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Identification of Shark Species in Seafood Products by Forensically Informative Nucleotide Sequencing (FINS)

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The identification of commercial shark species is a relevant issue to ensure the correct labeling of seafood products, to maintain consumer confidence in seafood, and to enhance the knowledge of the species and volumes that are at present being captured, thus improving the management of shark fisheries. The polymerase chain reaction was employed to obtain a 423 bp amplicon from the mitochondrial cytochrome *b* gene. The sequences from this fragment, belonging to 63 authentic individuals of 23 species, were analyzed using a genetic distance method. Nine different samples of commercial fresh, frozen, and convenience food were obtained in local and international markets to validate the methodology. These samples were analyzed, and sequences were employed for species identification, showing that forensically informative nucleotide sequencing (FINS) is a suitable technique for identification of processed seafood containing shark as an ingredient. The results also showed that incorrect labeling practices may occur regarding shark products, probably because of incorrect labeling at the production point.

KEYWORDS: Shark; cytochrome *b*; FINS; incorrect labeling; species identification

1. INTRODUCTION

Shark is the common name used for a group of fish with a cartilaginous skeleton; this feature differentiates them from the Osteichthyes or bony fishes. Sharks can be found in food markets in different presentations, depending upon the country. The main processed products from sharks include meat (fresh, frozen, salted/in brine, and smoked) and fins (mostly for shark-fin soup) (*1*).

Although the subclass Elasmobranch (including sharks and batoids) is comprised of 10 orders, 44 families, and 108 genera, representing nearly 480 species (*2, 3*), only about 12 shark species are commercially exploited (*4*). The importance of shark fisheries has been growing in the last few decades; thus, world catches of chondrichthyans increased 3-fold from 1950 to 2000 (*1*). This is probably because of the increase in demand for shark fins as well as the decline in catches of some traditional fish species.

During the past decade, the European Union has been issuing more strict food labeling and traceability regulations for fish and seafood products, specifying, for instance, the information that must be included on the labels. Thus, the EU labeling regulation (EC 104/2000) (*5*) specifies that both the scientific name and the approved commercial name must be included on the label of seafood products. For the purposes of this regulation, the Spanish government has published an updated list of the commercial designations accepted in the national territory for fish species, including several sharks. The list indicates the scientific name for each species, its commercial name, and other name or names accepted locally (*6*).

These new policies make it necessary to have adequate tools to verify, among other requirements, the authenticity of the species indicated on the label of seafood products and to correctly identify the shark captures and landed products at the species level. Confirming the correct labeling of seafood products in markets is therefore essential to avoid possible commercial fraud, to allow better control of commercialization of shark species, and also for conservation purposes.

Identification of shark species can be considered especially problematic because of several reasons. There is an ongoing debate regarding the phylogenetic relationships among elasmobranchs, which are still unclear and under discussion. Also, the way sharks are caught (multispecific fisheries), the practice of finning (cut the fins off and throw the carcass back in the water), the development of factory vessels with the capacity to process thousands of tons of fish, and the changes in consumer habits toward highly elaborated and convenience products are a considerable hurdle to species identification, making species identification during port inspections very difficult or even impossible (*7*−*9*).

Several methods of identifying fish species have been proposed. Some of them include the analysis of species-specific components of the fish, such as proteins, lipids, or nucleic acids. Because proteins become denatured when food products are

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Table 1. Authentic Fish Species

fish species	common name (FAO)	code	N^a	haplotypes	source of sequence
order Carcharhiniformes					
family Carcharhinidae					
Prionace glauca	blue shark	PGLA	8	5	this study
Carcharhinus obscurus	dusky shark	COBS	2	2	this study
Carcharhinus limbatus	blacktip shark	CLIM	2	1	this study
family Sphyrnidae					
Sphyrna lewini	scalloped hammerhead	SLEW	3	3	this study
Sphyrna mokarran	great hammerhead	SMOK	1	1	this study
Sphyrna tiburo	bonnethead	STIB	1	1	Genbank
family Triakidae					
Mustelus mustelus	smooth-hound	MMUS	1	1	this study
family Scyliorhinidae					
Galeus melastomus	blackmouth catshark	GMEL	4	4	this study
Apristurus sp.	blackroughscale catshark	APRI	2	1	this study
order Squaliformes					
family Squalidae					
Squalus acanthias	piked dogfish	SACA	2	2	this study
Etmopterus pusillus	smooth lanternshark	EPUS	1	1	this study
Centrophorus granulosus	gulper shark	CGRA	1	1	this study
Centroscyllium fabricii	black dogfish	CFAB	2	2	this study
Centrophorus squamosus	leafscale gulper shark	CSQU	1	1	this study
Centroscymnus coelolepμs	Portuguese dogfish	CCOE	1	1	this study
Deania calcea	birdbeak dogfish	DCAL	1	1	this study
Centroscymnus crepidater	longnose velvet dogfish	CCRE	1	1	this study
order Lamniformes					
family Alopidae					
Alopias superciliosus	bigeye thresher	ASUP	4	1	this study
Alopias vulpinus	thresher	AVUL	3	1	this study
family Lamnidae					
Isurus oxyrinchus	shortfin mako	IOXY	7	4	this study
Lamna ditropis	salmon shark	LDIT	1	1	Genbank
Lamna nasus	porbeagle	LNAS	12	6	this study
family Odontaspidae					
Carcharias taurus (*Eugomphodus taurus*)	sandtiger shark	CTAU	2	2	this study

