b and 16S RNA have been used to detect mislabelling where there have been records of substitution of high-priced fish with less desirable species in commercial fish species (Ardura et al., 2010; Chauhan & Rajiv, 2010; Pazartzi et al., 2019; Sarmiento-Camacho et al., 2018; van der Reis et al., 2018). Some applications of DNA barcoding has also been towards the validation of commercial fish species like salmon, hake, tilapia and sharks (Galal-Khallaf et al., 2014; Garcia-Vazquez et al., 2011; Rasmussen et al., 2009; Sarmiento-Camacho et al., 2018). The commercialisation of shark species has increased over the last years (Dent & Clarke, 2015) and the application of DNA barcoding for species identification will highly benefit these vulnerable marine species.

Global shark populations have drastically declined over the past decade because of climate change, destruction of marine environments by overfishing, ocean mining, pollution through dumping of waste and marketing of shark products (Dent & Clarke, 2015; Kibria et al., 2017; Sant & Welch, 2017). About 10 million sharks are captured annually for their fins and meat (Pérez Roda et al., 2019). World shark fin imports are estimated at USD 80 million from 2000 to 2011 by FAO based on statistics from 2011 to 2014 (Dent & Clarke, 2015). Sharks are characterised by low fecundity, slow growth, high migratory behaviours, delayed maturation and extended gestation periods which makes them extremely vulnerable to overexploitation (Dulvy et al., 2016; Klein et al., 2019). Due to their inability to sustain populations to meet the consumption demands of humans, the International Union for Conservation of Nature has listed most sharks species as critically Endangered, Endangered, Vulnerable or Near-threatened and further states that 'about a quarter of shark, ray, and chimaera are Threatened' (IUCN, 2014).

To deter IUU in the global seafood chain, regulations on certification and labelling needs to be provided and followed to ensure transparency in fisheries. A global meta-analysis of mislabelling conducted by Luque & Donlan (2019) showed that USA, Italy and Spain are the countries with most attempts to address mislabelling although "efforts to document mislabelling have been conducted in 38 countries". Spain, for instance, has regulations on labelling of seafood products regulated under The Spanish Agency for Food Security (AESAN - Agencia Española de Seguridad Alimentaria). Several legal regulations addressing the information display of seafood products include Boletin Oficial del Estado. (2004) and Olsen et al. (2019). However, labelling regulations in other countries such as Ghana are not as detailed as compared to that of Spain. Ghana Standards Authority General Labelling Rules, 1992 (L.I. 1541, 1992) is the only general labelling law in the country, where all the products are termed 'food and drugs'. The Fisheries Act 625 of 2002 and the Fisheries Regulation of 2010 (L.I. 1968) do not address the labelling issues of seafood products. Meanwhile, there seems to be much attention paid to high income earning seafood products, such as tuna, which under the management of the International Commission for the Conservation of Atlantic Tuna (ICCAT), provides specific regulations on the details of labelling of seafood by tuna purse seine vessels and longline vessels. The Fisheries Scientific Survey Division, (FSSD) of the Ministry of Fisheries and Aquaculture Development in Ghana is responsible for enforcing this regulation. There happens to be at the moment, no management plan for shark fisheries in Ghana (Vasconcellos et al., 2018).

The 550km coastline of Ghana provides the state with immense aquatic resources such as oil and gas, fisheries, and maritime activities from the 218 100 km^2 Exclusive Economic Zone (EEZ). The Fisheries sector in Ghana contributes over 10% to the labour force and generates an estimated US$1 billion in revenue yearly. The sector also contributes about 4.5% to the country's Gross Domestic Product (Asiedu et al., 2017; Government of Ghana, 2002) The artisanal fishery is the largest in the fisheries sector in Ghana, described as the most "important sector within the marine sector regarding the total volume of fish landed" (Cobbina, 2018) with a majority of the landings being Sardinellas, Croakers, Anchovy and mackerels (FAO, 2007). A survey by National Oceanic and Atmospheric Administration (NOAA) identified diverse Elasmobranchs and billfishes captured by fishermen in the coastal waters of Ghana which included the Bigeye Thresher-fin Shark (*Alopias superciliosus*), Blue Shark (*Prionace glauca*), Bull Shark (*Carcharhinus leucas*), Common Thresher-fin Shark (*Alopias vilpinus*), Scalloped Hammerhead Shark (*Sphyrna lewini*), Short-fin Mako Shark (*Isurus oxyrinchus*), Common Tiger Shark (*Galeocerdo cuvier*), Sand Tiger Shark(*Carcharius taurus*), and Great White Shark (*Carcharodon carcharias*) (Elasmobranchs & Billfishes caught in Ghana, Hen mpoano). The shark fisheries in Ghana are not regulated mainly because the species are caught as bycatch, and the meat is mostly used as bait for higher commercial species like tuna, anchovies and mackerels (Gelber, 2018). However, as fish stocks dwindle, fishers are shifting their attention to shark meat and fins due to their high economic value (Gelber, 2018). It is therefore imperative to manage shark fisheries in Ghana since many species caught in the Ghanaian waters for commercialisation are at the risk of extinction.

There is a need for authenticating fish and fish products, to ensure customers get value for their money and also to prevent health issues such as allergies, and its related accidents (Muñoz-Colmenero et al., 2016). The Ghanaian seafood industry can benefit significantly from implementing traceability in their supply chain systems with the use of genetic tools. No study in Ghana has applied molecular techniques to identify the degree of seafood mislabelling in the country. This study is thus the first of its kind in Ghana. Together with South Africa (Cawthorn et al., 2015), Egypt (Galal-Khallaf et al., 2014) and Guinea-Bissau (Minhós et al., 2013), there are few studies performed in African countries that apply DNA barcoding to the identification of species and to the assessment of the degree of mislabelling in seafood products.

In this study, we use the cytochrome c oxidase subunit I (COI) marker to evaluate, (a) the rate of mislabelling in shark species as well as other fish species commercialised in Ghanaian fish markets (b) the potential risk of selling different fish species under the same generic names, and (c), to assess the conservation status of all identified shark species in Ghana and Spanish fish markets and to discuss whether differences in strength of labelling regulations affect the rate of shark mislabelling in both regions.

2. MATERIALS AND METHODS

2.1 Sample Collection

Samples were obtained in two of the significant fishing communities in the Greater-Accra region of Ghana in February 2020. Fish samples were purchased in local fish markets known

to sell fish from small scale fishers to ensure the species were coming from Ghanaian waters. Shark samples were bought at the Jamestown landing site for artisanal fishers (5°32'06.0"N 0°12'32.6"W). This market receives catch from a wide range of fishers from neighbouring coastal villages, making it the ideal location for obtaining a variety of shark species. Mackerel, shrimps, anchovies, croakers and sardinella samples were purchased in the Tema Newtown fish market (5°38'35.6"N 0°01'01.8"E) for the same reasons. The labels of all the products were recorded for each sample. About 10g per sample was stored in Ziploc plastic bags and frozen at -20°C. The frozen samples were placed in an insulated bag before transportation to Oviedo, Spain. Upon receipt, the samples were already deteriorating. They were immediately washed with 70% ethanol, stored in absolute ethanol, and refrigerated at 4°C.

To compare the level of mislabelling in Ghana to other regions, 17 sequences belonging to sharks from Asturian fish markets in Spain were obtained from the Department of Functional Biology at the University of Oviedo, Spain. Labels of all samples purchased were recorded. See table 1 for details about the samples collected.

Table 1. List of the samples included in the present study. The number of samples n=85, region and location, the type of sample acquired and quantity of samples per species are reported.

