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DNA barcode authentication reveals highly fraudulent Cod commerce in Porto Alegre, Brazil

Bárbara B. Calegari [a,b,*], Eduardo F. Avila [a,c], Roberto E. Reis [a], Clarice S. Alho [a]

[a] Pontifícia Universidade Católica do Rio Grande do Sul, School of Sciences, Av. Ipiranga 6681, 90619-900 Porto Alegre, RS, Brazil
[b] Department of Vertebrate Zoology, National Museum of Natural History, Smithsonian Institution, 20013-7012 Washington, DC, USA
[c] Superintendência Regional de Polícia Federal no Rio Grande do Sul, Technical and Scientific Division, Federal Police, 90160-093, Porto Alegre, RS, Brazil

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ABSTRACT

Through the DNA barcoding technique using the cytochrome oxidase subunit I (*coI*) mitochondrial gene, a high degree of fraudulent commerce of Cod (*Bacalhau* in Portuguese) was detected in the city of Porto Alegre, Brazil. Tested products included frozen and dried salted fish during the Easter period, the season with the highest Cod consumption. The sale of Cod products in Brazil is controlled by governmental technical regulations restricting the Cod designation to salted and dried salted products made from three species of *Gadus*. The results of this investigation revealed a high level of fraud of various types against consumers. Of 10 tested products, seven were identified as fraudulent, including distinct species from the ones indicated in the labels, constituting cases of substitution for cheaper fish species, or species not allowed to be sold as Cod, or prepared in discordance with standard requirements of preparation (salted or dried salted), and were therefore considered mislabeled under Brazilian technical regulations.

1. Introduction

The sale of fraudulent fish is currently of great concern due to its common occurrence in food markets on a global scale [1,12,18]. Global fisheries' catches are constrained by the amount of primary marine production [4], and aquaculture is not a viable option of sustainable production [8] for most marine fish when compared to freshwater species; this contributes to the mislabeling of fish in an attempt to supply consumption demands or to increase economic gain. Records since the 1950s have been indicating overexploitation to such an extent that the proportion of fished stocks is above biologically sustainable levels and is consequently reducing the amount of fish available to fisheries [8].

In the face of the local depletion of fish stocks, fisheries are forced to seek new fishing grounds to supply the demand or to search for alternative species [12]. This problem tends to increase substitution fraud cases of particular types of fish product, especially those considered valuable, to meet consumer demand. Cod, Hake, and Haddock are included in the Order Gadiformes and represent some of the most important and expensive commercial fish in the world, accounting for approximately 18 % of the world's total marine fish catch [8]. For this reason, a high degree of fraud in Atlantic Cod (*Gadus morhua*) is seen in world fish markets due to the existence of distinct fish that are morphologically and palatably similar [1,11,12].

Culture and religion are sometimes closely related to food consumption, and some special holidays, such as Easter, Christmas, and the New Year, increase the demand for fish, especially Cod, in Brazil. The several types of Cod presentation, such as shredded, slivers, filleted, and the whole fish, and the various conservation methods (fresh, salted, dried salted, frozen, smoked, and ready-to-serve dishes) facilitate fraud by fish markets. According to Herrero et al. [12], the typical species substitutes for Atlantic Cod (*Gadus morhua*) are Pacific Cod (*G. macrocephalus*), Greenland Cod (*G. ogac*), Alaska Pollock (*G. chalcogrammus*), Ling (*Molva molva*), Blue Ling (*M. dypterygia*), Pollock (*Pollachius pollachius*), Saithe (*P. virens*), Haddock (*Melanogrammus aeglefinus*), Blue Whiting (*Micromesistius poutassou*), Hake (*Merluccius* spp.), and Whiting (*Merlangius merlangius*).

Accurate labeling of seafood products is vital for enabling proper identification and traceability, preventing illegal, unreported, and unregulated (IUU) fishing products from entering the market, combating overfishing, and enforcing sustainable fishing practices [22,26]. Brazilian technical regulations on the permission for use of the designation Cod (*Bacalhau* in Portuguese) established by the Ministry of Agriculture, Livestock, and Supply (MAPA) Ordinance #52 of December 29, 2000 [16] allow only salted and dried salted products made from the species Atlantic Cod (*Gadus morhua*), Pacific Cod (*G. macrocephalus*), and Greenland Cod (*G. ogac*) to be legally designated with this common name. In addition, a second legal requirement is for the scientific species name to appear together with the Cod designation on the product label.

* Corresponding author at: Pontifícia Universidade Católica do Rio Grande do Sul, School of Sciences, Av. Ipiranga 6681, 90619-900 Porto Alegre, RS, Brazil.
E-mail addresses: barbara.calegari@gmail.com (B.B. Calegari), efavilas@gmail.com (E.F. Avila), reis@pucrs.br (R.E. Reis), csalho@pucrs.br (C.S. Alho).

