Mitochondrial DNA

The Journal of DNA Mapping, Sequencing, and Analysis

ISSN: 1940-1736 (Print) 1940-1744 (Online) Journal homepage: informahealthcare.com/journals/imdn20

DNA barcoding unveils a high rate of mislabeling in a commercial freshwater catfish from Brazil

Daniel C. Carvalho, Danilo A. P. Neto, Bruno S. A. F. Brasil & Denise A. A. Oliveira

To cite this article: Daniel C. Carvalho, Danilo A. P. Neto, Bruno S. A. F. Brasil & Denise A. A. Oliveira (2011) DNA barcoding unveils a high rate of mislabeling in a commercial freshwater catfish from Brazil, Mitochondrial DNA, 22:sup1, 97-105, DOI: 10.3109/19401736.2011.588219

To link to this article: https://doi.org/10.3109/19401736.2011.588219

Published online: 27 Jun 2011.

Submit your article to this journal

Article views: 6130

View related articles

Citing articles: 11 View citing articles

Full Terms & Conditions of access and use can be found at
https://informahealthcare.com/action/journalInformation?journalCode=imdn21

Mitochondrial DNA, October 2011; 22(S1): 97–105

informa
healthcare

RESEARCH PAPER

DNA barcoding unveils a high rate of mislabeling in a commercial freshwater catfish from Brazil

DANIEL C. CARVALHO[1,2], DANILO A. P. NETO[1], BRUNO S. A. F. BRASIL[1], & DENISE A. A. OLIVEIRA[1]

[1]*Departamento de Zootecnia, Escola de Veterinária, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil, and* [2]*Molecular Ecology Laboratory, Flinders University, Adelaide, SA, Australia*

(Received 24 November 2010; revised 27 January 2011; accepted 28 March 2011)

Abstract

Background and Aims. Molecular markers have contributed to species authentication by flagging mislabeling and the misidentification of commercial landings. Such tools are of great value since the market substitution of fish of lower value for highly commercialized species is expected to become more pronounced due to a shortage of natural stocks.

Materials and Methods. Here we report on the molecular identification 4results from processed fish products (i.e. fillets) and whole fishes sold in Brazilian markets under the common name *surubim* (*Pseudoplatystoma* spp.).

Results. DNA barcoding revealed the incorrect labeling of around 80% of all samples analyzed, with mislabeling being more pronounced within fillets rather than whole fish.

Conclusion. To our knowledge, this is the first report correlating the rate of fraud with processed fish products. The establishment of an official list of acceptable common names for freshwater fish and seafood is urgently needed in Brazil for further trade regulations to take place.

Keywords: *DNA barcoding, catfish,* Pseudoplatystoma*, cytochrome oxidase* c *subunit I, São Francisco River, mislabeling*

Introduction

The effects of species substitution include economic fraud, health hazards, and the illegal trade of protected species. Therefore, the detection of species substitution has become an important topic within the food industry, and there is a growing need for rapid, reliable, and reproducible tests to verify species in commercial fish and other animal products (Rasmussen and Morrissey 2008). In addition, species identification is useful in ensuring honest trading exchanges for correct consumer information. Toward this end, regulatory organizations such as the European Union have established labeling laws for fish and aquaculture products, in which labels should bring traceability information (i.e. identification, origin of fish, and production method; Moretti et al. 2003; Martinez et al. 2005).

The substitution of a less valuable fish species and products (i.e. fillets and eggs) for a more valuable one represents a common commercial fraud (DeSalle and Birstein 1996; Civera 2003; Marko et al. 2004). It is known that fluctuations in the supply and demand of different fish species together with increases in international trade and fish consumption are important factors in intentional product mislabeling (Rasmussen and Morrissey 2008). However, the way fishes are processed for commercialization has not yet been correlated with the rate of fraud.