[a] N = number of individuals sequenced.

subjected to thermal treatments, their use is restricted to fresh or frozen seafood products; besides, the extraordinary development of molecular biology techniques in recent decades, such as the polymerase chain reaction (PCR), has made DNA sequences the main choice for species identification (*10*). Shark species identification using DNA has previously been developed to address particular fishery problems (*11*), to clarify phylogenetic relationships of elasmobranch (*12, 13*), or to reveal captures of threatened shark species (*14, 15*). Previous studies have demonstrated the usefulness of the forensically informative nucleotide sequencing (FINS) technique for fish species identification in seafood products (*16, 17*). This technique was first used by Barlett and Davidson (*18*), who demonstrated that the estimation of genetic distances between a group of reference sequences and an unknown sequence allows for the identification of the species of the latter. The unknown sequence shows the lowest genetic distance with the group of species sequences to which it belongs (*16, 19, 20*); this fact is reflected in a phylogenetic tree, where sequences of the same species will be grouped in the same clade.

The objective of this work was to study the applicability of FINS for the discrimination and identification of shark species used in foodstuffs and to validate this methodology with commercial samples.

2. MATERIALS AND METHODS

2.1. Elasmobranch Analyzed. A total of 63 authentic individuals belonging to 23 species were studied. Within these 23 species, 21 were sequenced in our laboratory and 2 were obtained from Genbank. The 23 species belong to 8 families, representing 3 orders (**Table 1**). Some specimens of authentic species were obtained fresh at the local fish auction, whereas others were obtained from commercial and research vessels. All of these animals were reliably classified by an experienced taxonomist. Samples of muscle were stored frozen (−20 °C) until analyzed. Nine samples of commercialized fresh, frozen, and convenience food were obtained in local and international markets. These samples were frozen shark slices (five samples), fresh shark slice (one sample), and precooked shark products (three samples).

2.2. DNA Extraction. The commercial samples were prepared as raw muscle with a prior rinse using sterile distilled water. For the DNA extraction, a tissue sample of 250 mg was placed in an Eppendorf tube and suspended in 860 μL of lysis buffer containing 2 mM of ethylenediaminetetraacetic acid (EDTA), 150 mM of NaCl, 1% of sodium dodecyl sulfate (SDS), and 10 mM Tris-HCl at pH 8. A total of 120 μL of guanidium thiocyanate (Sigma-Aldrich, St. Louis, MI) and 40 μL of proteinase K solution (20 mg/mL) (Gibco Invitrogen S.A., Prat de Llobregat, Spain) were added to this and incubated in a waterbath at 56 °C. After 2 h, extra 40 μL of proteinase K were added to the solution and left overnight in the waterbath at 56 °C. Then, the resulting digest was centrifuged, and the supernatant was collected.

For the isolation of the DNA, 500 μL of the collected solution was placed in a syringe barrel attached to a Wizard minicolumn, to which 1 mL of Wizard DNA clean-up resin (Promega, Madison, WI) was added. Then, vacuum was applied to draw the solution through the minicolumn. The column was washed with 2 mL of 80% isopropanol, reapplying the vacuum. The column was then transferred to a clean microfuge tube and spun for 2 min.

The DNA was eluted from the column by adding 50 μL of water prewarmed at 70 °C, left to stand 1 min, and centrifuged at 10000*g* for 20 s. The DNA solution was collected and stored at −20 °C.

2.3. DNA Quantification. DNA content in the extracts was quantified by measuring the absorbance of the DNA extracts at 260 nm and

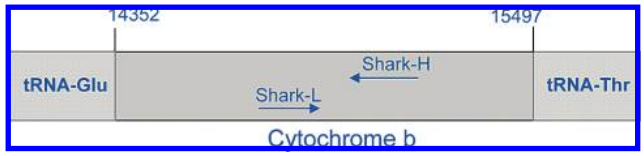

Figure 1. Cytochrome *b* fragment selected for studying the identification of shark species. Arrows Shark L and Shark H show the approximate position of primers used within the cytochrome *b* gene. Numbers 14 352 and 15 497 correspond to the starting and ending nucleotide position of the cytochrome *b* gene within a shark mitochondrial genome.

checking for protein impurities at 280 nm and RNA contamination at 234 nm. One optical density (OD) at 260 nm equaling 50 μg/mL DNA (*21*).

2.4. PCR Amplification of DNA Samples. The primers used for amplification were a modification of those described by Hoelzel (*22*) and Martin (*23*) and modified in our laboratory for this study. These primers were appropriately modified on the basis of alignments made with different shark sequences obtained from Genbank. The primers designed were for the forward primer Shark L: 5′-ACCATGAGGA-CAAATATT-3′ and reverse primer Shark H: 5′-AAGTATCAC-TCGGGTTTGATGTG-3′. The primers used amplify a region of 423 bp, located in the middle of the cytochrome *b* gene (**Figure 1**).

