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TECHNICAL NOTE

DNA barcode identification of shark fillet reveals fraudulent commerce in Brazil

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ABSTRACT

Fraudulent mislabeling of fish products has been reported with some frequency, representing an important problem in the food industry and regulatory agencies of many countries. This case reports a fraudulent substitution of shark fillets for the much cheaper Striped-catfish, in a large purchase for public elementary school meals in a Brazilian town. The economic and nutritional aspects involving such mislabeling demonstrate a serious fraud that should alarm governmental regulatory agencies to implement a more rigorous and frequent monitoring system on the species verification to guarantee the safety and quality of seafood.

RÉSUMÉ

Le mauvais étiquetage frauduleux des produits de la mer est fréquemment rapporté, ce qui représente un important problème pour l'industrie alimentaire et les organismes régulateurs de nombreux pays. Ce dossier rapporte la substitution frauduleuse de filets de requin pour la peu coûteuse barbue d'Amérique rayé (poisson-chat) lors d'un gros achat pour les repas d'une école publique élémentaire d'une ville brésilienne. Les aspects économiques et nutritionnels d'un tel mauvais étiquetage démontrent une sérieuse fraude ce qui devrait inquiéter les organismes régulateurs gouvernementaux. Ceux-ci devraient implanter un système de contrôle de vérification des espèces plus fréquent et plus rigoureux afin de garantir la sécurité et la qualité des poissons et fruits de mer.

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Introduction

Food safety and quality is currently of great concern since different types of fraudulent crimes in food commerce frequently occur. The substitution of a particular type of fish fillet, especially those considered noble fish, which are characterized by a distinctive market price, has been reported more frequently [1–6], particularly after the advent of new genetic technologies and the use of DNA barcoding identification. For fish fillet and other seafood commerce, the high market price can encourage traders

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to commit fraudulent substitutions in order to obtain higher profit. In fish, the use of morphological features to identify the species being sold is not always feasible due to the high external similarity between taxonomically related species. Fish fillets are even more difficult to identify by morphology alone, as the head, skin and fins are absent and cannot be used to check the species identification. In such cases, the DNA barcode study, a widely used molecular-based system to identify biological species, is capable of discriminating and precisely confirming the species identification, whether alive, dead, or even in the form of processed food. This identification tool allows for the sequence of the mitochondrial gene *coI* (subunit 1 of cytochrome c oxidase) of questioned samples to be verified against reference sequences in the global repository of the Barcode of Life Data System – BOLD (www.boldsystems.org).

Through this molecular tool, it was possible to investigate a fraud committed by a fish fillet supplier contracted by a Brazilian town to supply seven tons of shark fillet for meal preparation in public elementary schools. The result of this investigation reveals a crime against consumers, with an economic impact in governmental spending and loss of nutritional acquisition in schoolchildren.

Material and methods

Sample collection

Seven samples of distinct randomly chosen packages of frozen fish fillet labelled as shark were collected on 16 and 17 January 2013, from the industrial fridge warehouse of a specific southeastern Brazilian town. Representative pieces of muscle tissue were taken from the seven fish fillet packages at the investigated product location. The questioned samples were individually identified and fixed in 99% ethanol and posteriorly stored in a freezer at –20° C. Fish fillet package labels including information on origin, expiration dates, lot number, and inspection number of governmental agency control were used as evidence. The name of the product, the fish supplier, and the name of the town are not disclosed in this study due to ethical concerns.

Extraction, amplification, and sequencing

Total genomic DNA was isolated through the DNeasy Blood & Tissues Kit (QIAGEN®, Hilden, Germany). The polymerase chain reaction (PCR) was performed in 25 µl of 10 µM of each primer LCO1490 and HCO2198 [7], 1 × Master Mix Kit (QIAGEN®, Hilden, Germany), and 15–30 ng of DNA. The 610 bp fragment was amplified under the following thermocycler conditions: initial denaturation of 3 min at 96 °C, 35 cycles of 30 s at 95 °C, 30 s at 42 °C, and 2 min at 72 °C; followed by a final extension of 5 min at 72 °C. The amplicon was purified and sequenced in both directions at the Macrogen® Inc (South Korea) facility.