Region	**Location**	**Type of sample**	**Label**	**Quantity**
Ghana	Jamestown	fresh, filleted	Brown shark	16
Ghana	Jamestown	fresh, filleted	Nurse shark	1
Ghana	Jamestown	fresh, filleted	Hammerhead shark	2
Ghana	Jamestown	fresh, filleted	Bull shark	1
Ghana	Tema Newtown	fresh, whole	Mackerel	10
Ghana	Tema Newtown	fresh, whole	Croaker	10
Ghana	Tema Newtown	fresh, whole	Shrimps	10
Ghana	Tema Newtown	fresh, whole	Anchovy	10
Ghana	Tema Newtown	fresh, whole	Sardinella	8
Spain	Asturias	not frozen, whole	*Scyliorhinus sp*	3
Spain	Asturias	not frozen, N/A	*Raja sp*	3
Spain	Asturias	frozen, fillet with skin	*Prionace glauca* "tintorera."	11
				85

2.2 DNA Extraction

Genomic DNA was extracted from muscle tissues using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) by following the manufacturer's protocol in the laboratory of 'Grupo de Aula de Recursos Naturales (ARENA)', Department of Functional Biology, University of Oviedo, Spain. Completed extractions were stored at 4°C for further analysis. Genomic DNA products were visualised under UV light in 1% agarose gel stained with 2.5µl SimplySafe™ dye (EUR_X®).

2.3 PCR Amplification and Sequencing

Cytochrome c oxidase subunit I (COI) gene was selected for amplification due to its use as a standard molecular marker for the successful identification of a variety of metazoan species (Kochzius et al., 2010; Minhós et al., 2013; Rach et al., 2017; Sarmiento-Camacho et al., 2018; Ward et al., 2005). Samples were organised into three groups on the grounds of the application of different species-specific primers developed by Geller et al. (2013) and Ward et al. (2005) to optimise the success of PCR results. Negative controls were added to ensure that any kind of contamination could be identified. Ghanaian fish samples were organised into three groups. Group one contained Sharks (n=20), group two consisted of crustaceans (n=10), and group three comprised mixed fishes (anchovy, mackerel, croaker, and sardinella) (n=38).

PCR amplification for mitochondrial COI partial fragments for group one (sharks) occurred as follows: 0.5µM of primers Fish-F2/Fish-R2 (Ward et al., 2005), 0.25mM dNTPs, 2.5 mM $MgCl_2$, 1x Buffer GoTaq®Promega, 0.15µl of GoTaq® Polymerase (5u/µL), 2µL of DNA in a final volume of 20µL.

Samples in group two (crustaceans) were processed in a final volume of 20µL containing 0.5µM of primers jgLCO1490 and jgHCO2198 developed by (Geller et al., 2013), 0.25mM dNTPs, 2.5 mM $MgCl_2$, 1x Buffer GoTaq®Promega, 0.15µl of GoTaq® Polymerase (5u/µL), 1µL of DNA.

Mitochondrial COI genes for group three samples (mixed fishes) were amplified by using 0.5µM of primers COI-Fish forward and reverse (Ward et al., 2005), 0.25mM dNTPs, 2.5 mM $MgCl_2$, 1x Buffer GoTaq®Promega, 0.15µl of GoTaq® Polymerase (5u/µL), 2µL of DNA in a final volume of 20µL.

PCR products for groups one and three were run in a thermal cycler (Applied Biosystems, model 2720) following an initial denaturation step at 95°C for 5 minutes followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 53°C and 57°C for 40 seconds respectively, elongation at 72°C for 30 seconds and a final extension at 72°C for 15 minutes. For group two, amplification in a thermal cycler (Applied Biosystems, model 2720) was run at initial denaturation step at 95°C for 5 minutes followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 49°C for 40 seconds, elongation at 72°C for 45 seconds and a final extension at 72°C for 5 minutes.

Samples that failed PCR amplification due to bacterial contamination were corrected by using the 18s rRNA marker to eliminate any chances of amplifying bacterial genome. We chose this marker due to the absence of the 18S rRNA in prokaryotes, and also because the marker had been successfully used in the identification of fish species (Kaleshkumar & Rajaram, 2020; Zhan et al., 2013).

Final PCR results were visualised by gel electrophoresis in 2% agarose gel stained with 2.5µl SimplySafe™ dye (EUR_X®). The resulting amplicons were sent to Macrogen Inc, Madrid Spain for sequencing utilising the Sanger sequencing method.

2.4 Species Identification and Data analysis

Forward and reverse sequences for amplified samples were manually edited and aligned using the ClustalW tool in the BioEdit application (Hall, 1999). For species identification, consensus sequences obtained were compared to reference sequences using Basic Local Alignment Search Tool, BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) on GenBank database (Altschul et al., 1990) and cross-referenced on the Barcode of Life data systems (BOLD) (Ratnasingham & Hebert, 2007). Best scoring results were used for species identification. Conservation status of shark species was determined by consulting the International Union for Conservation of Nature and Natural Resources (IUCN) Red List (IUCN, 2020) and Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). A separate phylogenetic tree for sharks and all identified species were constructed separately using the Neighbour-Joining statistical method in the MEGA_X_10.1.8 software (Kumar et al., 2018). Using the Kimura-2 model, 1000 bootstrap replicates were run to obtain a visual depiction of the clustering format across species. A 655bp for *Chimaera opalescens* (GenBank ID: 283837981, Accession number: GU244534.1) and *Rhizostoma pulmo* (GenBank ID:1275887105, Accession number: KY131238.1) voucher sequences were obtained from GenBank for use as outgroups of the various species (Cunha et al., 2017). Verification of the results from the Neighbor-Joining method was done by building phylogenetic trees for shark species and another for the entire species identified using maximum-likelihood statistical method in MEGA_X_10.1.8. A matrix comparing the pairwise distances in five shark samples amplified with both COI and 18S markers were computed using a Gamma distributed (G) pattern based on the number of differences model to determine the level of accuracy in species identification using both markers in MEGA_X_10.1.8.