PCR reactions were carried out in volumes of 25 μL using ready-to-go PCR beads (Amersham Biosciences), which contain, when reconstituted, 200 μM of each dNTP in 50 mM KCl, 1.5−2 mM $MgCl_2$, 10 mM Tris-HCl at pH 9 and room temperature, and 1.5 units of Taq polymerase. Finally, 1.5 μL of each primer (20 μM) and 1 μL of the template DNA (150−200 ng/μL) were added to the reaction.

Amplifications were carried out in a GeneAmp 9700 PCR system (Applied Biosystems) with a preheating step of 5 min at 94 °C, then 35 cycles of 40 s at 94 °C, 80 s at 50 °C, 90 s at 72 °C, and a final extension step of 7 min at 72 °C.

2.5. Sequencing of PCR Fragments. Prior to the sequencing reactions, 20 μL of PCR product were treated with 3 μL of ExoSAP-IT (Amhersham Biosciences). The mixture was incubated at 37 °C for 30 min and then at 80 °C for another 15 min. Sequencing reactions were prepared with Big Dye (Applied Biosystems) following the instructions of the manufacturer. The conditions of the sequencing reactions were a preheating step of 3 min at 94 °C, then 25 cycles of 10 s at 96 °C, 5 s at 50 °C, and 4 min at 72 °C.

The extension products were purified using a precipitation procedure, and the pellet obtained was stored at −20 °C. Electrophoresis was carried out in an ABI PRISM 310 DNA sequencer (Applied Biosystems).

2.6. Data Analysis. Sequences of the fragment studied obtained from public databases, such as the GenBank, and the sequences obtained in our laboratory were analyzed using BIOEDIT (*24*) and CLUSTAL (*25*) to align the sequences and MEGA to calculate the genetic distances using the Tamura−Nei method (*26*). Phylogenetic trees for FINS were constructed using the calculated distances employing the neighbor−joining method (*27*), and a bootstrap test was performed for each tree using the MEGA program (*26*).

3. RESULTS

3.1. DNA Sequences and Genetic Analysis. Two primers previously described by Martin and Palumbi (*23*) and Hoelzel (*22*) were appropriately modified in our laboratory (Shark H and Shark L) using the PRIMER EXPRESS program (*28*), to allow for the amplification in all of the species used in this study.

DNA extracted from the species presented in **Table 1** was used to amplify the cytochrome *b* fragment shown in **Figure 1**, using the primers Shark H and Shark L. It was found that this set of primers amplifies a wide range of products from fresh or frozen to highly processed products. PCR products of enough quality to be sequenced were obtained, and the amplified cytochrome *b* fragment was sequenced and analyzed.

Sequences of specimens of the same species were analyzed to find haplotypes using the "number of differences" option from MEGA (*26*), and only these different haplotypes were used for analysis (see **Table 1**). **Table 1** presents the number of haplotypes found for each of the species.

Figure 2 shows the variable positions (196 positions) for all of the species and specimens used in this study. A total of 106 of these positions have two variants; 61 have three variants; and only 29 have four variants. These sequences were used for genetic distance measurement using the Tamura−Nei algorithm. Sequence divergence among haplotypes within species differs approximately 2 orders of magnitude from that among species within the genus (**Table 2**). Distance values ranged from 0.093 to 0.352 in interspecific comparisons between different genera, whereas distance values ranged from 0.099 to 0.159 in interspecific comparisons within the same genus. We also found that intraspecific comparisons ranged from 0.003 to 0.036.

On the basis of these distances, a phylogenetic tree was constructed using the neighbor-joining method. The sequence of a Chimaeriforme, *Chimaera monstrosa*, (Genbank accession number AJ310140.1) was used as an outgroup.

Figure 3 shows the phylogenetic tree constructed with haplotypes of authentic sequences of orders Carcharhiniformes, Lamniformes, and Squaliformes (including two sequences from Genbank: STIBL08043 and LDITU91438).

In **Figure 3**, the three orders are clearly differentiated with high bootstrap values; within each order, each family is separated into different clusters, thus allowing for the adequate differentiation of most of the species studied. Most clades containing multiple haplotypes within species were highly supported by bootstrap analyses. Those species represented by more than a single sequence were shown to be monophyletic groups in 100% of 2000 bootstrap replicates.

3.2. Identification of Commercial Samples of Elasmobranch Using FINS. DNA from different commercial products, including five different types of frozen fillets, one sample of fresh fillet, and three different types of processed fillets, was amplified with the primers Shark L and Shark H. Each commercial sample was identified as is shown in **Table 3**.

In this study, all commercial shark species obtained were successfully sequenced with the designed primers. All commercial samples were also effectively identified after measuring their level of similarity against the pool of our reference sequences (**Figure 4**). **Figure 4** shows an example of the phylogenetic tree obtained with one commercial product (PROD1), after the genetic distance measurement and phylogenetic tree construction. This sequence was grouped with the cluster of SLEW (*Sphyrna lewini*).

