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Research Note

Potential Use of DNA Barcodes in Regulatory Science: Applications of the *Regulatory Fish Encyclopedia*

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ABSTRACT

The use of a DNA-based identification system (DNA barcoding) founded on the mitochondrial gene cytochrome *c* oxidase subunit I (COI) was investigated for updating the U.S. Food and Drug Administration *Regulatory Fish Encyclopedia* (RFE; http://www.cfsan.fda.gov/~frf/rfe0.html). The RFE is a compilation of data used to identify fish species. It was compiled to help regulators identify species substitution that could result in potential adverse health consequences or could be a source of economic fraud. For each of many aquatic species commonly sold in the United States, the RFE includes high-resolution photographs of whole fish and their marketed product forms and species-specific biochemical patterns for authenticated fish species. These patterns currently include data from isoelectric focusing studies. In this article, we describe the generation of DNA barcodes for 172 individual authenticated fish representing 72 species from 27 families contained in the RFE. These barcode sequences can be used as an additional identification resource. In a blind study, 60 unknown fish muscle samples were barcoded, and the results were compared with the RFE barcode reference library. All 60 samples were correctly identified to species based on the barcoding data. Our study indicates that DNA barcoding can be a powerful tool for species identification and has broad potential applications.

All aquatic animals harvested, processed, distributed, and sold in the United States must be safe, wholesome, and properly labeled. Under the Federal Food, Drug, and Cosmetic Act, the Fair Packaging and Labeling Act, and the Public Health Service Act, the U.S. Food and Drug Administration (FDA) carries out a program that includes inspection, sampling, analysis, research, and education concerning seafood issues, safety, and labeling. The FDA also has oversight over economic fraud and food safety. Cases of consumer deception include the misbranding or improper labeling of a product and the substitution of an inferior product for a superior product. Seafood has garnered increasing attention because of potential health-related risks associated with misbranding. The major areas of concern and examples of species-specific hazards are listed in the hazard analysis and critical control point guide *(28)* (Table 1).

The Food Allergen and Protection Act requires unambiguous identity labeling of a food that is or contains an ingredient that is a major food allergen. Some of the aquatic species that may cause allergenic reactions are haddock, cod, hake, halibut, mackerel, tuna, salmon, orange roughy, shrimp, and crab. The act also identifies species that may not present a particular health concern, such catfish and basa, but that are covered by laws or regulations that require their identity to be monitored because of trade and tariff restrictions.

The FDA has been dealing with the problem of misbranding for many years. For example, the increase in seafood consumption and species substitution led the FDA and the National Marine Fisheries Service to recognize the need for a single source of market names to facilitate responsible trade in the marketplace and reduce confusion among consumers. In 1988, the FDA published the Fish List. Initially, the list contained only those fish species sold as part of interstate commerce, but it was revised in 1993 to include additional fish species and invertebrates and was renamed the Seafood List. Currently, this list is available as an updated searchable database on the FDA Center for Food Safety and Applied Nutrition Web site *(30).*

One challenge faced by both consumers and regulators is the detection of seafood substitution in the marketplace, a practice where low-value species (or species with potential toxins) are mislabeled and/or substituted in whole or in

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TABLE 1. *Economic deception and fish species substitution issues resulting in potential health hazards*

Actual fish species	Potential species-specific hazards	Species inappropriately labeled as:	Species-specific potential hazards of labeled species
Escolar (*Lepidocybium* spp.)	Gempylotoxin and histamine	Sea Bass or Grouper (*Dicentrarchus* spp. or *Epinephelus* spp.)	Parasites and ciguatoxin
Puffer Fish (*Sphoeroides* spp.)	Tetrodotoxin	Sole (*Microstomus* spp.)	Parasites, environmental hazards, chemical contaminants, and pesticides
Amberjack (*Seriola* spp.)	Histamine and ciguatera fish poisoning	Whitefish (*Coregonus* spp.)	Chemical contaminants and pesticides
Spanish Mackerel (*Scomberomorus* spp.)	Histamine and ciguatera fish poisoning	Kingfish (*Menticirrhus* spp.)	No listed potential hazard
Basa (*Lactarius* spp.)	Environmental hazards, chemical contaminants, and pesticides	Grouper (*Epinephelus* spp.)	Parasites and ciguatera fish poisoning

part and sold in place of or in a mixture with more expensive or nontoxic species. The detection of species substitution is important for reducing economic fraud and reducing the potential for health hazards. However, it is not always possible to tell by simple inspection that misbranding of an aquatic product has occurred. Processing often removes or damages diagnostic characteristics crucial for the identification of species by conventional taxonomic means. Therefore, traditional morphological methods are often insufficient for definitive species identification.