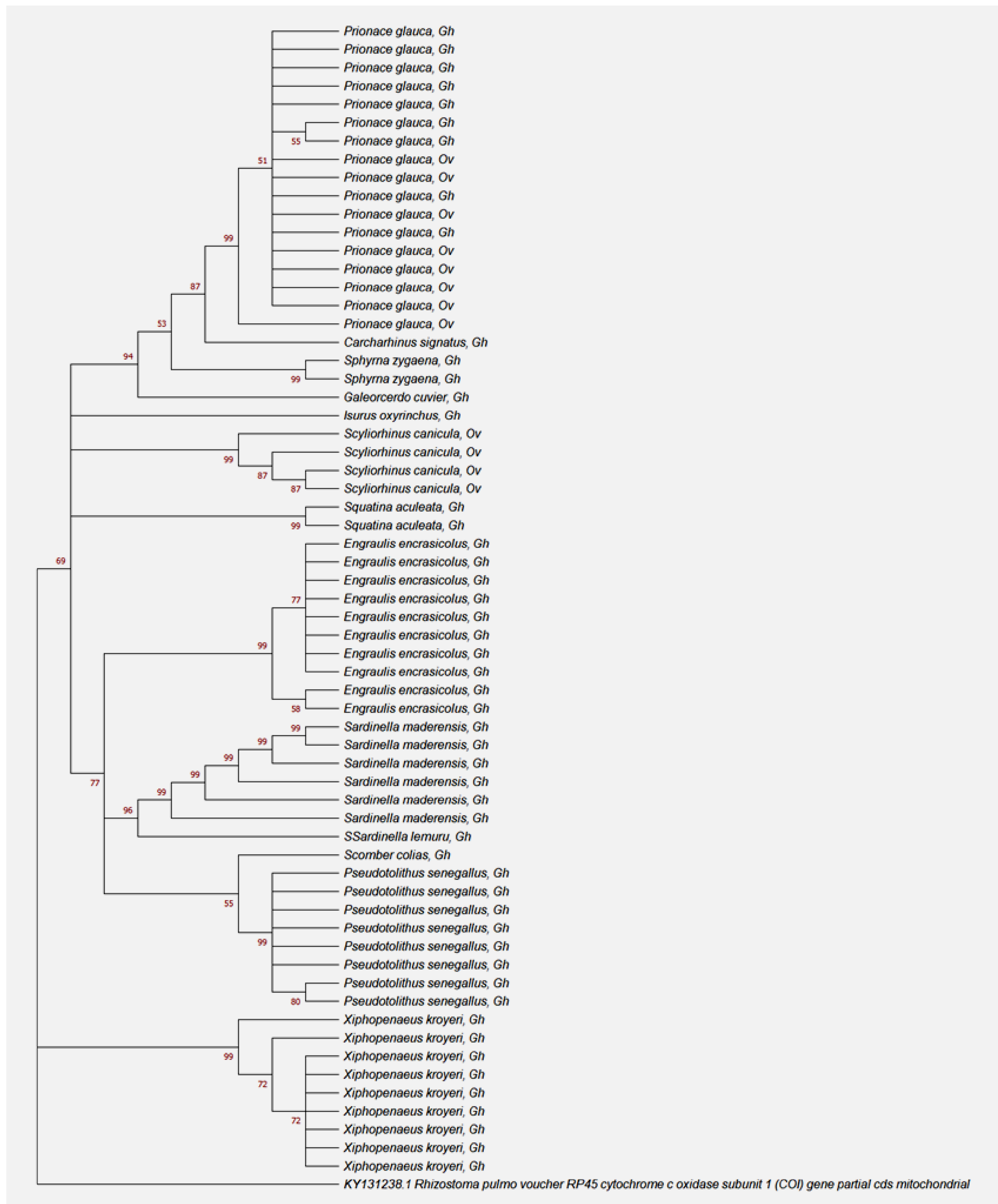
3. RESULTS

3.1 DNA Extraction, Amplification, and Sequencing

Positive PCR amplifications were obtained for all 85 samples using the cytochrome c oxidase subunit I gene (COI) marker. Sequencing results were aligned and edited to lengths ranging between 221 and 694 base pairs and queried for species identification using BLAST. Positive identities were obtained for 67 samples, and the remaining 18 were identified as bacteria (*Photobacterium damselae, Vibrio diabolicus, and Shewanella loihica*). Contamination by bacteria was likely due to an unpredicted delay in the delivery process coupled with poor preservation. Several attempts were undertaken to avoid bacteria amplification in the unsuccessful samples (sharks=7, mackerel=9, shrimp=1, and sardinella=1). New modification of amplification conditions with COI and selection of a substitute marker, 18S rRNA was amplified for the samples identified as bacteria to ensure a higher chance of obtaining positive identities. This marker was also chosen because it has previously been used to identify shark species (Kaleshkumar & Rajaram, 2020; Mallatt & Winchell, 2007). From the seven unsuccessful shark samples, five results were obtained with 18S and four with COI. In total, 71 positive identities were obtained, and the 14 that remained as bacteria were discarded.

3.2 Species Identification

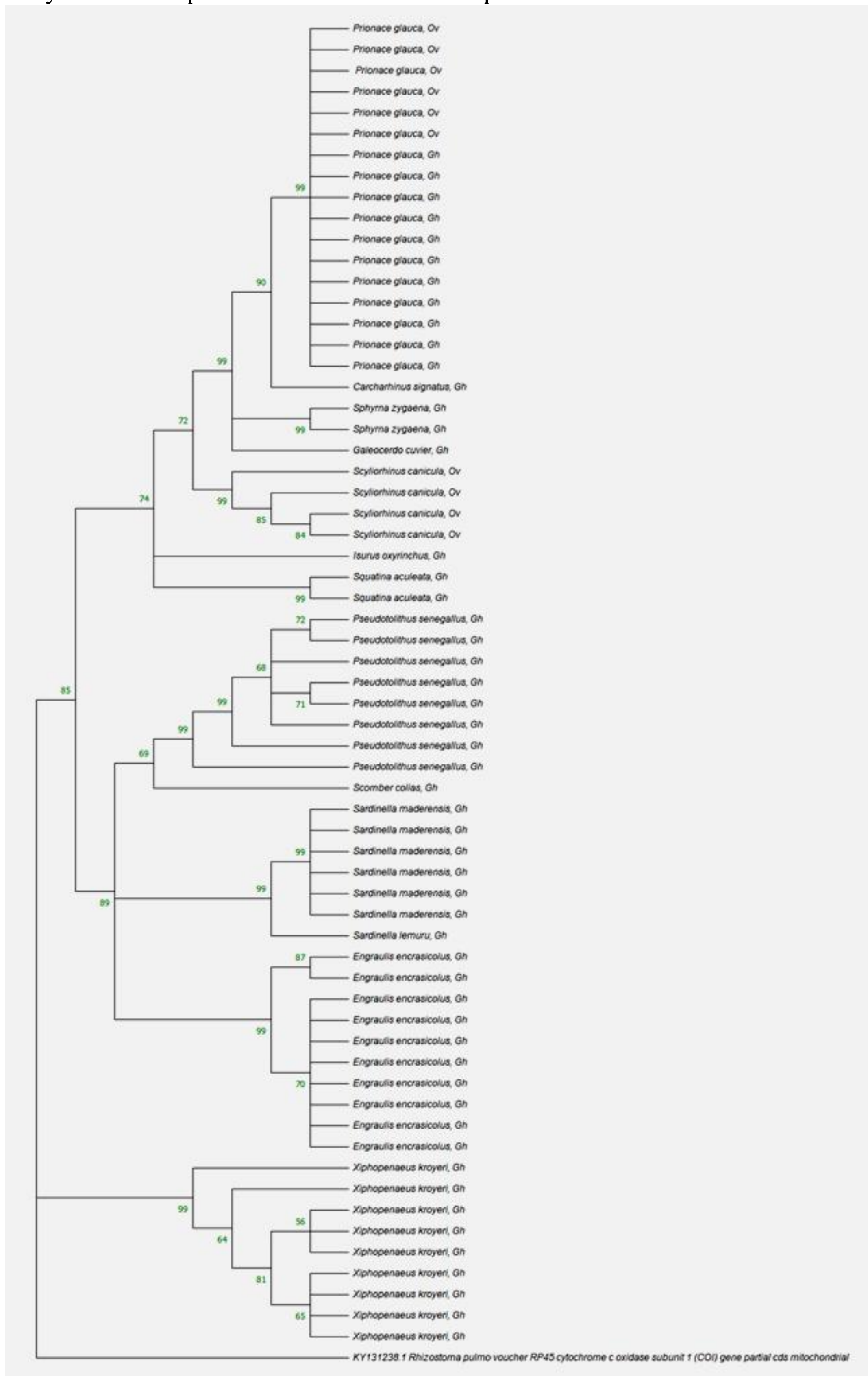
Identities of all the species were obtained with COI, where seven orders, (*Carcharhiniformes, Squatiniformes, Lamniformes, Scombriformes, Perciformes, Clupeiformes,* and *Decapoda*) and twelve genera (*Prionace, Sphyrna, Squatina, Isurus, Galeocerdo, Scomber, Pseudotolithus, Sardinella, Xiphopenaeus, Engraulis, Carcharhinus,* and *Scyliorhinus*) were identified. The complete list of species identified and their GenBank accession numbers for the top hit are presented in Table S1 and S2 in the supplementary material. Two phylogenetic trees of sequences 527bp in length were constructed to observe the diversity among species in the entire sample size and for sharks only. Figure 1 represents the phylogeny of the complete species identified in this study. The clear delimitation of clades identified by clusters of same species illustrates the robustness of COI as a genetic marker for species identification. The following samples, S8_Ghana, S4_Oviedo, S5_Oviedo, S17_Oviedo, S18_Oviedo and S21_Oviedo could not be aligned for the trees because the sequences were too short. There are, however, samples belonging to the same species from the region represented in the tree (S7-*Isurus oxyrinchus*_Gh, S7-S10 *Scyliorhinus canicula*_Ov and S27-S35 *Prionace glauca*_Ov). Figures one and two represent the clustering results of all species identified in this study from Neighbor-Joining (NJ) and Maximum Likelihood (ML) methods, respectively. In figures three and four, phylogenetic results obtained from NJ and ML methods are shown.



0.050

Fig.1. Neigbor-Joining tree estimated from 63 COI sequences of fish samples (sharks, anchovy, mackerel, shrimps, sardinella, and croaker) obtained from Ghanaian and Asturian fish markets. Species represented in the tree were obtained from 1000 bootstrap replicates with cut-off value at 50% using the Kimura 2-parameter model in MEGA X. Gh: Ghana and Ov: Oviedo.

As a means determine the accuracy of clustering in the NJ method, a maximum likelihood analysis was also performed for all 63 COI sequences.