4. DISCUSSION

Identification of shark species in seafood products, using DNA techniques, has been studied scarcely. Shark studies using DNA analysis are aimed at the study of population structure and phylogenetic relationships using sequence analysis (*29, 30*), restriction fragment length polymorphism (RFLP) (*31*), or microsatellites (*32, 33*). The identification of shark species in seafood products has been carried out mostly on fins (*15, 34, 22*). Fins reach remarkably high prices on the market, depending upon the species, because they are used mainly to prepare shark-fin soup, which traditionally have an exclusive market among ethnic Chinese groups.

In the Western markets, shark fins are not often consumed, but other shark products can be found. These products include frozen whole shark, which is by far the main product produced,

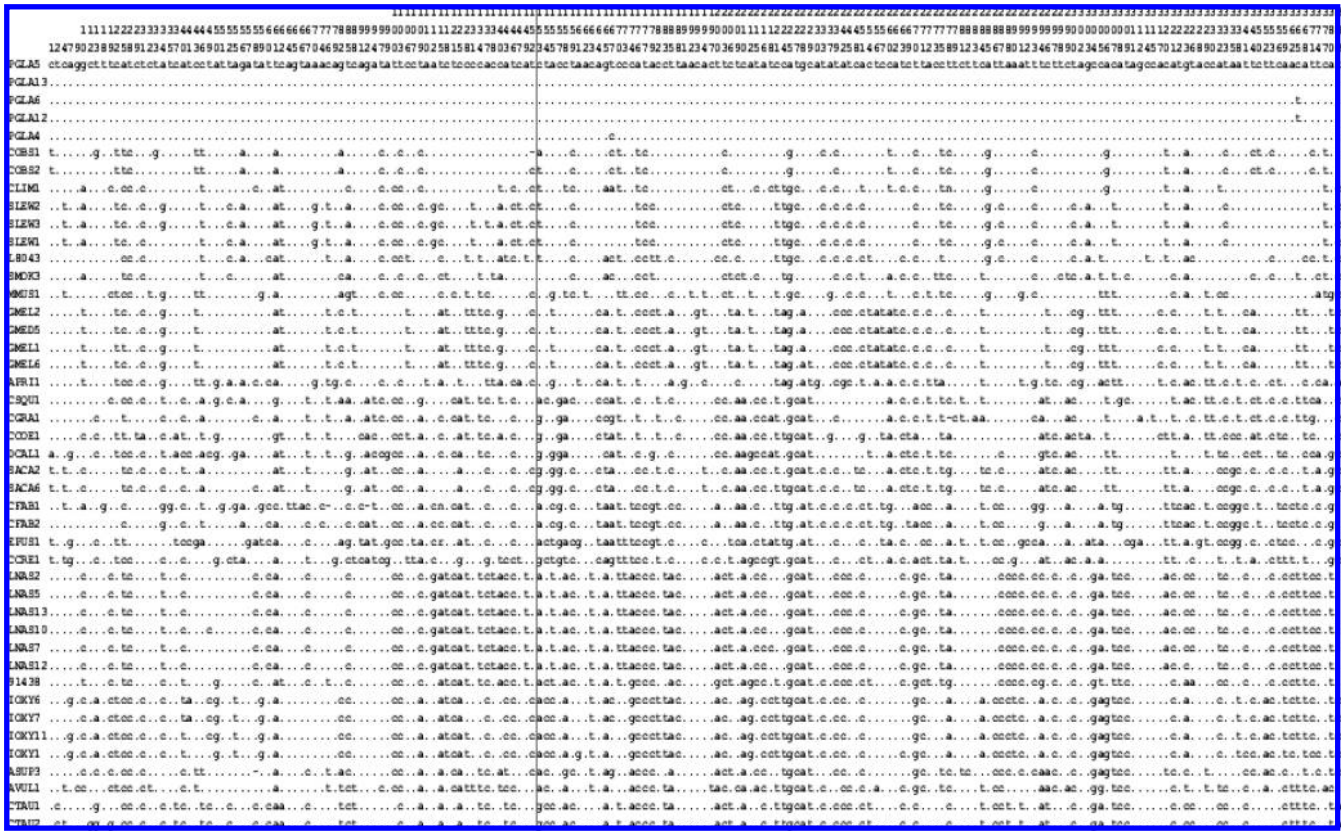

Figure 2. Alignment of partial cytochrome *b* region reference sequences from all individuals analyzed in the study. Dots indicate identity with consensus sequence (upper). Keys for species are provided in **Table 2**.

Table 2. Tamura−Nei Distance Matrix of 23 Different Haplotypes[a]