Moreover, it has become clear that most populations of fish are declining dramatically, and aquaculture efforts are not expected to compensate (Pauly et al. 2002). In this scenario, trade in animal species has contributed greatly to overall biodiversity crisis (Manel et al. 2002). For example, 77% of the

Correspondence: D. C. de Carvalho, Molecular Ecology Laboratory, School of Biology Sciences, Flinders University, Bedford Park, Adelaide, SA 5001, Australia. Tel: + 55 31 34092206. Fax: 61 882013015. E-mail: daniel.carvalho@flinders.edu.au; carvalhodcc@yahoo.com.br

ISSN 1940-1736 print/ISSN 1940-1744 online © 2011 Informa UK, Ltd.
DOI: 10.3109/19401736.2011.588219

fish sold in the USA as the highly threatened red snapper (*Lutjanus campechanus*) are in fact another species (Marko et al. 2004). Reliable and rapid molecular identification methods could help both to protect citizens from fraud and to protect endangered species from overexploitation and illegal trafficking (DeSalle and Birstein 1996; Teletchea et al. 2005).

To avoid mislabeling and commercial fraud, the US Food and Drug Administration has compiled an online Regulatory Fish Encyclopedia (http://www.fda.gov) listing acceptable market names, isoelectric focusing protein electrophoresis patterns, and DNA barcoding data (Yancy et al. 2008). These molecular data are also correlated with high-resolution images of whole fish and filleted fish species, plus geographic, taxonomic, and nomenclature information for imported and domestical species. All this information is on hand to assist government officials and purchasers in the correct identification and detection of species substitution and economic fraud. Another similar initiative is the European FishTrace Consortium (http://www.fishtrace.org), comprising 53 members from several institutions (Sevilla et al. 2007). Besides its rich biodiversity, no such initiative is yet currently ongoing in Brazil. Therefore, to implement laws against poaching and the trade of overexploited species and enforcing labeling regulations to prevent product substitution, there is a need for sensitive and reliable analytical methods that can be applied to determine the species of a fish, even when no detectable external features are present (Baker et al. 2000; Kyle and Wilson 2007). Not to mention the detrimental effects that fish adulteration can have on the commercial market, it can also put consumers at risk of purchasing potentially harmful and mislabeled products (Cohen et al. 2009; Lowenstein et al. 2010). The effectiveness of fish conservation and management programs can also be improved which aid in the protection of aquatic habitats and endangered species (Teletchea et al. 2005).

Molecular tools are advantageous for fish and fish products identification for three main reasons: large number of fish species from distinct live history stages (eggs, fry, and adults) can be examined; in addition, processed fish products lacking the morphological characteristics, such as frozen fillets and precooked fish, are also accessible (typically, these cannot be identified using the traditional identification procedure); and there are insufficient specialists in alpha taxonomy for fish identification, especially in the Neotropics.

Several protocols have been described for species identification of fish products, based on different technologies such as high-performance liquid chromatography, isoelectric focusing, and polyacrylamide gel electrophoresis (Martinez et al. 2005; Rasmussen and Morrissey 2008). Among these, DNA-based methodologies are one of the most promising approaches since they can be applied to all the different life stages of fish species and fish products (Rasmussen and Morrissey 2008; Smith et al. 2008; Vinas and Tudela 2009).

The mitochondrial DNA (mtDNA) cytochrome *c* oxidase subunit I gene (COI) has provided numerous examples as a reliable and universal tool for the identification of species such as the flatfish (Terol et al. 2002; Espineira et al. 2008), tuna (Terol et al. 2002; Lowenstein et al. 2010), anchovy (Jerome et al. 2008), sharks (Barbuto et al. 2010), and also wildlife forensics investigations (Dawnay et al. 2007; Nelson et al. 2007). Its application in molecular taxonomy has been criticized due to introgressive hybridization, mitochondrial pseudogenes in the nucleus, and the retention of ancestral polymorphisms (e.g. Rubinoff 2006; Rubinoff et al. 2006). However, species assignment failure rates do not typically exceed 5–10% (Hebert and Gregory 2005; Ward et al. 2005; Hubert et al. 2008; Valdez-Moreno et al. 2009). Therefore, a global effort to assemble a standardized reference DNA sequence library (using the COI region) for all fishes has been proposed. This initiative led to the establishment of international research collaboration, named the Fish Barcode of Life Campaign (FISH-BOL; Ward et al. 2009). The barcode data for thousands of freshwater fishes have been uploaded to the Barcode of Life Data Systems database (BOLD), including freshwater species from the Neotropics (data available on BOLD; Carvalho et al. 2011), allowing their application for the analysis of commercial fraud cases in Brazil.