0.050

Fig.2. Species divergence analysis for 63 COI sequences for all fish samples (sharks, anchovy, mackerel, shrimps, sardinella, and croaker) with consensus value of >50% inferred from the Maximum Likelihood method. Results of species clustering corresponding to 1000 bootstrap replicates were obtained from the Nearest-Neighbour Interchange using the Kimura-2 parameter model in MEGA X. Gh: Ghana and Ov: Oviedo.

A phylogenetic analysis was performed separately for only shark species identified in this study to determine if their clustering followed similar patterns observed across all taxa described by other publications. Two different clustering patterns in the shark species were identified when analysed with both NJ and ML methods. *I. oxyrinchus* is placed closer to the outgroup in NJ methods (Fig. 3), and in the ML methods, *S. aculeata* is placed close to the outgroup (Fig. 4)

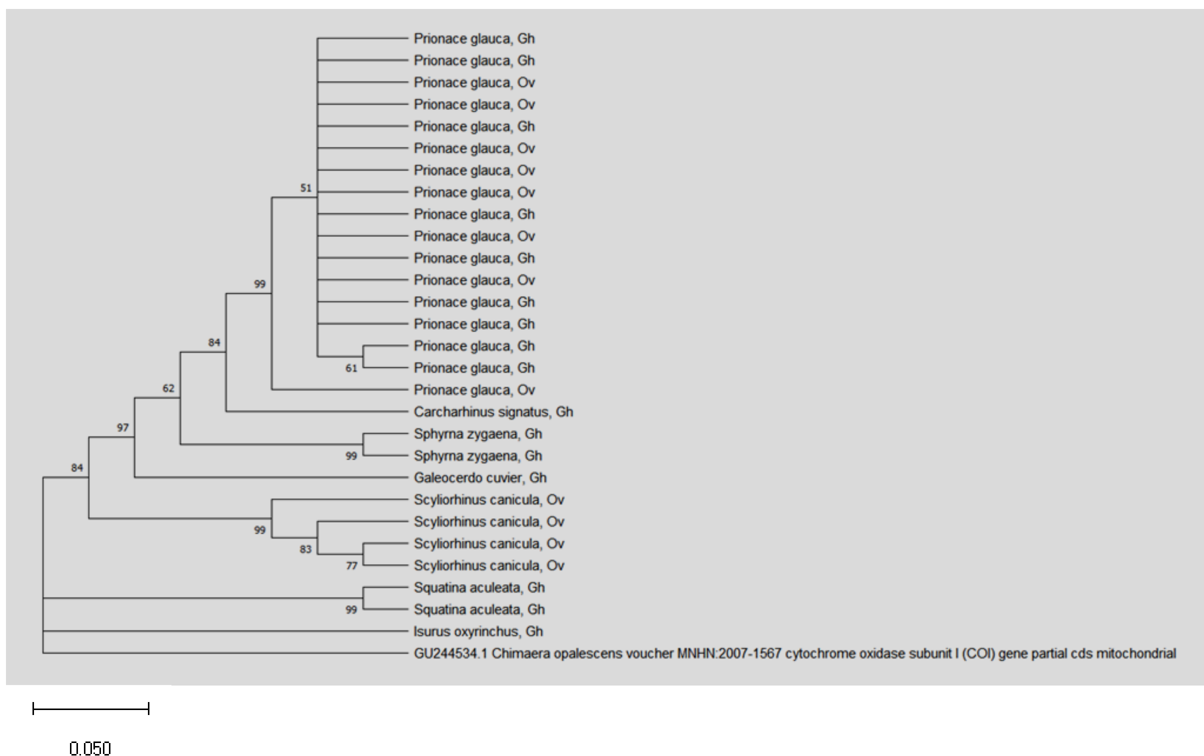


Fig.3. Neighbor-Joining tree of 28 shark COI nucleotide sequences of species identified in this study from both Ghanaian and Asturian fish market obtained from 1000 bootstrap replicates with cut-off value at 50%. Gh: Ghana and Ov: Oviedo.

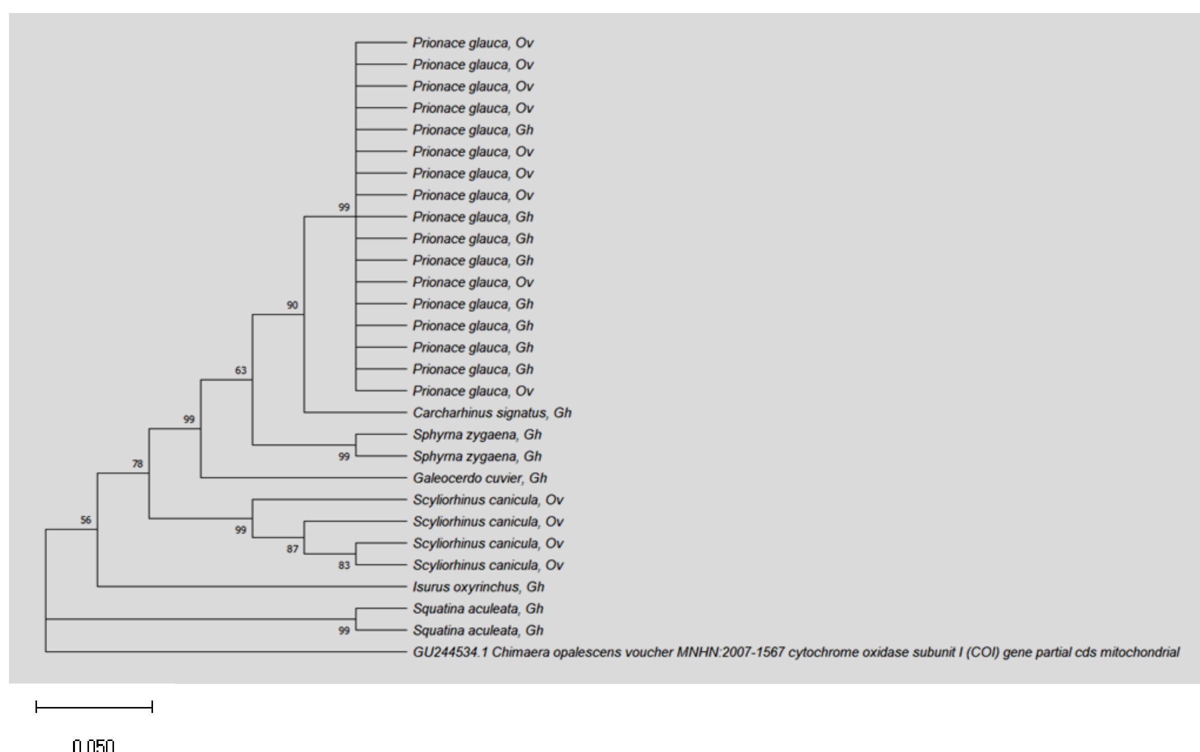


Fig.4. Phylogenetic analysis of 28 shark COI nucleotide sequences with the Maximum likelihood method. The tree represents species with results > 50% obtained from 1000 bootstrap replicates inferred from the Kimura-2 parameter method in MEGA X. Gh: Ghana and Ov: Oviedo.

3.3 Marker Resolution

The results obtained for 18S amplified species after performing BLAST were not specific as proposed. Matrices comparing the resolution of both 18S (Table 3b) and COI (Table 3a) markers were performed to determine the differences in nucleotide composition among the various sequences. All samples were aligned and trimmed to equal lengths of 343 basepairs, and pairwise distances based on the number of difference in sequences were determined as shown in Table 3a and 3b. The results conclude why 18S was not valid for the identification of the shark species.