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Over the past 10 years, large-scale seafood frauds have been identified by Brazilian law enforcement agencies, leading to administrative fines, product seizing, and even operational shutdown of some major fish-processing companies nationwide. Detected fraudulent activities have included several stages in fish handling and distribution, especially concerning packing and final product identification. Replacement of high-demand, locally valued species by morphologically or organoleptically similar substitutes and mislabeling of products composed of vulnerable species whose exploitation is regulated or prohibited by law have been observed at high rates [12]. Where proper fish species identification is vital, Brazilian federal police have been involved in preventing such practices, and this process has been accomplished through the proper use of molecular techniques.

The widely used genetic technique that permits seafood species identification is the DNA barcode sequencing of the mitochondrial cytochrome oxidase subunit I (*coI*) gene, which is commonly used to detect commercial substitution and mislabeling and has been validated for use in forensic species identification [7]. The present study used this authentication tool for testing the labeling of Cod products in fish markets of the city of Porto Alegre, Brazil during the 2018 Easter holiday period, when the demand for this fish is high, in an attempt to assess fraud occurrence in local Cod commerce.

2. Material and methods

2.1. Sample collection

A total of 10 fish products labeled as Cod were purchased from popular distinct retail outlets spanning supermarkets, grocery stores, and fishmongers during the 2018 Easter period. These retail outlets represent all the different wholesalers of Cod in Porto Alegre. The sample size was limited to avoid resampling the same suppliers and thus biasing the amount of fraud. The selection of fish samples considered two criteria based on the availability of product types: 1) cut fish pieces (i.e., whole, filleted, slivers, or shredded) and 2) preservation method (i.e., dried, salted, or frozen). To evaluate the correct identification of Cod products and labeling quality, only products properly labelled with a specific commercial designation were used (Table 1, second column). Photos of the samples' packaging, purchase receipts, and original tissue samples are currently stored at the School of Science, Pontifícia Universidade Católica

Table 1
Genetic identification of the questioned Cod products. Sample number; designation of the product as used in the original sale market; type cut of pieces of the products; permission to use the Cod designation according to Brazilian regulations; accession number for the products investigated deposited in GenBank; similarity of the product sequence to the reference sequences in GenBank; species identification based on the *coI* barcode; Genbank accession number of the reference sequences used to identify the products; and types of resulting frauds.

Sample number	Designation of Cod Product in Portuguese (English)	Type Cut of Pieces (Price US$/kg)	Species allowed to be designated Cod	GenBank Accession Number	% of the match to the Reference Sequence	Species identification by DNA Barcode (common name)	Closest match reference sequence Accession number	Type 1 fraud (Substitution)	Type 2 fraud (Mislabeling)	Type 3 fraud (Preparation)
S1	Bacalhau Saithe (Saithe)	Whole dried salted ($10.0)	**No**	MK241678	100%	*Pollachius virens* (Saithe)	KX119492.1	No	**Yes**	No
S2	Bacalhau Ling (Ling)	Whole dried salted ($12.6)	**No**	MK241679	100%	*Molva molva* (Ling)	KJ128552.1	No	**Yes**	No
S3	Bacalhau do Porto (Atlantic Cod)	Shredded dried salted ($23.7)	Yes	MK241680	100%	*Pollachius virens* (Saithe)	KC015819.1	**Yes**	**Yes**	No
S4	Bacalhau do Porto (Atlantic Cod)	Shredded dried salted ($7.9)	Yes	MK241681	100%	*Gadus chalcogrammus* (Walleye Pollock)	JF952737.1	**Yes**	**Yes**	No
S5	Bacalhau do Porto (Atlantic Cod)	Slivers dried salted ($23.8)	Yes	MK241682	100%	*Gadus morhua* (Atlantic Cod)	KX267087.1	No	No	No
S6	Bacalhau do Porto (Atlantic Cod)	shredded dried salted ($7.9)	Yes	MK241683	99.8%	*Gadus chalcogrammus* (Walleye Pollock)	KJ614772.1	**Yes**	**Yes**	No
S7	Bacalhau do Porto (Atlantic Cod)	Filleted dried salted ($15.8)	Yes	MK241684	100%	*Gadus morhua* (Atlantic Cod)	KX267087.1	No	No	No
S8	Bacalhau Ling (Ling)	Filleted dried salted ($7.4)	**No**	MK241685	100%	*Molva molva* (Ling)	KJ205059.1	No	**Yes**	No
S9	Bacalhau do Porto (Atlantic Cod)	Filleted unsalted frozen ($7.9)	Yes	MK241686	100%	*Gadus morhua* (Atlantic Cod)	KX267089.1	No	No	**Yes**
S10	Bacalhau do Porto (Atlantic Cod)	Shredded dried salted ($21.1)	Yes	MK241687	100%	*Gadus morhua* (Atlantic Cod)	KX267087.1	No	No	No

do Rio Grande do Sul. Muscle and skin samples were desalted through immersion in filtered water for 36 h, with the water changed every nine hours. Desalted tissues were then directly submitted to DNA extraction. Product designation, cut pieces and preservation, species identification, and GenBank accession numbers are shown in Table 1.