Molecular analyses have been a popular alternative to conventional morphology-based species identification. For more than 20 years, allozyme and DNA sequence data have been used to elucidate taxonomic relationships in groups in which morphology-based approaches are difficult *(1, 5, 17, 20).* Even for groups with well-established taxonomy, molecular approaches have frequently been applied in situations where traditional methods provide inconclusive data, e.g., linking various life stages *(3, 21),* recognizing prey items in predator gut contents *(25, 26),* and identifying tissue remains *(13, 15, 16).* Similar methods also have been recognized for their potential in forensic applications within the marketplace, e.g., forensically informative nucleotide sequencing *(2).*

Several years ago, a Web-based resource, the *Regulatory Fish Encyclopedia* (RFE), was developed for identification of 94 commercially important species of fishes *(27).* Organized in a series of species ''pages,'' the RFE contains high-resolution images of whole fish and their marketed product forms (e.g., fillets and steaks) and taxonomic, geographic, and other relevant tools for species identification. An example of an identification method listed on the Web site is protein identification by isoelectric focusing *(29).* The RFE was designed to be expandable to include additional data and newer analytical tools as they became available.

One new approach to species identification that could supplement the data in the RFE is DNA barcoding *(6),* a technique that exploits DNA sequence diversity to identify species based on similarities at a selected gene region. Although the idea of using sequence analyses to identify species is not new, the standardization of analysis of sequence diversity in a single gene region for recognition of species in large groups is new. The mitochondrial gene cytochrome *c* oxidase I (COI) has been adopted as the barcode region for the animal kingdom because it regularly delivers species-level resolution and it is more easily recovered across large assemblage of species than any other protein-coding region in the mitochondrial genome. Because of its adoption, sequence records are rapidly accumulating and barcode records are now available for over 250,000 individuals representing more than 28,000 species *(22).* This decision to focus sequence analysis on COI has been accepted by members of the fisheries community who have now joined forces to assemble DNA barcode records for all fish species. Although this work has been underway for only 2 years, records are in place for nearly 4,000 fish species, including many commercially important taxa. Because most of these records derive from vouchered specimens with carefully authenticated taxonomic assignments, they can potentially assist the FDA in monitoring and controlling species substitution in the marketplace. The purpose of this investigation was to evaluate the efficacy of DNA barcoding for identifying species listed in the RFE.

The success of a COI-based identification system is based on the satisfaction of two criteria: (i) the COI sequence for each specimen must be nearer to other COI sequences of that species than to sequences in other species and (ii) there must be a general correspondence between species assignments based on COI divergences and those based on conventional taxonomy. To test these criteria, COI barcodes were obtained from 72 authenticated species of marine and freshwater commercially relevant fish species listed in the RFE, representing approximately 75% of fishes currently recognized by the FDA as commercially harvested and traded in U.S. seafood markets. The practical applications of implementing a DNA barcoding system for regulatory control were evaluated by administering a blind test. COI sequences were derived from muscle samples of 60 undisclosed authenticated specimens and then identified using the online identification engine available in the Barcode of Life data system (BOLD) *(23).*

MATERIALS AND METHODS

Samples. Subsamples of muscle tissue from 172 authenticated aquatic specimens were obtained from the National Fish

TABLE 2. *Scientific and common names of* Regulatory Fish Encyclopedia *specimens used to generate a reference library of COI sequences*

