Fisheries Research 191 (2017) 30–35

Contents lists available at ScienceDirect

Fisheries Research

journal homepage: www.elsevier.com/locate/fishres

Nationwide Brazilian governmental forensic programme reveals seafood mislabelling trends and rates using DNA barcoding

Daniel Cardoso Carvalho[a,b,*], Danusa Guedes[e], Maria da Gloria Trindade[c], Regina Melo Sartori Coelho[c], Paulo Humberto de Lima Araujo[d]

[a] Programa de Pós-graduação em Biologia de Vertebrados, Laboratório de Genética da Conservação, PUC Minas, Minas Gerais, Brazil
[b] Myleus Biotecnologia Research Team, Minas Gerais, Brazil
[c] Laboratório Nacional Agropecuário em Goiás, Ministério da Agricultura, Pecuária e Abastecimento, Goiânia, Goiás, Brazil
[d] Serviço de Investigação de Violações e Notificações – SEIV/CGI/DIPOA/SDA Ministério da Agricultura, Pecuária e Abastecimento, Brasília, DF, Brazil
[e] Programa de Pós-Graduação em Ecologia de Biomas Tropicais, UFOP, Minas Gerais, Brazil

ARTICLE INFO

Article history:
Received 12 December 2016
Received in revised form 24 February 2017
Accepted 28 February 2017
Handled by George A. Rose
Available online 6 March 2017

Keywords:
Fish
COI
Mislabeling
Fraud
Enforcement
Molecular

ABSTRACT

The Brazilian federal government has adopted DNA barcoding (i.e. analysing about 650 base pairs of the COI mitochondrial gene) as a standardised method in a nationwide programme for routine and systematic regulation of processed seafood products. Here, we report rates and trends of a forensic programme which sampled products with twenty-eight commercial fish names (N = 255) confiscated by official governmental officers from 14 states, and which also included imports from 8 countries. A mislabelling rate of 17.3% (44 samples) was recovered when comparing the DNA barcode identification of fish products to the official Brazilian list of species and commercial names. No statistical differences in mislabelling rates between geopolitical regions was detected ($G = 2.4$, $N = 5$, $p = 0.66$). The number of mislabelled samples was not correlated to samples size per commercial name ($r = 0.34$, $N = 29$, $p = 0.07$), but instead, mislabelling was positively correlated to the number of species detected ($r = 0.75$, $N = 29$, $p < 0.00$), suggesting that more surveillance should be given to species with less well-defined commercial names. The programme resulted in financial penalties being applied according to the amount of mislabelling detected. Moreover, companies caught selling mislabelled products were further inspected until the company proved that their production was normalised according to Brazilian labelling regulations. A systematic nationwide forensic governmental programme may lead to more sustainable and trusted fisheries activities allowing consumers to make informed choices when buying seafood products.

1. Introduction

The DNA barcoding methodology (i.e. analysing 650 bp of the COI mitochondrial gene; Hebert et al., 2003) provides a standardised methodology for species identification, which has been extensively applied in forensic analysis (Carvalho et al., 2011, 2015; Cawthorn et al., 2012; Handy et al., 2011; Mariani et al., 2015). Thus, the US Food and Drug Administration (FDA) have validated the generation of DNA Barcodes for seafood product identification towards regulatory compliance (Handy et al., 2011).

Concerns over the collapse of wild-caught fisheries led to an intense focus on seafood certification using molecular tools resulting in consumer-driven support of sustainable seafood (Logan et al., 2008; Mariani et al., 2015). For example, seven species have been sold under the common name "Pacific red snapper" in the USA, even though 56% of them were listed as overfished (Logan et al., 2008). Therefore, generic common names may compromise the ability of consumers to make informed choices when buying seafood, which encouraged governmental regulatory agencies to use standardised methodologies (e.g. DNA barcoding) to inspect seafood products (Chang et al., 2016).

Detection of fraudulent commercialisation of seafood products has been extensively reported but results of governmental regulatory programmes are still scarce (Carvalho et al., 2015; Chang et al., 2016). The growing literature on seafood mislabelling is reported for market places worldwide, for instance in Brazil (Carvalho et al., 2015; Brito et al., 2015), Canada (Hanner et al., 2011), Europe (Mariani et al., 2015), USA (Khaksar et al., 2015; Wong and Hanner 2008), South-Africa (Cawthorn et al., 2012), and

* Corresponding author at: Rua Dom José Gaspar, 500, 30535-901 Belo Horizonte, MG, Brazil.
E-mail addresses: danielcarvalho@pucminas.br, carvalho.lgc@gmail.com (D.C. Carvalho).

http://dx.doi.org/10.1016/j.fishres.2017.02.021
0165-7836/

Taiwan (Chang et al., 2016). Nonetheless, there is evidence that mislabelling rates have been reducing in the European seafood market due to technology-based and policy-oriented actions (Mariani et al., 2015).

In Brazil, the first state governmental programme aiming to analyse seafood fraud detected 24% of mislabelling (Carvalho et al., 2015). However, they analysed only 30 samples of commercially important species collected from fishmongers, supermarkets, and restaurants in the city of Florianópolis. They found that highly priced species (flounder, pink cusk-eel, and cod) had been substituted for cheaper species such as basa and Alaska pollock, but due to the small sample size and restricted range of sample sites, the mislabelling rate of other Brazilian geopolitical regions still remains unknown.