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1	0.003																						
2	0.11	0.00																					
3	0.144	0.101	0.015																				
4	0.161	0.127	0.093	0.00																			
5	0.198	0.15	0.117	0.105	0.00																		
6	0.174	0.15	0.132	0.137	0.141	0.008																	
7	0.19	0.158	0.156	0.171	0.199	0.209	0.00																
8	0.199	0.26	0.242	0.234	0.233	0.207	0.206	0.004															
9	0.253	0.237	0.248	0.232	0.248	0.22	0.264	0.229	0.00														
10	0.257	0.232	0.224	0.271	0.216	0.234	0.243	0.285	0.255	0.003													
11	0.228	0.216	0.224	0.265	0.24	0.251	0.267	0.286	0.283	0.099	0.011												
12	0.265	0.254	0.243	0.267	0.25	0.293	0.318	0.274	0.298	0.166	0.146	0.00											
13	0.273	0.259	0.247	0.274	0.257	0.295	0.251	0.284	0.289	0.15	0.167	0.177	0.00										
14	0.247	0.219	0.193	0.205	0.22	0.222	0.234	0.258	0.273	0.162	0.173	0.165	0.16	0.003									
15	0.29	0.303	0.288	0.281	0.266	0.33	0.3	0.301	0.322	0.254	0.263	0.284	0.263	0.226	0.00								
16	0.338	0.311	0.317	0.342	0.332	0.324	0.322	0.351	0.372	0.28	0.267	0.296	0.291	0.238	0.206	0.00							
17	0.316	0.314	0.279	0.299	0.27	0.343	0.327	0.311	0.328	0.234	0.248	0.276	0.238	0.228	0.293	0.238	0.00						
18	0.288	0.282	0.253	0.272	0.25	0.243	0.269	0.298	0.329	0.251	0.259	0.312	0.285	0.265	0.256	0.304	0.314	0.00					
19	0.289	0.28	0.235	0.255	0.225	0.245	0.253	0.259	0.328	0.235	0.249	0.284	0.256	0.234	0.257	0.322	0.295	0.075	0.00				
20	0.291	0.303	0.266	0.292	0.288	0.295	0.306	0.294	0.34	0.262	0.279	0.303	0.276	0.242	0.289	0.311	0.292	0.172	0.146	0.008			
21	0.268	0.244	0.217	0.246	0.252	0.223	0.252	0.288	0.317	0.228	0.258	0.287	0.265	0.27	0.283	0.273	0.291	0.137	0.166	0.15	0.036		
22	0.299	0.316	0.265	0.248	0.273	0.286	0.255	0.258	0.32	0.23	0.289	0.309	0.26	0.257	0.286	0.352	0.326	0.172	0.179	0.17	0.16	0.00	
23	0.281	0.294	0.249	0.243	0.221	0.257	0.275	0.246	0.299	0.243	0.285	0.321	0.256	0.235	0.249	0.315	0.279	0.173	0.17	0.19	0.18	0.16	0.00

[a] Codes: 1, LNAS; 2, LDIT; 3, IOXY; 4, ASUP; 5, AVUL; 6, CTAU; 7, CLIM; 8, SLEW; 9, STIB; 10, PGLA; 11, COBS; 12, SMOK; 13, MMUS; 14, GMEL; 15, APRI; 16, CSQU; 17, CGRA; 18, CCOE; 19, DCAL; 20, SACA; 21, CFAB; 22, EPUS; 23, CCRE.

followed by dried, salted or shark in brine, as well as frozen shark fillets (*4*). DNA analysis allows the use of a single method for the whole range of products (*9*), and although it is necessary to obtain and analyze reference species beforehand, this is only performed once.

The combination of the PCR, which is a rapid, sensitive, specific technique, with FINS is currently one of the most reliable ways of identifying species. One of its main advantages is that results are not affected by intraspecific variability (*35*). Although some authors state that sequencing is a laborious technique, which requires very expensive equipment and highly qualified technicians, today the genomic revolution has brought cost-effective sequencing tools, making routine sequencing samples for species identification practical and optimal (*29*).

In this work, we present sequences of shark specimens belonging to three different orders as classified by Compagno (*2*).

The results obtained confirm that sharks show little intraspecific sequence variation (*23*). Also, our analysis of distances