Family	Species	Common name	FDA market name	No. of individuals
Achiridae: American Soles	*Pleuronectes americanus*	Blackback Flounder	Flounder (Sole)	2
	P. vetulus	English Sole	Sole	5
Anarhichadidae: Wolffishes	*Anarhichas lupus*	Atlantic Wolffish	Wolffish	2
Carangidae: Jacks	*Caranx caninus*	Pacific Crevalle Jack	Jack	2
	Selene peruviana	Pacific Moonfish	Jack	5
	Seriola lalandi	Yellowtail	Amberjack or Yellowtail	1
	Trichiurus lepturus	Atlantic Cutlassfish (Largehead Hairtail)	Cutlassfish	2
Centropomidae: Snooks	*Centropomus undecimalis*	Common Snook	Snook	1
Chanidae: Milkfishes	*Chanos chanos*	Milkfish	Milkfish	2
Cichlidae: Cichlids	*Oreochromis mossambicus*	Mozambique Tilapia	Tilapia	7
	O. niloticus	Nile Tilapia	Tilapia	2
Gadidae: Cods	*Gadus macrocephalus*	Pacific Cod	Cod (Alaska Cod)	5
	G. morhua	Atlantic Cod	Cod	3
	Melanogrammus aeglefinus	Haddock	Haddock	1
	Pollachius virens	Atlantic Pollock	Pollock	3
	Theragra chalcogramma	Walleye Pollock	Pollock (Alaska Pollock)	3
Gempylidae: Snake Mackerels	*Lepidocybium flavobrunneum*	Escolar	Escolar	4
	Ruvettus pretiosus	Oilfish	Oilfish	3
Hexagrammidae: Greenlings	*Ophiodon elongatus*	Lingcod	Lingcod	1
Ictaluridae: Bullhead Catfishes	*Ictalurus furcatus*	Blue Catfish	Catfish	3
	I. punctatus	Channel Catfish		4
Labridae: Wrasses	*Semicossyphus pulcher*	California Sheephead	Sheephead	2
Lotidae: Hakes and Burbots	*Brosme brosme*	Cusk	Cusk	3
Lutjanidae: Snappers	*Lutjanus apodus*	Schoolmaster	Schoolmaster	2
	L. campechanus	Red Snapper	Snapper	3
	L. peru	Pacific Snapper	Snapper	2
	L. purpureus	Caribbean Red Snapper	Snapper	1
	L. synagris	Lane Snapper	Snapper	3
	L. vivanus	Silk Snapper	Snapper	3
	Ocyurus chrysurus	Yellowtail Snapper	Snapper	1
Notothenidae: Antarctic Cods	*Dissostichus eleginoides*	Patagonian Toothfish	Patagonian Toothfish	2
Oreosomatidae: Oreos	*Zeus faber*	European John Dory	Dory	2
Pangasiidae: Shark Catfishes	*Pangasius bocourti*	Basa	Basa, Bocourti, Bocourti Fish, Basa Fish	1
	P. conchophilus	Conchophilus		1
	P. hypophthalmus	Sutchi	Swai, Sutchi, Striped Pangasius	1
	P. larnaudii	Spot Pangasius	Spot Pangasius	1
Pleuronectidae: Righteye Flounders	*Atheresthes stomias*	Arrowtooth Flounder	Flounder, Arrowtooth	1
	Eopsetta jordani	Petrale Sole	Sole (Flounder)	2
	Glyptocephalus cynoglossus	Gray Sole (Witch Flounder)	Sole (Flounder)	2
	G. zachirus	Rex Sole	Sole (Flounder)	3
	Limanda ferruginea	Yellowtail Flounder	Flounder	2
	Microstomus pacificus	Dover Sole	Sole	2
	Platichthys stellatus	Starry Flounder	Flounder	4
Salmonidae: Salmon and Trout	*Oncorhynchus gorbuscha*	Pink Salmon	Salmon, Pink (Humpback)	3
	O. mykiss	Rainbow Trout	Trout, Rainbow (Steelhead)	8
	Salmo salar	Atlantic Salmon	Salmon, Atlantic	2
Sciaenidae: Drums and Croakers	*Pogonias cromis*	Black Drum	Drum	4
	Roncador stearnsii	Spotfin Croaker	Croaker or Corvina	2
	Sciaenops ocellatus	Red Drum	Drum (Redfish)	2
Scombridae: Mackerels	*Euthynnus affinis*	Kawakawa	Tuna	2
	Katsuwonus pelamis	Skipjack Tuna	Tuna	1
	Sarda chiliensis	Pacific Bonito	Bonito	2
	Scomber japonicus	Chub Mackerel	Mackerel, Chub	2
	Thunnus alalunga	Albacore	Tuna	3
	T. albacares	Albacore	Tuna	2