Analysis and species identification

The chromatograms of molecular data were visualized and edited using the software Geneious® 6.0.5 (http://www.geneious.com) [8]. The sequences were aligned in this

same software using automatic assembly in the implemented MUSCLE with the default parameters, and each contig pair was visually inspected and edited before consensus sequences were extracted. The codon positions of the protein-codifying gene were tested based on amino acid translation.

The DNA barcode samples identification was conducted using primarily the "Identification Engine Tool" (IDS) approach on the BOLD database (www.barcodinglife.com), which consists of searching and matching reference barcode sequences considering only those specimens matching above 98% of the sequence. Thus, the cut-off value for identity match search was >98% and the species identification levels were considered those with genetic distance equal to zero. Additionally, species identification was also investigated by a comparison to the reference sequences deposited in the GenBank (www.ncbi.nlm.nih.gov/genbank), and FISH-BOL (Fish Barcode of Life, [9]; www.fishbol.org) databases using the BLASTn algorithm. Subsequently, all aligned sequences from questioned and reference samples were used to produce a phenogram, grouping specimen sequences by similarity based on nucleotide genetic distances calculated using the kimura-2-parameters distance model [10] under Neighbor-Joining, implemented in BOLD.

Results

The *coI* barcode sequences of the questioned samples submitted to the GenBank and BOLD databases and their accession numbers are shown in Table 1. The supposed shark fillet samples were 100% matched with reference samples of *Pangasianodon hypophthalmus*, an Asian freshwater catfish popularly known as the Striped Catfish, Swai, or Panga in Brazil. All questioned samples had identical DNA sequences, lacking any variant sites (except by the position 274 in sample MH358380), and were clearly distinguishable from any reference sequences representing shark species or related chondrichthyan taxa. According to the BOLD similarity tree (Figure 1), the

Table 1. Genetic identification of the questioned shark fillet samples. Common name of the species as identified in the original fish package; accession number for the samples deposited in GenBank; species identification based on *coI* barcode; GenBank accession number of reference sequences with 100% match; and mislabeling result.

Common name of expected species	Sample accession number	% of the match to the reference sequences	Species identification	Closest match reference sequence (some of many accession numbers)	Mislabeling
Shark	MH358383	100%	*Pangasianodon hypophthalmus*	EF609427, JF292409,	yes
Shark	MH358382	100%	*Pangasianodon hypophthalmus*	JF292407, JF292403,	yes
Shark	MH358381	100%	*Pangasianodon hypophthalmus*	JF292401, JF292399,	yes
Shark	MH358380	99.84%	*Pangasianodon hypophthalmus*	JF292395, JF292410, [. . .]	yes
Shark	MH358379	100%	*Pangasianodon hypophthalmus*		yes
Shark	MH358378	100%	*Pangasianodon hypophthalmus*		yes
Shark	MH358377	100%	*Pangasianodon hypophthalmus*		yes