2.2. Extraction, amplification, and sequencing

Total genomic DNA was isolated through the DNeasy Blood & Tissues Kit (QIAGEN®, Hilden, Germany). DNA quantification was performed using Qubit Fluorometer (ThermoFisher Scientific). Polymerase chain reactions (PCR) were carried out in a total volume of 25 μl containing 2 μl (15−30 ng) of DNA template, 8 μl of HotStarTaq Master Mix Kit (QIAGEN®, Hilden, Germany), 1.25 μl (10 μM) of each primer LCO1490 and HCO2198 [10], 0.7 μl of $MgCl_2$ and water to adjust final volume. The *coI* fragment was amplified under the following thermocycler conditions: initial denaturation 15 min at 96 °C, 35 cycles of 30 s at 94 °C, 30 s at 42 °C, and 2 min at 72 °C; followed by a final extension of 10 min at 72 °C. The amount of amplified fragments per sample was quantified through comparison with Low DNA Mass Ladder (Invitrogen), using horizontal electrophoresis to test the amplification success. Amplicons were purified and sequenced in both directions at the Functional Biosciences facility (Madison, USA).

2.3. Analysis and species identification

Chromatograms of molecular data were visualized and edited using Geneious® 6.0.5 software (http://www.geneious.com) [13]. The sequences were aligned in this same program, using automatic assembly in the implemented multiple sequence comparison by log-expectation (MUSCLE) algorithm with default parameters, and each contig pair was visually inspected and edited before consensus sequences were extracted. Codon positions of the protein-codifying gene were tested based on the vertebrate mitochondrial genetic code of amino acid translation.

The sequences' identity was tested by the Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information (NCBI, Bethesda, USA), using the GenBank database (www.blast.ncbi.nlm.nih.gov), and the Identification Engine Tool (IDS) in BOLD (Barcode of Life Database, Biodiversity Institute of Ontario, University of Guelph, Guelph, Canada, www.barcodinglife.com) (Fig. 1). The cutoff value for the identity match search was >98 %, and at the species identification level, the sequences with genetic distance equal to or very close to zero were considered. Additionally, species identification was confirmed by comparing to reference sequences deposited in GenBank. All aligned sequences from supposed Cod samples and reference samples from GenBank were used to produce a phenogram clustering specimen sequences by similarity based on nucleotide genetic distances calculated using the kimura-2-parameters distance model [14] under the Neighbor-Joining approach implemented in BOLD.

3. Results

Ten dried, salted, and frozen fish samples (S1 to S10) of questioned Cod products were tested to validate the label identification, using *coI* barcode sequences, which were submitted to GenBank. The DNA sequences ranged from 531 to 619 base pairs, with no stop codons detected. The DNA barcoding technique associated with compliant reference sequences of GenBank was used to identify all 10 samples at the species level (Fig. 1).

Seven product samples were detected as fraudulent (see Table 1). These frauds belonged to one or more of three fraud types (Fig. 2): 1) species substitution (type 1 fraud), in which the species does not match the identification in the product label and usually a cheaper fish species has replaced the more expensive announced species; 2) mislabeling (type 2 fraud), that is, illegal use of the Cod designation for unauthorized species according to Brazilian technical regulations, or 3) disqualified preparation (type 3 fraud), in which the fish product does not follow the salted or dried salted standard preparation necessary to receive the Cod designation. In all cases, the trader action represents fraud under the law, *per se*, indicating deliberately misleading the customer. Even if incorrect use of the Cod designation is unintentional, due to a lack of information related to current technical regulations, customers are misled not only in terms of price but also in terms of quality and distinct characteristics of fish meat.

Among the seven verified frauds in the Cod commerce in Porto Alegre, all three distinct fraud types were observed. The identified species in three samples (S3, S4, and S6) did not correspond to the species declared in the product label information, representing substitution fraud (Table 1). In addition to substitution fraud, these samples are also considered mislabeled because they represent species not allowed to be designated as Cod. Two of these cases were substitutions at the intrageneric level (S4 and S6), where Atlantic Cod (*Gadus morhua*) was replaced with

Query ID	Best ID	Top %	Graph	Low %
S1	*Pollachius virens*	100		92.2
S2	*Molva molva*	100		88.7
S3	*Pollachius virens*	100		91.9
S4	*Gadus chalcogrammus*	100		99.7
S5	*Gadus morhua*	100		99.8
S6	*Gadus chalcogrammus*	99.8		99.7
S7	*Gadus morhua*	100		99.8
S8	*Molva molva*	100		88.7
S9	*Gadus morhua*	100		99.8
S10	*Gadus morhua*	100		99.8

Fig. 1. Barcode identification of questioned Cod products based on BOLD (Barcode of Life) databases. Graph indicating genetic similarity scores (%) of 99 sequence matches, ranked from maximum similarity (Top) to minimum similarity (Low) for each questioned sample.