Table 3a. Pairwise sequence divergence between shark species identified with COI (sequence length = 343 bp)

	S. zygaena	*C.signatus*	*P.glauca*	*S.aculeata*	*P.glauca*
S. zygaena	-				
C. signatus	39.0	-			
P. glauca	33.0	28.0	-		
S. aculeata	118.0	124.0	118.0	-	
P. glauca	33.0	28.0	0.0	118.0	-

Table 3b. Pairwise sequence divergence between shark species identified with 18S rRNA (sequence length = 343 bp)

	S. zygaena	*C.signatus*	*P. glauca*	*S.aculeata*	*P.glauca*
S. zygaena	-				
C. signatus	0.0	-			

P. glauca	0.0	0.0	-		
S. aculeata	2.0	2.0	2.0	-	
P. glauca	0.0	0.0	0.0	1.0	-

3.4 Mislabelling

Genetically identified species were compared to the name of species stated on the packages to determine the rate of mislabelling in the samples. Where there was no complete harmony between the stated name on a label and identified name, the product was declared mislabelled. A high level of mislabelling was identified in the shark samples (Table 3). 94% mislabelling was identified in the Ghanaian shark species (n=17) while mislabelling was 18% in the Asturian shark samples (n=17). Of the seven species of sharks identified, *Prionace glauca* accounted for the majority (58%); followed by *S. canicula* (18%), *I. oxyrinchus*, *S. zygaena*, *S. aculeata* (6% each), *C. signatus* and *G. cuvier* (3% each). Similar trends were observed by Almerón-Souza et al. (2018) in a mislabelling study of sharks consumed in a Brazilian market. Despite observing no mislabelling in the mixed fish samples, we identified samples sold under "umbrella" names. *Sardinella* which comprised two different species: *Sardinella lemuru* and *Sardinella maderensis* were not differentiated on the labels. *Xiphopenaeus kroyeri,* the Atlantic seabob is also sold generally as a shrimp, but genetically identified as a prawn.

Table 4. Summary of samples, initial names on package, and mislabelling remarks. JT represents Jamestown fish market, TN represents Tema Newtown fish market and AS represents Asturias fish market. n is the number of species Identified. Initial samples with common names have been replaced with scientific names.

Market	**Sample**	**Label on package**	**Identified as**	**Mislabelling**
JT, Ghana	S1-S16	*Carcharhinus plumbeus*	*Prionace glauca* (n=8)	Yes
			Squatina aculeata (n=2)	Yes
			Vibrio diabolicus (n=3)	N/A
			Isurus oxyrinchus (n=2)	Yes
			Carcharhinus signatus (n=1)	Yes
JT, Ghana	S17	*Ginglymostoma cirratum*	*Sphyrna zygaena* (n=1)	Yes
JT, Ghana	S18	*Carcharhinus leucas*	*Galeocerdo cuvier* (n=1)	Yes
JT, Ghana	S19	*Sphyrna* spp.	*Sphyrna zygaena* (n=1)	No
JT, Ghana	S20	*Sphyrna* spp.	*Prionace glauca* (n=1)	Yes
TN, Ghana	S21-S30	*Scomber* spp.	*Photobacterium damselae* (n=9)	N/A
			Scomber colias (n=1)	No
TN, Ghana	S31-S40	*Pseudotolithus* spp.	*Pseudotolithus senegallus* (n=10)	No

TN, Ghana	S41-S48	*Sardinella* spp.	*Madeiran sardinella* (n=6) *Bali sardinella* (n=1)	No
			Shewanella loihica (n=1)	N/A
TN, Ghana	S58-S67	*Caridea* spp.	*Xiphopenaeus kroyeri* (n=9)	No
			Photobacterium damselae (n=1)	N/A
TN, Ghana	S68-S77	*Engraulis* spp.	*Engraulis encrasicolus* (n=10)	No
AS, Spain	S4, S5, S7	*Scyliorhinus* spp.	*Scyliorhinus canicula* (n=3)	No
AS, Spain	S8, S9, S10	*Raja* spp.	*Scyliorhinus canicula* (n=3)	Yes
AS, Spain	S17, S18, S21	Prionacea glauce "Caella"	*Prionace glauca* (n=3)	No
AS, Spain	S27, S28, S30-S35	*Prionace glauca* "tintorera"	*Prionace glauca* (n=8)	No

3.5 Conservation Status of Shark Samples

The seven shark species consisting of 34 individuals identified in this study were compared to IUCN listed species to estimate their degree of exploitation. All seven species but one, *S. canicula* (Small-spotted catshark) was reported to have decreasing populations globally. Figure five portrays the status of each identified shark species according to the nine categories provided by the IUCN.

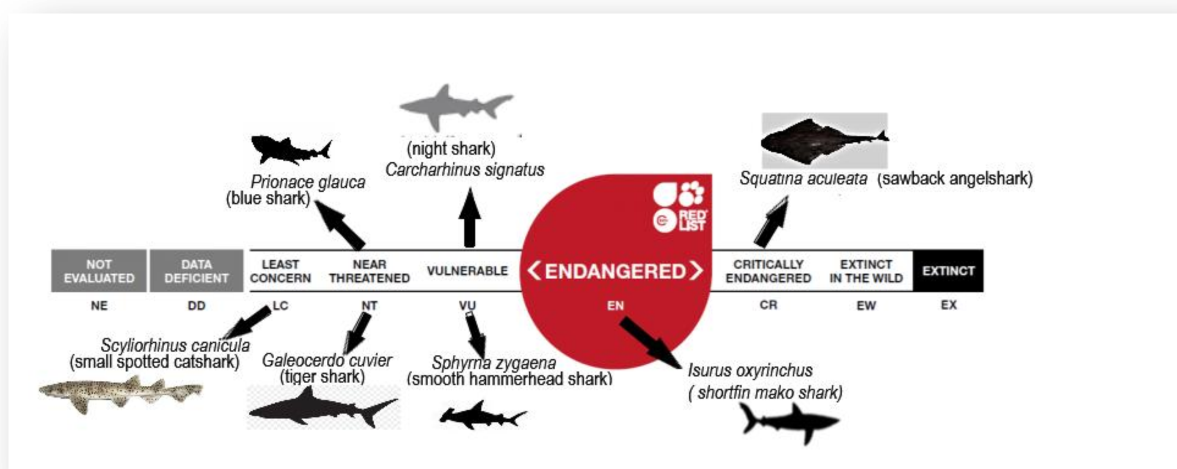


Fig 5. Conservation status of identified sharks showing the exploitation level of the various species along the nine categories used by the IUCN. Two shark species, *I. oxyrinchus* (Shortfin mako shark) and *S. zygaena* (Smooth hammerhead shark) are both listed as Appendix II species by CITES

4. DISCUSSION

4.1 Species Identification

The identities of all species could be accurately distinguished as seen in the phylogenetic analysis results in Fig.1, Fig.2, Fig.3, and Fig.4. Results obtained from the pairwise analysis of sequence divergence showed a highly variable value for *I. oxyrinchus*, Table 3a. The positioning of *I. oxyrinchus* close to the outgroup can be explained by the long-branch attraction effect described by Bergsten. (2005). The species clustering found in the ML phylogenetic tree are in concordance with those identified by Cunha et al. (2017), and Pavan-Kumar et al. (2014).

4.2 Marker Resolution

After comparing the divergence of five shark sequences obtained by COI and 18S rRNA amplification (Table 3a and 3b), 18S showed very low sequence divergence among species, which can make intraspecies identification problematic. Although 18S marker had been successfully used in the identification of shark species (Kaleshkumar & Rajaram, 2020; Mallatt & Winchell, 2007), results obtained in this study showed the poor resolution of 18S for shark species identification. Porter & Hajibabaei. (2018) also made similar remarks, following a study which compares the low number of 18S sequences in the GenBank database to COI which has over 2.5 million sequences. The highly conservative nature of the 18s marker has also been described to affect species identification (Kaleshkumar & Rajaram, 2020; Wu et al., 2015). On the other hand, the COI marker showed enough inter-species variation, which can be explained in the highly supported grouping of species (Fig. 1, 2, 3, and 4).

4.3 Mislabelling Levels in the Study Locations

4.3.1 Ghana

This study is the first in Ghana to use DNA barcoding to determine the level of mislabelling in seafood products. The result shows a high rate of mislabelling in the Ghanaian shark samples compared to the other fish samples (sardinella, anchovy, mackerel, shrimp and croaker) from the Ghanaian fish market. A trend was observed in the cost of fish and the level of mislabelling. Also, an observation of an apparent relationship between the availability of regulations and mislabelling rates in the Ghanaian samples was identified.