This work was conducted to identify which species have been sold in Brazil labeled as *surubim* or *pintado* using DNA barcode data. These vernacular names are applied to *Pseudoplatystoma corruscans* and *Pseudoplatystoma reticulatum* (former *Pseudoplatystoma fasciatum*; Froese and Pauly 2011); however, Brazil does not have an official list of commercial and Latin names for these fishes. *P. corruscans* is considered the most valuable commercial and recreational freshwater fish in the São Francisco River (fourth biggest river in Brazil); however, *surubim* harvest has shown clear indications of collapse, since harvesting of *P. corruscans* has declined from 10.3 to 0.8 kg captured fish per day from 1987 to 1999 (Godinho et al. 2007). Moreover, the genus *Pseudoplatystoma* consists of at least eight species, five of which were recently described (Buitrago-Suarez and Burr 2007). *P. reticulatum* and *P. corruscans* are sympatric in the Paraná basin (southern Brazil), whereas only the latter has been described in the São Francisco basin. A recent molecular phylogeny of the genus *Pseudoplatystoma* did not find any correlation between morphological and molecular data (Control Region) in two species (*P. reticulatum* and *Pseudoplatystoma punctifer*; Torrico et al. 2009). For all other species, including

P. corruscans (*surubim*), the molecular data supported the morphological classification.

From this work, we reported a fish market DNA barcode survey of processed (i.e. fillets) and whole fishes sold in Brazil under the common name *surubim*. We tested the hypothesis that substitutions would be more frequent among fillets than within whole fish because of the difficulty of visual identification. Our results showed a strong correlation between the methods in which fishes are commercialized (i.e. fillets or whole fish) and fraud, since the highest number of substitutions was detected within fillets.

Materials and methods

Samples labeled as *surubim* were purchased at nine supermarkets in the city of Belo Horizonte (Minas Gerais state, Brazil) in 2009–2010. Samples consisted of two types: whole fish ($n = 30$) and fillets ($n = 33$). Upon collection, tissues were stored in ethanol 95% and details of brand, price, and pictures of the product were taken for documentation purposes. DNA was isolated by homogenization and digestion with proteinase K at 37°C overnight, followed by standard phenol/chloroform purification (Sambrook et al. 1989). A fragment of 658 bp of COI was amplified using the primers FishF1 and FishR1 (Ward et al. 2005). The 25 μl PCR mixes included 19.5 μl ultrapure water, 2.5 μl of 10 × PCR buffer, 2.5 mM $MgCl_2$, 0.35 μl each primer (10 mM), 2.5 μl dNTP (1 mM), 0.25μl PHT *Taq* polymerase (5 U/μl), and 1.0 μl DNA template (50–100 ng/μl). Thermal cycling conditions consisted of an initial denaturation step at 94°C for 2 min, 35 cycles at 94°C for 30 s, 54°C for 30 s, and 72°C for 1 min, with a final extension at 72°C for 10 min. PCR products (1–2 μl) were visualized on an agarose gel and selected for direct sequencing. Sequences were determined bi-directionally using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, California, USA), following the manufacturer's protocol on an ABI PRISM 310 Genetic Analyzer.