TABLE 2. *Continued*

Family	Species	Common name	FDA market name	No. of individuals
Scophthalmidae: Turbots	*Scophthalmus aquosus*	Windowpane	Flounder	2
Sebastidae: Rockfish	*Sebastes aleutianus*	Rougheye Rockfish	Rockfish	1
	S. alutus	Pacific Ocean Perch	Perch, Ocean	1
	S. entomelas	Widow Rockfish	Rockfish	2
	S. flavidus	Yellowtail Rockfish	Rockfish	5
	S. maliger	Quillback Rockfish	Rockfish	1
	S. norvegicus	Golden Redfish	Perch, Ocean	2
	S. pinniger	Canary Rockfish	Rockfish	2
	S. ruberrimus	Yelloweye Rockfish	Rockfish	1
Serranidae: Sea Basses	*Cephalopholis fulva*	Coney	Grouper	2
Siganidae: Rabbitfishes	*Archosargus probatocephalus*	Sheepshead	Sheepshead	4
	Chrysophrys auratus	Red Hawaiian Porgy (Squirefish)	Porgy	2
	Siganus javus	Java Rabbitfish (Streaked Spinefoot)		2
Sphyraenidae: Barracudas	*Sphyraena argentea*	Pacific Barracuda (California)	Barracuda	2
	S. barracuda	Great Barracuda	Barracuda	5
Stromateidae: Butterfishes	*Pampus argenteus*	Silver Pomfret	Butterfish	2

Tissue Collection located at the FDA Seafood Products Research Center (Bothell, Wash.). These 172 authenticated samples were used to construct the reference library of COI sequences (Table 2), and 60 undisclosed authenticated samples were used as blind tests. All tissues were initially stored at −80°C for 4 to 12 years and subsequently subsampled into 95% ethanol for shipment to the Canadian Center for DNA Barcoding (CCDB, Guelph, Ontario, Canada) for molecular analysis.

DNA analysis. DNA extracts were prepared using the automated glass fiber method described by Ivanova et al. *(10).* Subsequently, the barcode region was amplified in a Mastercycler Eppendorf gradient thermal cycler (Brinkmann Instruments, Inc., Westbury, N.Y.) under the following thermal conditions: 2 min at 95°C; 35 cycles of 0.5 min at 94°C, 0.5 min at 52°C, and 1 min at 72°C; 10 min at 72°C. The final products were held at 4°C until needed. The 12.5-μl PCR mixes included 6.25 μl of 10% trehalose, 2.00 μl of ultrapure water, 1.25 μl of 10× PCR buffer (200 mM Tris-HCl, pH 8.4, 500 mM KCl), 0.625 μl of 50 mM $MgCl_2$, 0.125 μl of each primer cocktail (0.01 mM), 0.062 μl of each deoxynucleoside triphosphate (10 mM), 0.060 μl of Platinum *Taq* polymerase (Invitrogen, Carlsbad, Calif.), and 2.0 μl of DNA template. The barcode region of the COI gene was amplified using three primer combinations published by Ivanova et al. *(11)*: FF2d-FR1d, VF2_t1-VR1_t1, and C_FishF1-C_FishR1. Initially, amplification was attempted for all samples with a single primer combination (FF2d-FR1d). Those samples that failed to amplify were subjected to a second PCR using VF2_t1-VR1_t1. Remaining failures were then recovered using a combination of primer cocktails, C_FishF1-C_FishR1. Before sequencing, PCR products were visualized on a 1.2% agarose gel with an E-Gel precast agarose electrophoresis system (Invitrogen). Products were labeled with the BigDye Terminator (version 3.1) Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, Calif.) and sequenced bidirectional with an ABI 3730 capillary sequencer (Applied Biosystems) following the manufacturer's instructions *(4).* Primer combinations used to amplify each sample, sequence data, electropherograms, and specimen details are all available within the project files ''RFE FDA'' and ''Blind Study'' under the subdirectory ''US Food and Drug Administration'' on the BOLD site *(22).*

Sequences were aligned using SeqScape (version 2.0) software (Applied Biosystems). Sequence divergences were calculated using the Kimura two-parameter distance model *(14),* the most suitable model of molecular evolution when distances are low *(18).* Neighbor-joining trees were generated using tools available on the BOLD site.