Sardinella, mackerel, croaker, anchovy, shrimp and tuna are Ghana's dominant commercially landed fish species accounting for 70% of total marine production (Tall & Failler, 2012). However, these fishes are of low economic value, fetching between $2-4 per kilo compared to shark products that cost around $20-30 per kilo (Tall & Failler, 2012). Declining fish stocks of the leading commercial fisheries make the trade of sharks a lucrative business for local fishers, especially since there are no regulations such as total allowable catch (TACs) rates set for them. The sharks are mostly caught as bycatch and represent 1.6% of annual fish catch (Vasconcellos et al., 2018). Shark fisheries provide fishers with alternate fast source of income due to the high demand for shark products. Another potential

explanation for the high level of mislabelling in the Ghana shark samples can be the inadequate knowledge about shark species identification. The result of low knowledge about sharks can be seen in the identities of the substitute species, which are morphologically different from the 13 samples labelled as brown sharks (Table 4). It is noteworthy, however, to mention how the commercial presentation of these sharks can also explain the high level of mislabelling. All shark samples were sold as fillets under the same umbrella term: Shark. Since there are no laws regarding the labelling of seafood products in Ghana, it is reasonable to observe fish products sold by just a common name, mostly of local origin (Gelber, 2018).

Regulations for fishery resources in Ghana vary across species, with high importance placed on commercial fish species (Vasconcellos et al., 2018). Due to their high economic importance in export value to the country, catch quotas are set for fishers by the Ministry of Fisheries and Aquaculture Development (MoFAD) for Sardinella, mackerel, anchovy, shrimps and tuna resources which allow the ministry to observe fish population trends (FAO Fishery and Aquaculture Country Profiles 2016). This likely explains the labelling accuracy of these species. In the mixed fish samples (all samples minus sharks), however, issues connected with the sale of seafood products under generic names were observed. The croaker fish (*Pseudotolithus senegallus*) and *Sardinella maderensis* are listed by the IUCN as vulnerable, with decreasing populations. *Sardinella lemuru* is also listed as near threatened, with decreasing global populations. Conservation statuses of all identified species are presented in Table S1 comments in supplementary materials.

The conservation measures supposed to be afforded to these species are likely to be ignored when they are generally sold under umbrella terms as seen in the croaker species. Sardinella samples also consisted of two different species: *S. lemuru* and *S. maderensis*. Sale of products under generic names highly impedes the effective management of the species (Cawthorn et al., 2018) and as such all measures that can contribute to IUU as the ones mentioned above needs to be addressed. For these reasons, the application of DNA barcoding for detecting mislabelling in seafood products can offer valuable insights into such developments.

4.3.2 Spain

A mislabelling rate of 18% was observed in the Asturian shark samples. Three samples that were labelled as *Raja spp* were identified to be *S. canicula,* commonly called the small-spotted catshark. Although Spain has specific labelling regulations for products of seafood origin (Olsen et al., 2019), high rates of mislabelling have been identified in various seafood (Garcia-Vazquez et al., 2011; Machado-Schiaffino et al., 2008; Muñoz-Colmenero et al., 2016; Pardo & Jimenez, 2020). The mislabelled species, however, is of least concern under the IUCN Red List categories. There was no mislabelling in the remaining 14 samples that were genetically identified to match the name on the labels. It is worth mentioning that the mislabelled samples identified were sold under a generic name (Raja spp) which goes against the labelling regulations of Spain (EC 2065/2001, 2001).

A brief meta-analysis of shark species mislabelling conducted showed similar exploitation of endangered shark species in the UK, where there was a widespread sale of sharks under umbrella terms and mislabelled species were identified as *P. glauca* (Hobbs et al., 2019). In Greek shark samples, analysis by Pazartzi et al. (2019) observed a high mislabelling rate of 55.81%, with *S. canicula* as one of the most mislabelled species. Almerón-Souza et al. (2018) observed in their study of shark exploitation in Southern Brazilian fish markets that *P. glauca* (23.8%) and *Sphyrna lewini* (22.2%) were the most widely traded.

4.4 Conservation Status of Identified Shark Samples

Shark species observed in this study are among coastal shark species traded globally in large quantities. The Blue shark, *P. glauca*, is identified as the most traded pelagic shark species worldwide (Clarke et al., 2006; Coelho et al., 2020). Global landings of *P. glauca* in 2017 alone was 103,528 mt (Okes & Sant, 2019). The lack of specific regulations and catch limits on blue sharks globally explains the high quantities of blue sharks identified in both Ghanaian and Asturian fish markets. 25,000 tonnes out of 42,000 tonnes of annual shark catch by European fleets are made up of blue sharks (The Shark Alliance, 2012). A decreasing trend in the global catch for *P. glauca*, however, shows the rate of overexploitation in the species (Ferretti et al., 2010). Although *P. glauca* species are traded in high quantities, at the time of sample collection, there were no trade regulations for the species by CITES. However, *P. glauca* became listed on Appendix II of the Convention on the Conservation of Migratory Species of Wild Animals (CMS), which came into effect in May 2020.

Two of the seventeen shark samples from Ghana were identified as *S.aculeata*. The sale of Sawback angelshark (*S. aculeata*) which is a critically endangered species in the Ghanaian fish market is of great concern. Shortfin mako sharks (*I. oxyrinchus*) were also identified as part of the traded species in Ghana. *I. oxyrinchus* and *S. zygaena* are widely traded and have been listed in Appendix II of the CITES document which has been in effect since 2014. Both species are also endangered and vulnerable, according to the IUCN Red List.

Ghana is a member and signatory to several international and regional legislation (FAO Port State Measures Agreement (PSMA), ICCAT, and Fisheries Committee for West Central Gulf of Guinea (FCWC)). There are also national regulations such as the Fisheries Act 625 of 2002 and the Fisheries Regulation of 2010 (L.I. 1968) which provides a legal framework for the operations of shark fisheries in Ghana. Regardless of all of these provisions, management of shark fisheries in the country is poor. Regular surveys conducted by the Fisheries Scientific Survey Division of MoFAD to determine catch trends of sharks species show that catch data for sharks are lacking and whenever available, species of different taxonomic groups are lumped together (Vasconcellos et al., 2018). The lack of proper monitoring of sharks can partially explain the results found in this study. Although an equal number of shark samples from both regions were genetically examined in this research, six out of the seven species were from Ghana while only two were from Spain. Even though Spain ranks in the top three in global Shark and Ray catches (The Shark Alliance, 2012),

direct regulations set to protect vulnerable shark species have immensely benefitted these vulnerable species. Regulations such as the setting of Total Allowable Catches (TACs) by the EU ensured fishing pressure on sharks and other endangered species was reduced. TACs for deep-sea sharks were set at zero in 2012 (The Shark Alliance, 2012), and coastal species were monitored by improving data reporting. The EU plan of action on sharks (EUPOA) has been a useful document used to manage shark fisheries in the EU to ensure fishing limits are set in precautionary measures.

This study purposed to see the importance of using genetic tools such as DNA barcoding in identifying discrepancies in the labelling of seafood products. Even though a lot of effort and procedures like sampling, costly laboratory work, extensive data mining, and research are involved in this type of seafood authentication, the results have shown its importance. Technological tools are being applied in the management of marine resources, like the use of electronic monitoring on fishing vessels and seafood traceability applications (Commission et al., 2020; Lewis & Boyle, 2017). The use of molecular techniques in fisheries is however a sure way of correctly identifying trends in seafood consumptions on a global level. The use of DNA barcoding in this study as an authentication tool did not only portray the high level of mislabelling in the Ghanaian shark fisheries but also for the first time identified endangered marine species that are being exploited in Ghanaian waters, even in areas where surveys have failed to detect them (Vasconcellos et al., 2018).

5. CONCLUSIONS AND RECOMMENDATIONS

DNA barcoding has proven to be a relevant genetic tool for species identification and detection of mislabelling in seafood product of diverse origin. Detailed labelling regulations are needed to ensure traceability in the fisheries industry. Laws on conservation of marine species and labelling regulations in developing countries are generally low, and where present, unimplemented (Cawthorn et al., 2015). The use of genetic tools needs to be highly considered for application in species management to ensure the sustainability of marine resources. Data deficient gaps in reporting of shark catch as observed in Ghanaian shark fisheries needs to be addressed. Public education of seafood consumers on the state of fish they consume needs to be highly improved and where necessary, alternatives provided to enable them to make more sustainable choices.