Data analysis

COI sequences recovered from samples labeled as *surubim* were identified by searching the GenBank database using the BLASTN algorithm (http://blast.ncbi.nlm.nih.gov) and by BOLD (http://www.boldsystems.org) identification engine to search DNA barcode records within BOLD (Ratnasingham and Hebert 2007). The best-scoring matches obtained from both databases for each sample were registered. Genetic divergence was calculated using Kimura's two-parameter (K2P) nucleotide substitution model (Kimura 1980). A neighbor-joining tree (Saitou and Nei 1987) of K2P distances was generated in MEGA 3 (Kumar et al. 2004) to provide a graphic representation of divergence among analyzed samples. Node support was assessed with 10,000 bootstrap replicates (Felsenstein 1985).

Brazil does not have a list of acceptable common names; therefore, the FishBase (http://www.fishbase.org) nomenclature was used to identify the scientific names that may correspond to the commercial name *surubim* (*P. corruscans* and *P. reticulatum*), especially since BOLD and GenBank rely on FishBase as a taxonomic authority for valid fish species names (Froese and Pauly 2011). It is interesting to note that hybrids obtained by crossing a female *P. reticulatum* and a male *P. corruscans* (Carvalho et al. 2008; Bignotto et al. 2009) are of the most common freshwater catfishes commercialized in Brazil. A standard DNA barcode sequence for *P. corruscans* (Carvalho et al. 2011; BOLD record number BSB400-10 and GenBank accession number HM405206) was included as a reference in the alignment dataset.

Results

Genetic identification of samples commercialized as surubim using the GenBank and BOLD search engines

All amplified sequences that exceeded 600 nucleotides in length with no insertions, deletions, or stop codons were observed, thus reducing the possibility of mtDNA copies in the nucleus. The sequences obtained from the samples were deposited on GenBank (accession numbers HQ689323–HQ689385) and compared against the BOLD and GenBank databases. Successful matches varied from 89 to 100% pairwise sequence identity (Table I). Only three samples could not be identified in the BOLD Species Reference database. Nonetheless, the BOLD Full database returned hits with a percentage of identity as high as GenBank, with the advantage of being a more reliable source of taxonomic identification. Our results showed that only one *P. corruscans* haplotype (sample BS1) was detected among the 63 commercial samples analyzed (Table I). Most of the samples had 100% similarity with the catfish species *Pseudoplatystoma tigrinum* and *P. reticulatum*. However, high-identity matches were also obtained with marine species *Genidens barbus* (100% match) and *Cynoscion virescens* (89–100% match), clearly showing a case of mislabeling (Table I). Lower identity scores were also returned, and samples were provisionally identified; for example, *Netuma thalassina* (sample 24, Table I) with a 90% match (BOLD database) and *Cynoscion jamaicensis* (sample 66) with an 89% match in BOLD. The latter species (sample 66) also had a GenBank top match with the spotfin croaker *Roncador stearnsii* (87%), a species from the eastern Pacific ocean (Table I).

Table I. Identification of commercial *surubim* samples using the GenBank and BOLD search engines.