RESULTS AND DISCUSSION

Reference library of COI sequences. The effectiveness of a DNA barcoding system is based on the assumption that no two species share an identical COI sequence. Thus, COI sequences derived from one species must more closely resemble the COI sequences of other individuals of that same species (conspecifics) than those of other closely related sister taxa (congenerics). A simple method of screening for possible sequence sharing is to compare histograms of conspecific and congeneric divergences (Fig. 1). In a perfect system, the plots do not overlap, creating a complete bifurcation between conspecific and congeneric divergences. This pattern is typical for most animal groups; within- and between-species comparisons of sequence divergence largely do not overlap and are generally separated by an arbitrary threshold *(8, 12).* The value of the threshold likely varies among taxonomic groups, but empirical evidence suggests that 2% is a useful first approximation *(8).* Conspecific and congeneric values that fall below and above this threshold, respectively, represent cases that are easily diagnosable using DNA barcodes. Pairwise values that represent outliers from their respective distributions (i.e., areas of overlap between conspecific and congeneric plots) indicate possible disagreements between barcodes and morphology (Fig. 2). Although such cases do represent

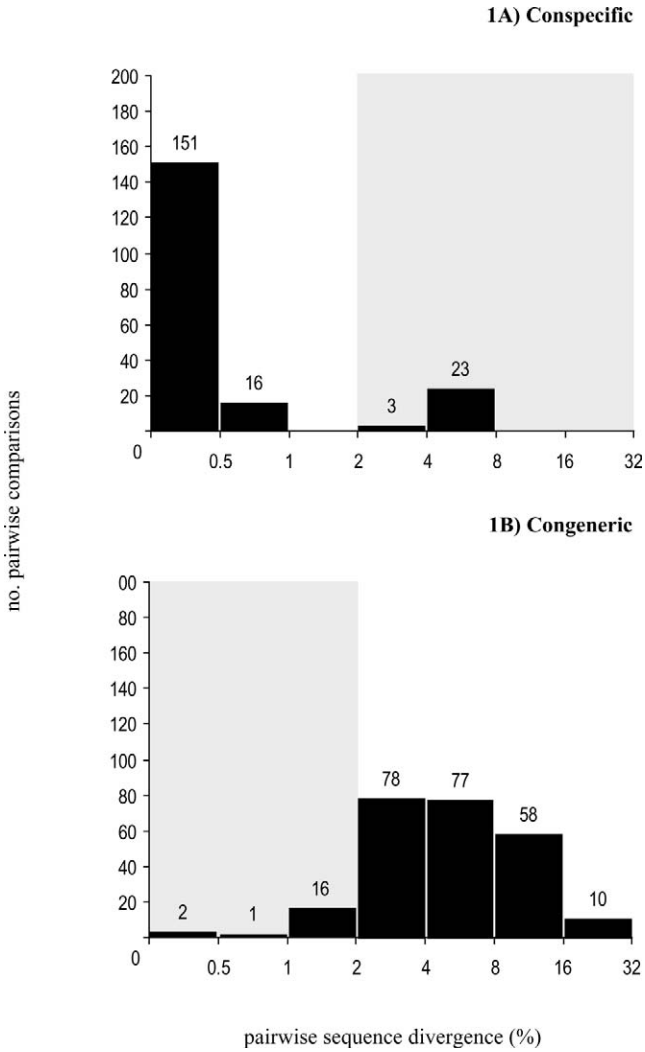

FIGURE 1. *Distributions of pairwise sequence divergence for conspecific (A) and congeneric (B) fish pairs. Atypical divergence values are highlighted.*

exceptions, they do not necessarily preclude the effectiveness of a DNA barcoding system for accurate species identification. For example, the conspecific and congeneric distributions reported by Ward et al. *(31)* overlapped for 10% of comparisons, but 100% of the analyzed fish species possessed unique DNA barcodes. Overlaps were due to overlooked cryptic taxa and unusually low variation among tuna species belonging to the genus *Thunnus.*