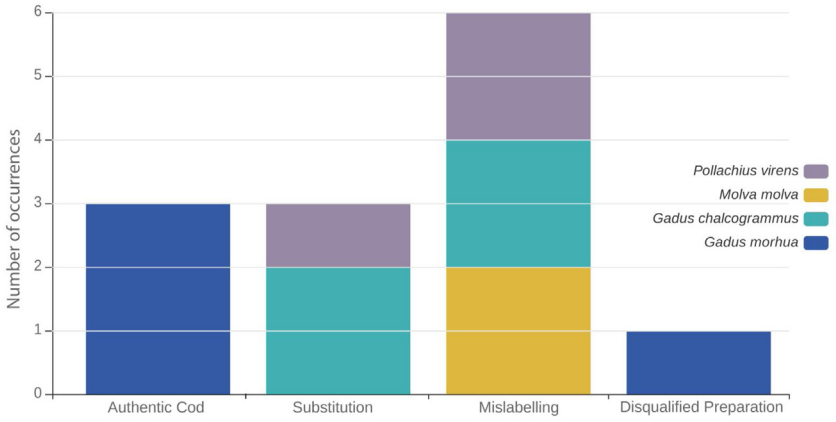

Fig. 2. Authenticity of Cod products. The series refers to authentic Cod and the different types of Cod fraud—substitution, mislabeling, and disqualified preparation—detected for each species indicated in the product label according to the color pallet.

Walleye Pollock (*Gadus chalcogrammus*), while S3 represented a more severe fraud, replacing the supposed Atlantic Cod with Saithe (*Pollachius virens*). Three other samples (S1, S2, and S8) declared the correct scientific name of the species in the product label information but represented commercial fraud through mislabeling errors, in which species not allowed to be designated as Cod (*Polachius virens* and *Molva molva*) were sold under this name (Table 1). Finally, one sample (S9) was commercialized as unsalted frozen Cod, a preparation type in discordance with technical regulations restricting this designation to salted or dried salted preparation (Table 1).

An important finding associated with Cod substitution and mislabeling, generally unnoticed or unreported in the literature, is related to cutting type. The present results revealed a high incidence of substitution in shredded products commerce (three of four samples) when compared to the filleted or whole fish (no substitution fraud), with all substitution cases occurring in shredded samples (Table 1). The reasons for the dealer preference for substituting Cod in shredded products is that macroscopic substitution detection is considerably more difficult in shredded meat than in larger pieces of fillet or whole fish.

4. Discussion

Cod commerce in Porto Alegre exhibits a severe fraud scenario present in Brazil (Fig. 2), with significant losses for consumers both financially, since Cod can reach twice or three times the price of similar species, and in terms of food quality. Other studies focused on fish barcode investigation and mislabeling of fish products have also reported high levels of substitution and mislabeling fraud involving Cod and other seafood products in Brazil [1,2], Europe [5,6,9], North America [15,17,25], Asia [3,20,23], and worldwide [12].

Local Atlantic Cod stocks have declined dramatically, and the species is currently depleted worldwide [19,21,18] and assessed as threatened with extinction in the IUCN category Vulnerable [24] because of the long-term intensive harvest to which this species has been exposed. Mislabeling has been acknowledged as an additional factor that can exert a harmful effect on the conservation of harvested fish because it creates a false perception of market availability, causing consumers to believe that stocks must be healthy [5,18]. Lack of transparency in processing and labeling of seafood by the industry and marketplaces is a serious problem that affects the entire production chain, from fishery operations to restaurants, consumers, and species conservation itself [18,26].

The exposed fraud scenario of substitution and mislabeling of seafood products revealed in Porto Alegre has also been reported in 14 other Brazilian states by Carvalho et al. [1,2] and indeed on a global scale [12,18]. This prevalent situation is a consequence of ineffective seafood inspection, in spite of MAPA's adoption of the DNA barcoding methodology as a standard for routine inspection and regulation of seafood products in Brazil. Based on these findings, to enhance the traceability of seafood products and to minimize the incidence of IUU fishing products entering the market, Brazilian authorities must assess regulatory weaknesses and take necessary measures to permit systematic monitoring, including the use of rapid DNA barcoding technologies, which are becoming increasingly sophisticated and affordable, and the expansion of inspection capacity to precisely identify substitution and mislabeling practices.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.fsir.2020.100072.

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