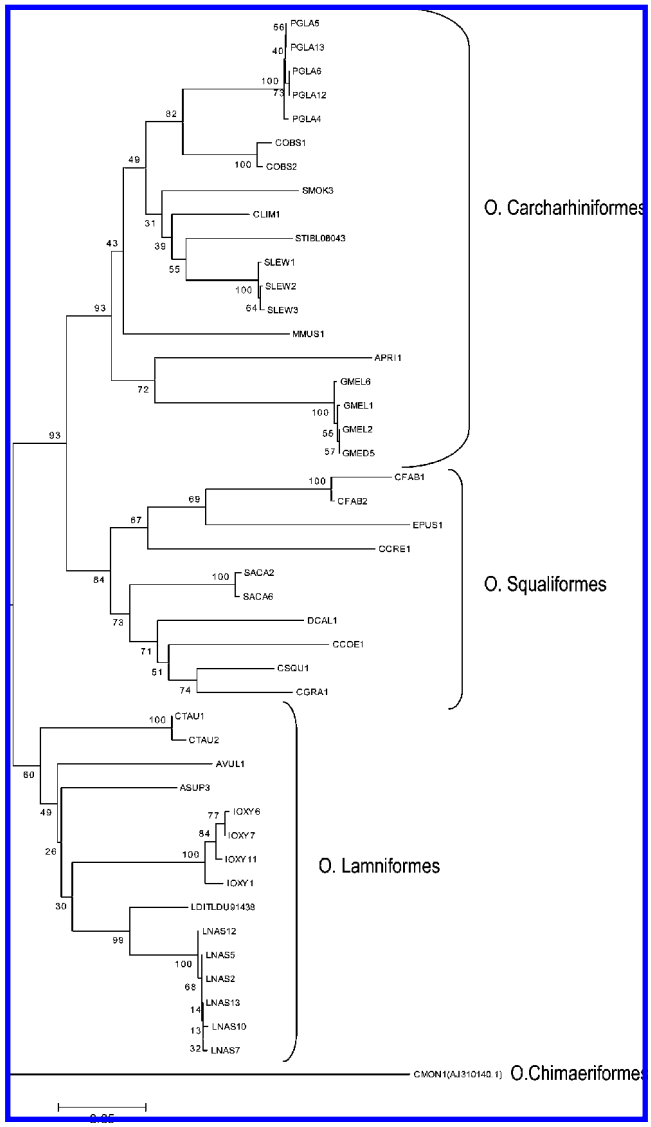

Figure 3. Phylogenetic tree of partial cytochrome *b* fragment sequences using Tamura−Nei distances and the neighbor-joining method. The significance of each branch is indicated by the bootstrap test. Four orders (Carcharhiniformes, Squaliformes, Lamniformes, and Chimaeriformes) with their correspondent sequences are shown.

Table 3. Species Identification in Nine Different Seafood Products Analyzed

type of seafood product	species identification by FINS	code
frozen shark slice lot 1	*Sphyrna lewini*	PROD1
frozen shark slice lot 2	*Prionace glauca*	PROD2
frozen shark slice lot 3	*Prionace glauca*	PROD3
frozen shark slice lot 4	*Prionace glauca*	PROD4
frozen shark slice lot 5	*Prionace glauca*	PROD5
fresh shark slice lot 6	*Isurus oxyrinchus*	PROD6
precooked shark lot 1	*Squalus acanthias*	PROD7
precooked shark lot 2	*Squalus acanthias*	PROD8
precooked shark lot 3	*Lamna ditropis*	PROD9

(**Table 2**) shows that sequence divergence within species is minor compared to divergences at the interspecific level: intraspecific distance values are 2 orders of magnitude bigger than those of interspecific comparisons. Therefore, the combination of low intraspecific variability with enough interespecific divergence indicates that the fragment studied is suitable and allows for unambiguous differentiation at the species level. This

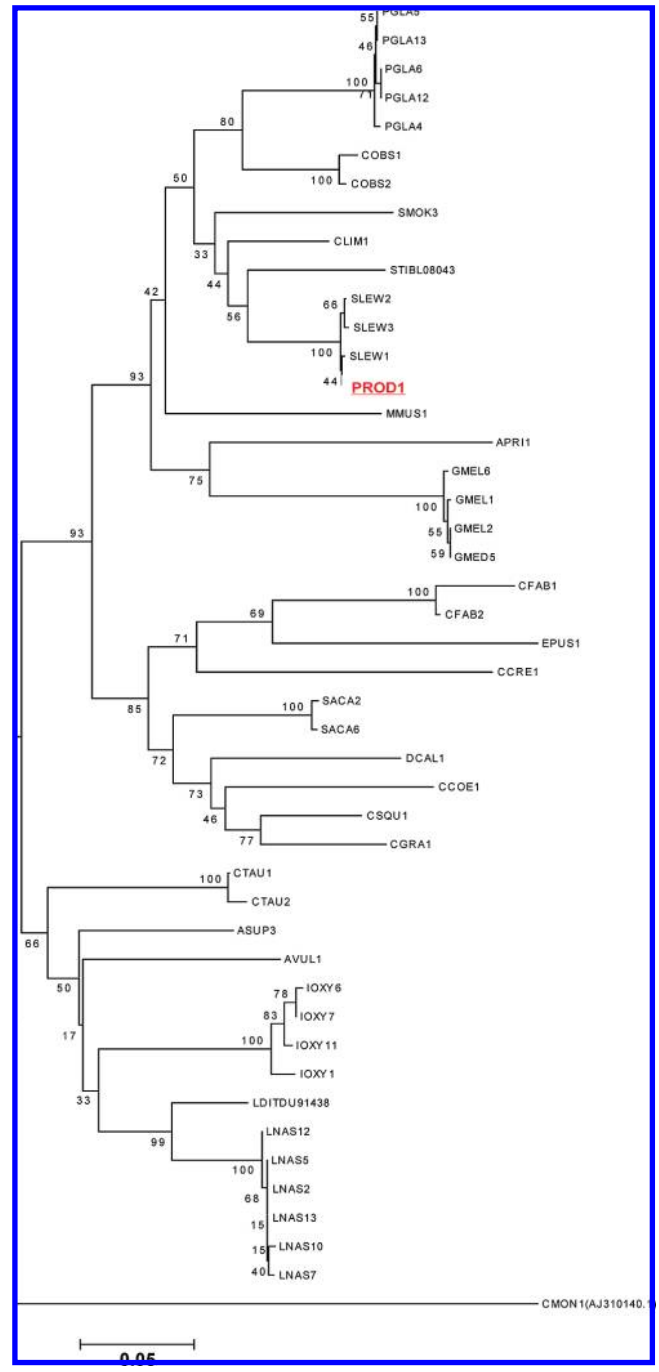

Figure 4. Identification of the commercial sample "frozen shark slices lot 1" (PROD1) by FINS. The significance of each branch is indicated by the bootstrap test. Keys for species are provided in **Table 2**.

finding reassures the value of this cytochrome *b* locus, used previously (*22*), to successfully differentiate shark species.