The mean conspecific divergence obtained in the current study (0.99%) was approximately threefold higher than the mean value reported in a similar study involving Australian fishes *(31).* The differences reflected unusually deep splits within four species: *Oreochromis mossambicus* (3.7%), *Oreochromis niloticus* (7.4%), *Lepidocybium flavobrunneum* (2.7%), and *Oncorhynchus mykiss* (7.3%) (Fig. 2). All four of these species have deep intraspecific divergences, all of which exceeded the upper limit of 2.0% conspecific sequence divergence recognized by Hebert et al. *(8).* Omitting these four cases, the conspecific mean falls to 0.19%. Although such deep conspecific structure often is due to overlooked diversity *(7, 9),* such overlooked diversity is unlikely in commercially important taxa. A more likely explanation is that these deep splits represent misidentifications. The inadvertent inclusion of two closely related taxa under the same species name can create cases of apparent deep intraspecific splits (Fig. 2). This explanation appears very likely for the rainbow trout (*O. mykiss*), where the BOLD identification engine identified one cluster as the closely related Coho salmon (*Oncorhynchus kisutch*). Such misidentifications are plausible considering the morphological similarity of these species during river spawning migrations; Coho salmon often are confused with other salmonid species *(19).* We suspect that the other three deep conspecific splits also represent cases of misidentifications because they all involve genera in which species assignments are difficult. We were not able to critically test this hypothesis because voucher specimens were not available for reexamination (sources of DNA used for barcode analysis represented only subsamples, not whole fishes). Despite a few exceptions to the general patterns of conspecific divergences, DNA barcodes are generally reliable for assigning proper species identifications.

Congeneric divergences were much greater than those differences detected within species, averaging approximately 34 times that of the adjusted conspecific mean. However, a few species pairs had much lower divergences between COI barcodes: *Lutjanus campechanus* versus *Lutjanus purpureus, Thunnus alalunga* versus *Thunnus albacares,* and *Sebastes alutus* versus *Sebastes norvegicus,* all together forming the lower bound of pairwise congeneric sequence divergences detected in this study. The low variation within these pairs was not unexpected considering the low level of variation in mitochondrial DNA reported in other genetic studies involving *Lutjanus* spp. *(16), Thunnus* spp. *(31),* and *Sebastes* spp. *(24).* However, despite their low sequence divergences, each species pair examined in our study was distinguishable using the barcode region of COI.

Overall, the patterns of sequence divergence reported in this study support the effectiveness of the use of the barcode region of the COI gene for species identification. Each species possessed a unique and therefore diagnostic COI sequence, rendering this data set compliant with the criteria required to act as a reference library for species identification.

Blind study. Whole intact specimens of fishes are rarely available when species substitution is suspected. More often, only limited tissue samples of the organism are available for examination by regulatory agencies. Therefore, the accuracy of DNA barcoding had to be evaluated in a practical setting with no visual cues for identification under controlled conditions. To create a realistic test scenario, a blind test was administered by the Center for Veterinary Medicine to evaluate the capacity of DNA barcoding to correctly identify muscle tissue from a 60-sample test group. By utilizing the reference library of COI sequences generated in this study, the CCDB was able to correctly place species-level identifications on all blind samples (Table 3). All 60 unknown samples were identified correctly to species for fishes contained in the RFE.