This pilot study, despite being based on a limited number of samples, has provided insights into the state of shark mislabelling rates in both Ghanaian and Spanish fish markets. The result of this study identifies shark species that are critically endangered, near threatened, vulnerable and endangered being traded in both regions as well as commercial trade of the vulnerable law croaker fish and sardinella fish species in Ghana. Hopefully, the main results of this study can be used towards the implementation of informed decisions on effective regulations in both shark and other commercial fisheries by the Ghana Government.

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SUPPLEMENTARY MATERIALS

Table S1. List of all samples, the initial label on package, species identified, GenBank accession numbers, final length in basepairs, result of mislabelling, and conservation status of samples

Sample ID	Name on label	Genetically identified species	GenBank accession number	Length	Mislabelling	IUCN Status	CITES Listing
Ghana samples							
S1	Brown shark	*Prionace glauca*	MH194481.1	657	YES	Near threatened	Not listed
S2	Brown shark	*Prionace glauca*	KF590237.1	630	YES	Near threatened	Not listed
S3	Brown shark	*Squatina aculeata*	KR610532.1	626	YES	Critically endangered	Not listed
S4	Brown shark	*Prionace glauca*	KJ146042.1	599	YES	Near threatened	Not listed
S5	Brown shark	*Prionace glauca*	KJ146042.1	630	YES	Near	Not listed

						threatened	
S6	Brown shark	*Prionace glauca*	KJ146042.1	630	YES	Near threatened	Not listed
S7	Brown shark	*Isurus oxyrinchus*	KJ146030.1	600	YES	Endangered	Appendix II
S8	Brown shark	*Isurus oxyrinchus*	KJ146030.1	570	YES	Endangered	Appendix II
S9	Brown shark	*Prionace glauca*	KJ146042.1	611	YES	Near threatened	Not listed
S10	Brown shark	*Prionace glauca*	KJ146042.1	631	YES	Near threatened	Not listed
S11	Brown shark	*Vibrio diabolicus*					
S12	Brown shark	*Squatina aculeata*	KR610532.1	641	YES	Critically endangered	Not listed
S13	Brown shark	*Vibrio diabolicus*					
S14	Brown shark	*Prionace glauca*	MH719984.1	577	YES	Near threatened	Not listed
S15	Brown shark	*Vibrio diabolicus*					
S16	Brown shark	*Carcharhinus signatus*	FJ519159.1	599	YES	Vulnerable	Not listed
S17	Nurse shark	*Sphyrna zygaena*	MH194504.1	621	YES	Vulnerable	Appendix II
S18	Bull shark	*Galeocerdo cuvier*	MH911012.1	549	YES	Near threatened	Not listed
S19	Hammerhead shark	*Sphyrna zygaena*	MH194422.1	680	NO	Vulnerable	Appendix II
S20	Hammerhead shark	*Prionace glauca*	MH194480.1	641	YES	Near threatened	Not listed
S21-S29	Chub mackerel	*Photobacterium damselae*					
S30	Chub mackerel	*Scomber colias*	KT074092.1	630	NO	Least concern	
S31	Croaker	*Pseudotolithus senegallus*	KP722769.1	630	NO	Vulnerable	
S32	Croaker	*Pseudotolithus senegallus*	KP722769.1	650	NO	Vulnerable	
S33	Croaker	*Pseudotolithus senegallus*	KP722769.1	630	NO	Vulnerable	
S34	Croaker	*Pseudotolithus senegallus*	KP722769.1	610	NO	Vulnerable	
S35	Croaker	*Pseudotolithus senegallus*	KP722769.1	620	NO	Vulnerable	
S36	Croaker	*Pseudotolithus senegallus*	KP722769.1	610	NO	Vulnerable	
S37	Croaker	*Pseudotolithus senegallus*	KP722769.1	630	NO	Vulnerable	
S38	Croaker	*Pseudotolithus senegallus*	KP722769.1	630	NO	Vulnerable	
S39	Croaker	*Pseudotolithus senegallus*	KP722769.1	640	NO	Vulnerable	
S40	Croaker	*Pseudotolithus senegallus*	KP722769.1	630	NO	Vulnerable	
S41	Sardinella	*Sardinella maderensis*	AP009143.1	630	NO	Vulnerable	
S42	Sardinella	*Shewanella loihica*					
S43	Sardinella	*Sardinella*	AP009143.1	630	NO	Vulnerable	

		maderensis					
S44	Sardinella	*Sardinella maderensis*	AP009143.1	630	NO	Vulnerable	
S45	Sardinella	*Sardinella maderensis*	AP009143.1	630	NO	Vulnerable	
S46	Sardinella	*Sardinella maderensis*	AP009143.1	620	NO	Vulnerable	
S47	Sardinella	*Sardinella maderensis*	AP009143.1	630	NO	Vulnerable	
S48	Sardinella	*Sardinella lemuru*	MT294005.1	630	NO	N/A	
S58	Shrimp	*Xiphopenaeus kroyeri*	KY449139.1	621	NO	N/A	
S59	Shrimp	*Xiphopenaeus kroyeri*	KY449157.1	631	NO	N/A	
S60	Shrimp	*Xiphopenaeus kroyeri*	KY449139.1	611	NO	N/A	
S61	Shrimp	*Xiphopenaeus kroyeri*	KY449139.1	621	NO	N/A	
S62	Shrimp	*Xiphopenaeus kroyeri*	KY449139.1	621	NO	N/A	
S63	Shrimp	*Xiphopenaeus kroyeri*	KY449139.1	631	NO	N/A	
S64	Shrimp	*Xiphopenaeus kroyeri*	LC477202.1	671	NO	N/A	
S65	Shrimp	*Xiphopenaeus kroyeri*	KY449139.1	578	NO	N/A	
S66	Shrimp	*Xiphopenaeus kroyeri*	KY449139.1	621	NO	N/A	
S67	Shrimp	*Photobacterium damselae*					
S68	Anchovy	*Engraulis encrasicolus*	KP136718.1	630	NO	Least concern	
S69	Anchovy	*Engraulis encrasicolus*	KU056680.1	678	NO	Least concern	
S70	Anchovy	*Engraulis encrasicolus*	KP940607.1	670	NO	Least concern	
S71	Anchovy	*Engraulis encrasicolus*	KY176470.1	650	NO	Least concern	
S72	Anchovy	*Engraulis encrasicolus*	KP940607.1	674	NO	Least concern	
S73	Anchovy	*Engraulis encrasicolus*	MN893191.1	677	NO	Least concern	
S74	Anchovy	*Engraulis encrasicolus*	KU056680.1	676	NO	Least concern	
S75	Anchovy	*Engraulis encrasicolus*	KP940607.1	686	NO	Least concern	
S76	Anchovy	*Engraulis encrasicolus*	KP940607.1	679	NO	Least concern	
S77	Anchovy	*Engraulis encrasicolus*	KP940607.1	694	NO	Least concern	
Asturias samples							
S4	Scyliorhinus sp	*Scyliorhinus canicula*	KY949095.1	314	NO	Least concern	Not listed
S5	Scyliorhinus sp	*Scyliorhinus canicula*	KY949053.1	385	NO	Least concern	Not listed
S7	Scyliorhinus sp	*Scyliorhinus canicula*	KJ205429.1	601	NO	Least concern	Not listed
S8	Raja sp	*Scyliorhinus*	KJ205429.1	601	YES	Least	Not listed

		canicula				concern	
S9	Raja sp	*Scyliorhinus canicula*	KJ205182.1	601	YES	Least concern	Not listed
S10	Raja sp	*Scyliorhinus canicula*	KJ205180.1	601	YES	Least concern	Not listed
S17	Prionacea glauce "Caella"	*Prionace glauca*	MN641801.1	278	NO	Near threatened	Not listed
S18	Prionacea glauce "Caella"	*Prionace glauca*	MN641800.1	221	NO	Near threatened	Not listed
S21	Prionacea glauce "Caella"	*Prionace glauca*	MN641800.1	236	NO	Near threatened	Not listed
S27	Prionace glauca "tintorera"	*Prionace glauca*	MH719984.1	601	NO	Near threatened	Not listed
S28	Prionace glauca "tintorera"	*Prionace glauca*	MH719984.1	591	NO	Near threatened	Not listed
S30	Prionace glauca "tintorera"	*Prionace glauca*	MH719984.1	601	NO	Near threatened	Not listed
S31	Prionace glauca "tintorera"	*Prionace glauca*	MH719984.1	601	NO	Near threatened	Not listed
S32	Prionace glauca "tintorera"	*Prionace glauca*	MG703523.1	601	NO	Near threatened	Not listed
S33	Prionace glauca "tintorera"	*Prionace glauca*	MH719984.1	591	NO	Near threatened	Not listed
S34	Prionace glauca "tintorera"	*Prionace glauca*	MH719984.1	591	NO	Near threatened	Not listed
S35	Prionace glauca "tintorera"	*Prionace glauca*	MH719984.1	601	NO	Near threatened	Not listed