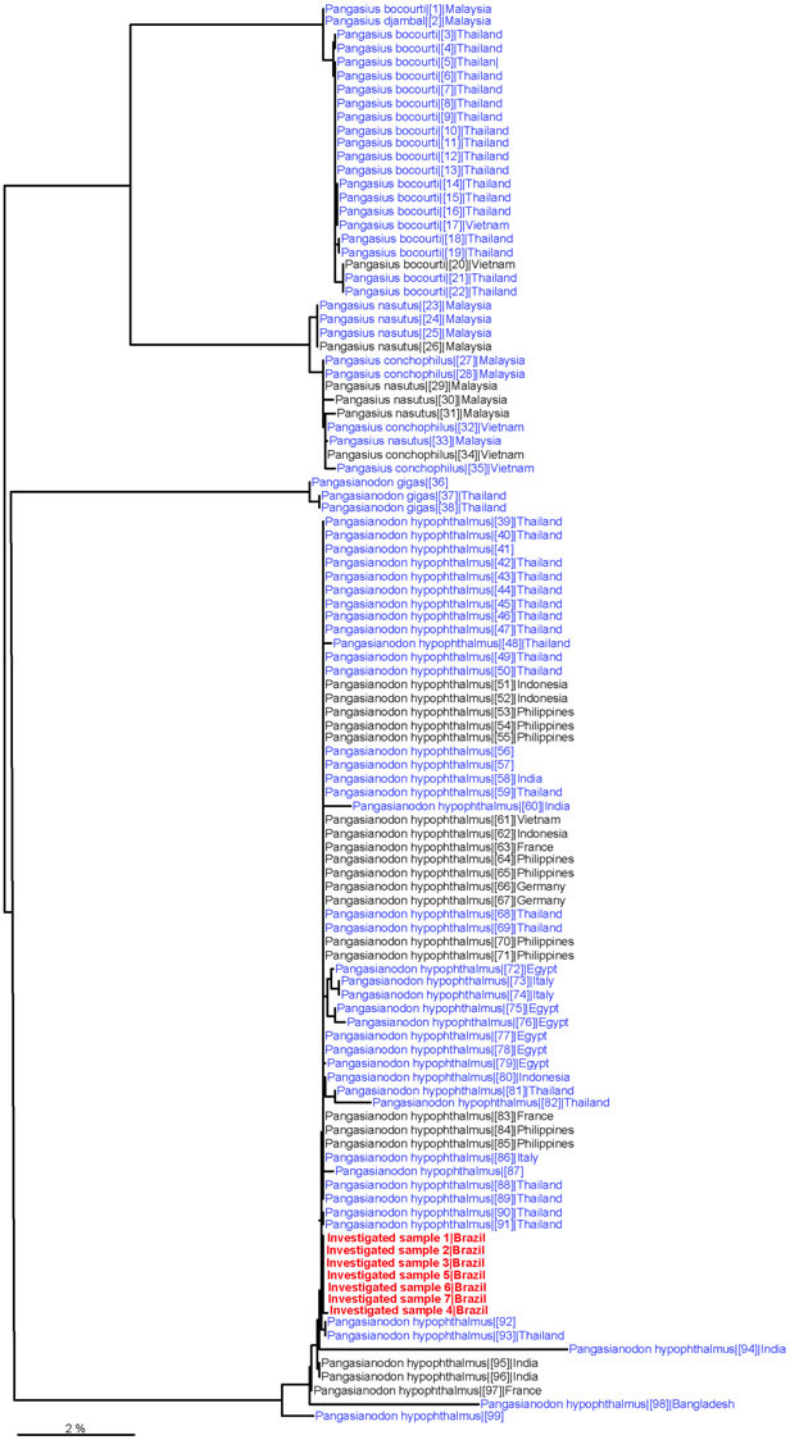

Figure 1. Tree based on genetic similarity of the seven questioned sample sequences (in red), and 99 reference sequences deposited on BOLD (black) and GenBank (blue) databases. Name of species in the diagram is followed by the number of the searched ranked samples, and locality. [To view this figure in colour, please see the online version of this journal.]

closest sequences to questioned samples were those obtained from specimens from Thailand, indicating a possible origin for the fish fillets analyzed in the current study.

Conclusion

The fraud reported herein represents an additional example of a lower priced species being marketed as a more expensive and valuable one. This constitutes a clear case of commercial crime, consisting of serious fraud in both economically and nutritionally. Panga is significantly cheaper than shark in the global fish market (wholesale price 0.5 to 0.7 as compared with shark), likely due to the high production of fillets at low cost by aquaculture in Southeast Asia, as opposed to open sea exploratory fisheries. Panga is commonly farmed in freshwater basins in Vietnam and Thailand, and exported to many European, American, Australian, and South American markets. The nutritional quality of Panga fillet was reported to be considered low. This species has a high saturated fatty acid meat (41.1–47.8% of total fatty acids) [11], a fat known to potentially affect risk factors for chronic diseases in certain heart and cardiovascular diseases [12]. Despite the low cholesterol levels found in Panga fillets [11], this species contains a fatty acid profile unusual for most fish, being devoid of the typical nutritional characteristics present in fish and seafood (namely the polyunsaturated fatty acids (PUFA), particularly the *n*-3 (omega-3), which makes fish potentially the only significant dietary source of omega-3 in the human diet).

The markedly different nutritional properties of shark and Panga will affect the main meal of public school children. In Brazil, public elementary schools are mostly attended by low-income family children, and in some places the school meal represents the main, or sometimes the only daily meal. Nutritional deficiencies affect the overall development of children, and may have severe individual and collective consequences. Considering the high financial and social investment that the Brazilian government has made to promote better development of children from low-income families, it is believed that molecular tests can be good allies in the fight against fraud, as reported here.

Disclosure statement

No potential conflict of interest was reported by the authors.

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