It is well-known that consumer habits have changed considerably nowadays with an increasing demand for convenience and ready-to-eat seafood. Most of these products are made from raw material, which often lacks morphological characteristics, thus hindering the process of species identification (*9*). The need becomes obvious for analytical procedures, which enable the precise identification of fish species employed for convenience seafood, to comply with labeling regulations. In the case of sharks, it is even more important because various species are often classified and labeled with the generic term "shark". Here, we have demonstrated not only the accuracy and efficiency of the combination of our primers with the FINS technique to

differentiate between several shark species but also its usefulness in identifying nine commercial seafood products. In this study, it is shown that the primers used generated strong amplification products with DNA isolated from a variety of processed products, from fresh and frozen muscle to convenience food.

The increase in the consumption of shark species as seafood is documented not only by the increased volume of shark captures but also by the amount of shark products that can be seen in Western markets at present.

Therefore, the correct classification of sharks will allow not only for enhancement of the knowledge of the species and the volumes that are in fact being captured, thus improving the management of shark fisheries, but also for compliance with labeling regulations, in turn maintaining consumer confidence in seafood. Cases have been documented of fish substitution, generally substituting cheaper fish for more expensive fish (*9*). Such practices are likely to be more common with bony fish. Regarding sharks, misidentification may occur partly because of the unclear status of the classification of Chondrichthyes, both morphologically and genetically, as well as the lack of records of catches of many exploited species (*15, 7*). This is consistent with our results, because two of the shark products used in the study (products 1 and 2) have misleading labels. These labels showed disparity between commercial and scientific names.

In summary, we have found that incorrect labeling practices may occur regarding shark products, probably because of incorrect labeling at the production point (vessels, fish market, etc.). If these problems need to be solved to comply with labeling regulations, there is a necessity to assess shark species identification at different levels: fishing vessels, fish auctions, fish processing companies, and fish markets.

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LITERATURE CITED

(1) Food and Agriculture Organization (FAO). Fisheries Circular No. 990, World markets and industry of selected commercially exploited aquatic species with an international conservation profile, 2004.

(2) Compagno, L. J. V. FAO species catalogue. Vol. 4. Sharks of the world. An annotated and illustrated catalogue of shark species known to date. Part 1. Hexanchiformes to Lamniformes. FAO Fish Synopsis (125), 1984, Vol. 4, pp 1−249.

(3) Compagno, L. J. V. Checklist of living elasmobranch, 1999 (http://www.reefquest.com/topics/checlis_res.htm).

(4) Vannuccini, S. Shark utilization, marketing and trade. FAO Fisheries Technical Papers 389, 1999.

(5) Council Regulation (EC) No. 104/2000 of Dec 17, 1999 on the common organization of the markets in fishery and aquaculture products.

(6) Spanish regulation Feb 7, 2008 from Secretaría General de Pesca Marítima (BOE No. 56, Mar 5, 2008).

(7) Abercrombie, D. L.; Clarke, S. C.; Shivji, M. S. Global-scale genetic identification of hammerhead sharks: Application to assessment of the international fin trade and law enforcement. *Conserv Genet.* **2005**, *6*, 775–788.

(8) Walker, T. I. Can shark resources be harvested sustainably? A question revisited with a review of shark fisheries. *Mar. Freshwater Res.* **1998**, *49*, 553–572.

(9) Calo-Mata, P.; Sotelo, C.; Pérez-Martín, R. I.; Rebíen, H.; Hola, G. L.; Russell, V. J.; Pryde, S.; Quinteiro, J.; Rey-Méndez, M.; Carla Rosa; Santos, A. T. Identification of gadoid fish species using DNA-based techniques. *Eur. Food. Res. Technol.* **2003**, *217* (3), 259–264.

(10) Sotelo, C. G.; Pérez-Martín, R. I. *Food Authenticity and Traceability. Species Identification in Processed Seafood*; Woodhead Publishing Limited: Cambridge, U.K., 2003; Chapter 14, pp 323−346.

(11) Keeney, D. B.; Heupel, M.; Hueter, R. E.; Heist, E. J. Genetic heterogeneity among blacktip shark, *Carcharhinus limbatus*, continental nurseries along the U.S. Atlantic and Gulf of Mexico. *Mar. Biol.* **2003**, *143*, 1039–1046.

(12) Kitamura, T.; Takemura, A.; Watabe, S.; Taniuchi, T.; Shimizu, M. Molecular phylogeny of the sharks and rays of the superorder Squalea based on mitochondrial cytochrome *b* gene. *Fish. Sci.* **1996**, *62*, 344–343.

(13) Winchell, C. J.; Martin, A. P.; Mallatt, J. Phylogeny of elasmobranch base don LSU and SSU ribosomal RNA genes. *Mol. Phylogenet. Evol.* **2004**, *31*, 214–224.

(14) Chan, R. W. K. Application of DNA-based techniques for the identification of whaler sharks (*Carcharhinus* spp.) caught in protective beach meshing and by recreational fisheries off the coast of New South Wales. *Fish. Bull.* **2003**, *101*, 910–914.