Table S2. BLAST details of species identified. E-value, percent identities, total score and query cover of sequences submitted to GenBank.

Sample	Genetically identified species	Common name of identified species	E-value	Percent identity %	Score	Query cover %
S1	*Prionace glauca*	Blue shark	0.0	99.85	1206	99
S2	*Prionace glauca*	Blue shark	0.0	100.00	1144	98
S3	*Squatina aculeata*	Sawback angelshark	0.0	100.00	1120	96
S4	*Prionace glauca*	Blue shark	0.0	99.00	1074	100
S5	*Prionace glauca*	Blue shark	0.0	99.52	1146	99
S6	*Prionace glauca*	Blue shark	0.0	100.00	1158	99
S7	*Isurus oxyrinchus*	Shortfin mako	0.0	99.83	1103	100
S8	*Isurus oxyrinchus*	Shortfin mako	0.0	95.09	898	100
S9	*Prionace glauca*	Blue shark	0.0	97.71	1051	100
S10	*Prionace glauca*	Blue shark	0.0	99.68	1153	100
S11	*Vibrio diabolicus*	Bacteria				
S12	*Squatina aculeata*	Sawback angelshark	0.0	99.69	1168	99
S13	*Vibrio diabolicus*	Bacteria				

S14	*Prionace glauca*	Blue shark	0.0	96.01	931	99
S15	*Vibrio diabolicus*	Bacteria				
S16	*Carcharhinus signatus*	Night shark	0.0	98.83	1068	100
S17	*Sphyrna zygaena*	smooth hammerhead	0.0	99.84	1140	100
S18	*Galeocerdo cuvier*	Tiger Shark	0.0	100.00	1014	100
S19	*Sphyrna zygaena*	smooth hammerhead	0.0	100.00	1256	100
S20	*Prionace glauca*	Blue shark	0.0	99.69	1160	99
S21-S29	*Photobacterium damselae*					
S30	*Scomber colias*	Atlantic chub mackerel	0.0	94.25	929	99
S31	*Pseudotolithus senegallus*	Law croaker	0.0	99.84	1149	99
S32	*Pseudotolithus senegallus*	Law croaker	0.0	99.39	1179	100
S33	*Pseudotolithus senegallus*	Law croaker	0.0	99.84	1153	99
S34	*Pseudotolithus senegallus*	Law croaker	0.0	91.93	848	99
S35	*Pseudotolithus senegallus*	Law croaker	0.0	100.00	1147	100
S36	*Pseudotolithus senegallus*	Law croaker	0.0	89.72	780	99
S37	*Pseudotolithus senegallus*	Law croaker	0.0	100.00	1164	99
S38	*Pseudotolithus senegallus*	Law croaker	0.0	99.84	1155	99
S39	*Pseudotolithus senegallus*	Law croaker	0.0	99.37	1147	98
S40	*Pseudotolithus senegallus*	Law croaker	0.0	99.84	1151	99
S41	*Sardinella maderensis*	Madeiran sardinella	0.0	100.00	1157	99
S42	*Shewanella loihica*	Bacteria				
S43	*Sardinella maderensis*	Madeiran sardinella	0.0	100.00	1157	99
S44	*Sardinella maderensis*	Madeiran sardinella	0.0	99.68	1149	99
S45	*Sardinella maderensis*	Madeiran sardinella	0.0	99.68	1153	100
S46	*Sardinella maderensis*	Madeiran sardinella	0.0	99.52	1131	100
S47	*Sardinella maderensis*	Madeiran sardinella	0.0	100.00	1158	99
S48	*Sardinella lemuru*	Bali sardinella	0.0	99.05	1131	100
S58	*Xiphopenaeus kroyeri*	Atlantic seabob	0.0	88.42	708	94
S59	*Xiphopenaeus kroyeri*	Atlantic seabob	0.0	88.47	713	93
S60	*Xiphopenaeus kroyeri*	Atlantic seabob	0.0	87.01	652	95
S61	*Xiphopenaeus kroyeri*	Atlantic seabob	0.0	88.42	708	94
S62	*Xiphopenaeus kroyeri*	Atlantic seabob	0.0	88.42	708	94
S63	*Xiphopenaeus kroyeri*	Atlantic seabob	0.0	88.42	708	93
S64	*Xiphopenaeus kroyeri*	Atlantic seabob	0.0	86.34	717	98
S65	*Xiphopenaeus kroyeri*	Atlantic seabob	0.0	87.48	662	99
S66	*Xiphopenaeus kroyeri*	Atlantic seabob	0.0	88.42	708	94
S67	*Photobacterium damselae*	Bacteria				
S68	*Engraulis encrasicolus*	European anchovy	0.0	99.84	1146	99
S69	*Engraulis encrasicolus*	European anchovy	0.0	100.00	1203	96
S70	*Engraulis encrasicolus*	European anchovy	0.0	99.85	1203	97
S71	*Engraulis encrasicolus*	European anchovy	0.0	100.00	1184	98
S72	*Engraulis encrasicolus*	European anchovy	0.0	99.85	1203	97
S73	*Engraulis encrasicolus*	European anchovy	0.0	100.00	1206	96
S74	*Engraulis encrasicolus*	European anchovy	0.0	100.00	1203	96
S75	*Engraulis encrasicolus*	European anchovy	0.0	99.85	1203	95
S76	*Engraulis encrasicolus*	European anchovy	0.0	100.00	1208	96
S77	*Engraulis encrasicolus*	European anchovy	0.0	100.00	1208	94

S4	*Scyliorhinus canicula*	Small-spotted catshark	0.0	100.00	1092	100
S5	*Scyliorhinus canicula*	Small-spotted catshark	0.0	100.00	1092	100
S7	*Scyliorhinus canicula*	Small-spotted catshark	0.0	100.00	1110	100
S8	*Scyliorhinus canicula*	Small-spotted catshark	0.0	100.00	1110	100
S9	*Scyliorhinus canicula*	Small-spotted catshark	0.0	99.67	1098	100
S10	*Scyliorhinus canicula*	Small-spotted catshark	0.0	100.00	1110	100
S17	*Prionace glauca*	Blue shark	1E-141	100.00	514	100
S18	*Prionace glauca*	Blue shark	4E-110	100.00	409	100
S21	*Prionace glauca*	Blue shark	2E-118	100.00	436	100
S27	*Prionace glauca*	Blue shark	0.0	100.02	1109	99
S28	*Prionace glauca*	Blue shark	0.0	100.04	1050	99
S30	*Prionace glauca*	Blue shark	0.0	100.06	1110	100
S31	*Prionace glauca*	Blue shark	0.0	100.08	1110	100
S32	*Prionace glauca*	Blue shark	0.0	100.10	1110	100
S33	*Prionace glauca*	Blue shark	0.0	100.12	1092	100
S34	*Prionace glauca*	Blue shark	0.0	100.14	1092	100
S35	*Prionace glauca*	Blue shark	0.0	100.16	1110	100