The Ministry of Agriculture, Livestock, and Food Supply (MAPA – Ministério da Agricultura, Pecuária e Abastecimento), responsible for ensuring accurate labelling of foodstuff at the federal level in Brazil, have adopted and implemented the DNA barcoding methodology as a standardised method for routine and systematic regulation of seafood products. Due to the megadiverse fish fauna present in the Neotropics (approximately 5600 described species) (Reis et al., 2016), it is challenging to regulate all seafood commercialised, but an official list of legal commercial names and Latin scientific names was produced in order to facilitate market regulation (MAPA, 2015). Here, we report rates and trends from the first country-wide forensic programme aiming to regulate the seafood market and compare mislabelling rates between Brazilian geopolitical regions.

2. Material and methods

2.1. Sample collection

We analysed 255 fish products, consisting of twenty-nine commercial fish names, confiscated by official governmental officers from fishmongers, markets, and supermarkets located in 14 Brazilian states. For mislabelling rates comparisons we used 240 samples presenting sampled site information, which were grouped into five geopolitical regions: Central-west (states of Goiás, Mato Grosso do Sul, Mato Grosso, Distrito Federal – n = 33), Northeast (states of Bahia, Ceará, Pernambuco, Rio Grande do Norte n = 20), North (states of Amapá, Pará – n = 16), Southeast (states of Rio de Janeiro, São Paulo – n = 70), South (sates of Paraná, Rio Grande do Sul, Santa Catarina – 63), and eight countries (Argentina, Chile, China, Spain, Italy, New Zealand, Portugal, Uruguay – n = 38). Samples were conserved in ethanol 70% and sent to the official Laboratory of the Ministry of Agriculture, Livestock and Food Supply (LANAGRO-GO) for molecular identification.

2.2. Molecular identification

DNA extraction was conducted using a modified CTAB protocol (Sambrook et al., 2001). Barcode sequences consisting of approximately 700 base pairs of the COI mitochondrial gene were obtained by a polymerase chain reaction (PCR) consisting of 1.25 μl of each primer (10 μmol) (FISHCO1LBC: 5–TCA ACY AAT CAY AAA GAT ATY GGC AC, and FISHCO1HBC: 5–ACT TCY GGG TGR CCR AAR AATCA) described elsewhere (Handy et al., 2011) with a M13 tail, 2.5 μl of buffer (10x), 2.0 μl of $MgCl_2$ (25 mmol/l), 1 μl of dNTP (2.5 mmol/l), 1.0 μl of hot-start Taq DNA polymerase (5U/μl), and 5 μl of template DNA. PCR conditions comprised an initial denaturation step of 95 °C for 12 min followed by 40 cycles of 30 s at 95 °C, 30 s at 64 °C, 30 s at 72 °C and a final step of 72 °C for10 min. Positive PCR amplification was verified by electrophoresis in an agarose gel. PCR products were purified using the ExoSAP-IT® (Affymetrix) and sequenced using BigDye® 3.0 (Thermofisher) according to the manufacture's instructions. The Genetic Analyzer 3500 was used to obtain DNA sequences. Since all barcode sequences obtained were not from voucher samples, they were not deposited in the GenBank or BOLD databases.

2.3. Data analysis

DNA sequences were compared to the BOLD database for molecular identification. Top matches recovered from searches using the Species Level Barcode Records on BOLD identification website were annotated, considering a threshold of 99% for species identification. When Barcodes had a similarity lower than 95%, the sample was classified as unidentified species.

Latin scientific names were associated to the corresponding market names following the official governmental regulatory list of species: *Instrução Normativa nº 29, de 23 setembro de 2015* (IN 29 – MAPA, 2015). Other vernacular names derived from FishBase (Froese and Pauly, 2016) were used when Latin names of species were not found in the official governmental list.

The Pearson correlation test (Sokal and Rohlf, 1995) was applied to test the correlation between: (1) sample size and mislabelled samples, and (2) number of species detected and mislabelled samples.

To compare the number of mislabelled samples between Brazilian geopolitical regions (namely: South, Southeast, North, Northeast, and Central-west) and samples originating from other countries (namely "Imported" samples), we applied the G test (Sokal and Rohlf, 1995). First, a Pearson correlation test between the number of samples and the number of mislabelled detected per region was performed to check if the number of mislabelled samples was correlated to the number of samples from each site. Since this correlation was not significant ($r = 0.51$, $N = 5$, $p = 0.37$), the expected number of mislabelled samples was estimated as the total number of mislabelled samples divided by the number of regions (Sokal and Rohlf, 1995).

The SPSS® software was used to conduct all statistical tests at the significance level of 5%.

3. Results and discussion

DNA barcode sequence lengths ranged from 479 to 692 bp and comparisons with the BOLD System database resulted in matches with a similarity of 90.51% to 100%. There were 22 cases whose similarities to the reference library (BOLD database) did not reach the threshold of >99%, and therefore, they were identified only to genus or family level (Table 1). As 33 samples had similarity lower than 95% to any entry in Genbank, they were classified as unidentified, exemplifying a significant lack of DNA barcodes for Neotropical fish species. Therefore, 200 samples cold be unambiguously identified to the species level.

From the twenty-nine commercial fish names, we detected at least 80 species from the 255 confiscated products (Table 1). The number of species per commercial name ranged from 1 to 5, and the highest number of species detected were found within: linguado (5 species), merluza (5 species), pescada-branca (5 species), surubim/pintado (5 species), abadejo (4 species), bacalhau (4 species), corvine (4 species), dourada (4 species), garoupa (4 species), and sardinha (4 species). On the other hand, within the samples labelled as bagre, cacao, filhote, haddock, mapará, pescada-cambucu, pirarucu, and tambaqui only one species was detected.

Considering all geopolitical regions analysed, a mislabelling rate of 17.3% was recovered when comparing the Barcode identification of fish products to the official Brazilian list of species and their commercial names (IN 29 – MAPA, 2015). The number of mislabeled