(15) Shivji, M. S.; Chapman, D. D.; Pikitch, E. K.; Raymond, P. W. Genetic profiling reveals illegal international trade in fins of the great white shark *Carcharodon carcharias*. *Conserv. Genet.* **2005**, *6*, 1035–1039.

(16) Sotelo, C. G.; Calo-Mata, P.; Chapela, M. J.; Pérez-Martín, R. I.; Rehbein, H.; Georgina, L.; Russell, V. J.; Pryde, S.; Quinteiro, J.; Izquierdo, M.; Rey-Mendez, M.; Rosa, C.; Santos, A. T. Identification of Flatfish (*Pleuronectiforme*) species using DNA-based techniques. *J. Agric. Food Chem.* **2001**, *49*, 4562–4569.

(17) Jérôme, M.; Lemaire, C.; Verrez-Bagnis, V.; Etienne, M. Direct sequencing method for species identification of canned sardine and sardine-type products. *J. Agric. Food Chem.* **2003**, *51*, 7326–7332.

(18) Barlett, S. E.; Davidson, W. S. FINS (forensically informative nucleotide sequencing): A procedure for identifying the animal origin of biological specimens. *BioTechniques* **1992**, *12* (3), 408–411.

(19) Quinteiro, J.; Sotelo, C. G.; Rehbein, H.; Pryde, S. E.; Medina, I.; Pérez-Martín, R. I.; Rey-Mendez, M.; Mackie, I. M. Use of mtDNA direct polymerase chain reaction (PCR) sequencing and PCR-restriction fragment length polymorphism methodologies in species identification of canned tuna. *J. Agric. Food Chem.* **1998**, *46* (4), 1662–1669.

(20) Quinteiro, J.; Vidal, R.; Izquierdo, M.; Sotelo, C. G.; Chapela, M. J.; Pérez-Martín, R. I.; Rehbein, H.; Hold, G. L.; Russell, V. J.; Pryde, S. E.; Rosa, C.; Santos, A. T.; Rey-Mendez, M. Identification of *hake* species (*Merluccius* genus) using sequencing and PCR−RFLP analysis of mitochondrial DNA control region sequences. *J. Agric. Food Chem.* **2001**, *49*, 5108–5114.

(21) Sambrock, J.; Fritsch, E. F.; Maniatis, T. *Molecular Cloning—A Laboratory Manual*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, 1989; p 6.15.

(22) Hoelzel, A. R. Shark fishing in fin soup. *Conserv. Genet.* **2001**, *2*, 69–72.

(23) Martin, A. P.; Palumbi, S. R. Protein evolution in different cellular environments: Cytochrome *b* in sharks and mammals. *Mol. Biol. Evol.* **1993**, *10* (4), 487–891.

(24) Hall, T. http://www.mbio.ncsu.edu/BioEdit/bioedit.html.

(25) Thompson, J. D.; Higgins, D. G.; Gibson, T. J. Clustal W: Improving the sensibility of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **1994**, *22*, 4673–4680.

(26) Kumar, S.; Tamura, K.; Nei, M. MEGA 3.1: Integrated software for molecular evolutionary genetics analysis and sequence alignment. *Briefings Bioinf.* **2004**, *5*, 150–163.

(27) Saitou, N.; Nei, M. The neighbour-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **1987**, *4*, 406–425.

(28) Primer Express. Version 2.0. Applied Biosystems.

(29) Greig, T. W.; Moore, M. K.; Woodley, C. M. Mitochondrial gene sequences useful for species identification of western North Atlantic Ocean sharks. *Fish. Bull.* **2004**, *103*, 516–523.

(30) Winchell, C. J.; Martin, A. P.; Mallatt, J. Phylogeny of elasmobranches based on LSU and SSU ribosomal RNA genes. *Mol. Phylogenet. Evol.* **2004**, *31*, 214–224.

(31) Heist, E. J.; Musick, J. A.; Graves, J. E. Genetic population structure of the shortfin mako inferred from restriction fragment length polymorphism analysis of mitochondrial DNA. *Can. J. Fish. Aquat. Sci.* **1995**, *53*, 583–588.

(32) Feldheim, K. A.; Gruber, S. H.; Ashley, M. V. Population genetic structure of the lemon shark (*Negaprion brevirostris*) in the western Atlantic: DNA microsatellite variation. *Mol. Ecol.* **2001**, *10*, 295–303.

(33) Schrey, A. W.; Heist, E. J. Microsatellite markers for the shortfin mako and cross-species amplification in lamniformes. *Conserv. Genet.* **2002**, *3*, 459–461.

(34) Abercrombie, D. L.; Clarke, S. C.; Shivji, M. S. Global-scale genetic identification of hammerhead sharks: Application to assessment of the international fin trade and law enforcement. *Conserv. Genet.* **2005**, *6*, 775–788.

(35) Chapela, M. J.; Sotelo, C. G.; Pérez-Martín, R. I. Molecular identification of cephalopod species by FINS and PCR−RFLP of a cytochrome *b* gene fragment. *Eur. Food. Res. Technol.* **2003**, *217*, 524–529.

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