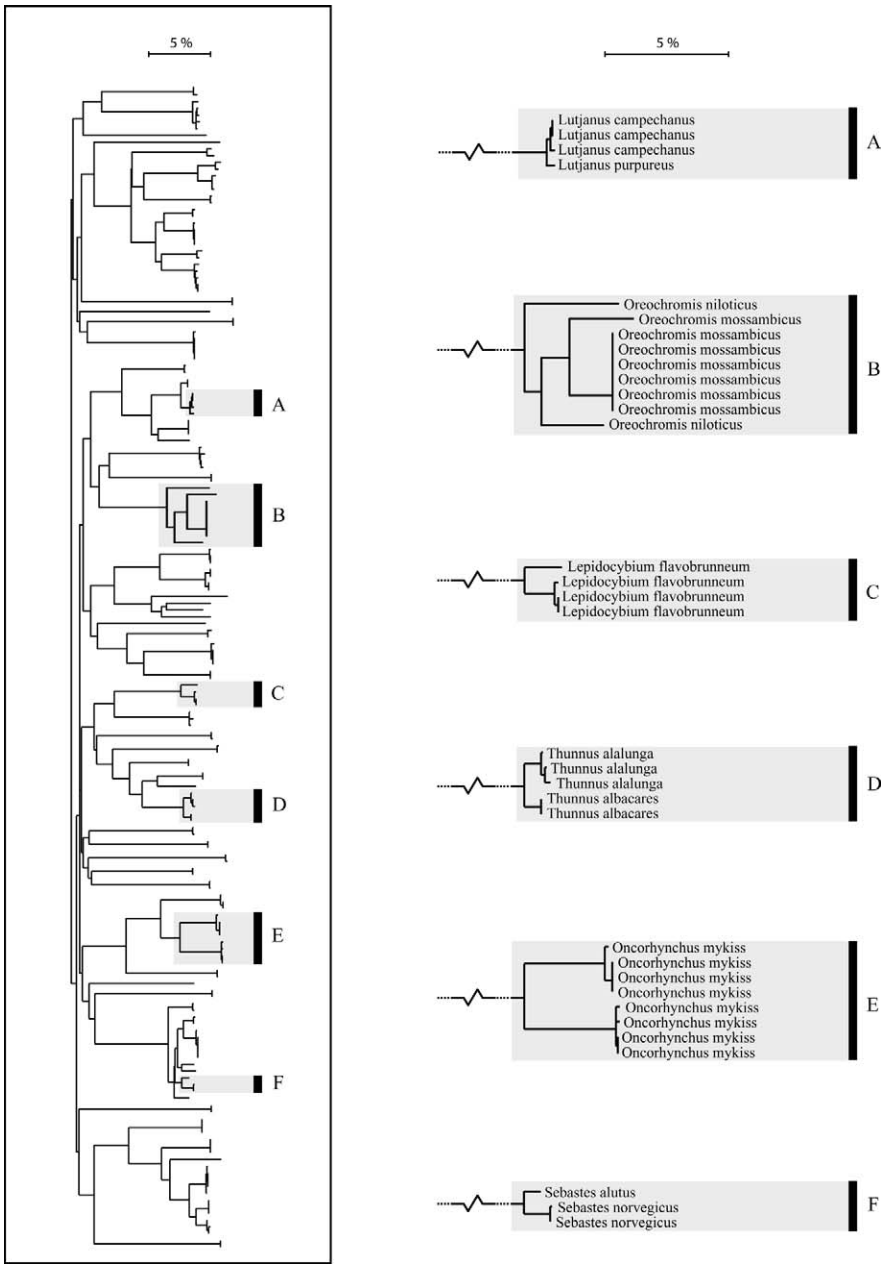

FIGURE 2. *Neighbor-joining tree of 172 COI sequences. Highlighted regions of the tree correspond to atypical divergence values for conspecific and congeneric pairs indicated in Figure 1.*

Both the food industry and the consumer must be protected against species substitution. Consumers need information about fish and fish products, and the industry needs to maintain and improve its ability to distinguish among species so they can properly label their products. The Seafood List and the RFE were developed to provide more information and promote consistency in labeling among various areas in the United States, enhancing the ability of consumers to make informed choices among seafood products and increasing compliance with U.S. food labeling requirements. The DNA barcode library of RFE fishes is one more tool that can be used for monitoring and controlling seafood substitution in the marketplace. The advantage of a DNA barcode system is that it is all-encompassing; DNA barcodes can be used for routine identifications, as supplemental data for morphologically difficult groups, or for species diagnoses when traditional diagnostic characters have been removed (e.g., for fins, steaks, or fillets) or have not yet developed (e.g., for eggs, larvae, and juveniles). Because only minute amounts of DNA are required for barcode analysis, the applications of this system are likely extendable to processed seafood products for which all other identification methods are uninformative.

The patterns of sequence divergence reported in this study support the effectiveness of COI as a suitable marker for distinguishing among various fishes commercially important in the United States. The 100% accuracy of the blind test further supports the practical applications of barcoding to help monitor and control cases of product substitution. We hope to continue to expand the COI database to generate a comprehensive library of DNA barcodes for every fish species that may enter the market under either legitimate or illegitimate names. A further goal includes expansion of this system to include

TABLE 3. *Species identifications for blind study*

Sample no.	Species identification[a]	Barcode library
Blind-01	*Hoplostethus atlanticus*	RFE
Blind-02	*Gadus macrocephalus*	RFE
Blind-03	*Theragra chalcogramma*	RFE
Blind-04	*Alopias vulpinus*[b]	*(31)*
Blind-05	*Coryphaena hippurus*[b]	Unpublished
Blind-06	*Carcharhinus dussumieri*[b]	*(31)*
Blind-07	*Scomberomorus munroi*[b]	*(31)*
Blind-08	*Tetrapturus angustirostris*[b]	*(31)*
Blind-09	*Girella tricuspidata*[b]	*(31)*
Blind-10	*Parupeneus heptacanthus*[b]	*(31)*
Blind-11	*Cubiceps baxteri*[b]	*(31)*
Blind-12	*Pseudopentaceros richardsoni*[b]	*(31)*
Blind-13	*Euthynnus affinis*	RFE
Blind-14	*Katsuwonus pelamis*	RFE
Blind-15	*Thunnus alalunga*	RFE
Blind-16	*T. albacares*	RFE
Blind-17	*Acanthopagrus berda*[b]	*(31)*
Blind-18	*Pampus argenteus*	RFE
Blind-19	*Xiphias gladius*[b]	*(31)*
Blind-20	*Pleuronectes vetulus*	RFE
Blind-21	*Atheresthes stomias*	RFE
Blind-22	*Eopsetta jordani*	RFE
Blind-23	*Microstomus pacificus*	RFE
Blind-24	*Ophiodon elongatus*	RFE
Blind-25	*Sebastes alutus*	RFE
Blind-26	*S. alutus*	RFE
Blind-27	*S. pinniger*	RFE
Blind-28	*S. alutus*	RFE
Blind-29	*S. flavidus*	RFE
Blind-30	*S. entomelas*	RFE
Blind-31	*P. vetulus*	RFE
Blind-32	*Lutjanus apodus*	RFE
Blind-33	*G. macrocephalus*	RFE
Blind-34	*G. macrocephalus*	RFE
Blind-35	*Lutjanus peru*	RFE
Blind-36	*S. pinniger*	RFE
Blind-37	*S. flavidus*	RFE
Blind-38	*E. jordani*	RFE
Blind-39	*Glyptocephalus zachirus*	RFE
Blind-40	*M. pacificus*	RFE
Blind-41	*Platichthys stellatus*	RFE
Blind-42	*P. stellatus*	RFE
Blind-43	*Ictalurus punctatus*	RFE
Blind-44	*Sphyraena barracuda*	RFE
Blind-45	*S. barracuda*	RFE
Blind-46	*T. alalunga*	RFE
Blind-47	*T. chalcogramma*	RFE
Blind-48	*Oncorhynchus mykiss*	RFE
Blind-49	*O. mykiss*	RFE
Blind-50	*O. mykiss*	RFE
Blind-51	*T. albacares*	RFE
Blind-52	*T. chalcogramma*	RFE
Blind-53	*Chanos chanos*	RFE
Blind-54	*Scomber japonicus*	RFE
Blind-55	*Caranx caninus*	RFE
Blind-56	*Euthynnus affinis*	RFE
Blind-57	*Dissostichus eleginoides*	RFE
Blind-58	*O. mykiss*	RFE
Blind-59	*O. mykiss*	RFE
Blind-60	*O. mykiss*	RFE

[a] Identifications were achieved using the BOLD identification engine (www.boldsystems.org/views/idrequest.php).

[b] Reference sequences for these species were not available in the RFE barcode library.

all commercially important aquatic foods (e.g., crustaceans and mollusks).

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