Sample number	Genbank accession number	Type	BOLD full database	BOLD reference database	Genbank	Mislabeled*
23	HQ689374	Fillet	*Brachyplatystoma platynemum* (91.45)	No sequence	*Brachyplatystoma filamentosum* (92)	Yes
24	HQ689375	Fillet	*Netuma* cf. *thalassina* (90.69)	No sequence	*Siluriformes* sp. BOLD:AAC3439 (90)	Yes
37	HQ689376	Fillet	*Genidens barbus* (99.75)	*Genidens barbus* (99.75)	*Siluriformes* sp. BOLD:AAF4992 (99)	Yes
38	HQ689377	Fillet	*Genidens barbus* (100)	*Genidens barbus* (100)	*Siluriformes* sp. BOLD:AAF4992 (100)	Yes
39	HQ689378	Fillet	*Genidens barbus* (100)	*Genidens barbus* (100)	*Siluriformes* sp. BOLD:AAF4992 (100)	Yes
40	HQ689379	Fillet	*Genidens barbus* (99.75)	*Genidens barbus* (99.75)	*Siluriformes* sp. BOLD:AAF4992 (99)	Yes
41	HQ689380	Fillet	*Genidens barbus* (100)	*Genidens barbus* (100)	*Siluriformes* sp. BOLD:AAF4992 (100)	Yes
42	HQ689381	Fillet	*Genidens barbus* (100)	*Genidens barbus* (100)	*Siluriformes* sp. BOLD:AAF4992 (100)	Yes
43	HQ689382	Fillet	*Pseudoplatystoma fasciatum* (100)	*Pseudoplatystoma fasciatum* (100)	*Siluriformes* sp. BOLD:AAA2588 (99)	No
44	HQ689383	Fillet	*Genidens barbus* (99.75)	*Genidens barbus* (99.75)	*Siluriformes* sp. BOLD:AAF4992 (99)	Yes
45	HQ689384	Fillet	*Genidens barbus* (99.75)	*Genidens barbus* (99.75)	*Siluriformes* sp. BOLD:AAF4992 (99)	Yes
46	HQ689385	Fillet	*Pseudoplatystoma tigrinum* (100)	*Pseudoplatystoma tigrinum* (100)	*Brachyplatystoma filamentosum* (100)	Yes
48	HQ689353	Fillet	*Pseudoplatystoma fasciatum* (100)	*Pseudoplatystoma fasciatum* (100)	*Siluriformes* sp. BOLD:AAA2588 (100)	No
49	HQ689354	Fillet	*Pseudoplatystoma fasciatum* (100)	*Pseudoplatystoma fasciatum* (100)	*Siluriformes* sp. BOLD:AAA2588 (99)	No
50	HQ689355	Fillet	*Pseudoplatystoma fasciatum* (100)	*Pseudoplatystoma fasciatum* (100)	*Siluriformes* sp. BOLD:AAA2588 (100)	No
51	HQ689356	Fillet	*Pseudoplatystoma fasciatum* (100)	*Pseudoplatystoma fasciatum* (100)	*Pseudoplatystoma fasciatum* (98)	No
52	HQ689357	Fillet	*Pseudoplatystoma fasciatum* (100)	*Pseudoplatystoma fasciatum* (100)	*Pseudoplatystoma fasciatum* (100)	No
53	HQ689358	Fillet	*Pseudoplatystoma fasciatum* (100)	*Pseudoplatystoma fasciatum* (100)	*Pseudoplatystoma fasciatum* (100)	No
54	HQ689359	Fillet	*Pseudoplatystoma fasciatum* (100)	*Pseudoplatystoma fasciatum* (100)	*Pseudoplatystoma fasciatum* (100)	No
55	HQ689360	Fillet	*Pseudoplatystoma fasciatum* (100)	*Pseudoplatystoma fasciatum* (100)	*Pseudoplatystoma fasciatum* (100)	No
56	HQ689361	Fillet	*Pseudoplatystoma fasciatum* (100)	*Pseudoplatystoma fasciatum* (100)	*Pseudoplatystoma fasciatum* (100)	No
57	HQ689362	Fillet	*Pseudoplatystoma fasciatum* (100)	*Pseudoplatystoma fasciatum* (100)	*Pseudoplatystoma fasciatum* (99)	No
58	HQ689363	Fillet	*Pseudoplatystoma fasciatum* (100)	*Pseudoplatystoma fasciatum* (100)	*Pseudoplatystoma fasciatum* (100)	No
59	HQ689364	Fillet	*Pseudoplatystoma fasciatum* (100)	*Pseudoplatystoma fasciatum* (100)	*Pseudoplatystoma fasciatum* (98)	No
66	HQ689365	Fillet	*Cynoscion jamaicensis* (89.73)	No sequence	*Roncador stearnsii* (87)	Yes
67	HQ689366	Fillet	*Cynoscion virescens* (99.58)	*Cynoscion virescens* (99.58)	*Perciformes* sp. BOLD:AAJ3285 (99)	Yes
68	HQ689367	Fillet	*Cynoscion virescens* (99.16)	*Cynoscion virescens* (99.16)	*Perciformes* sp. BOLD:AAJ3285 (100)	Yes
69	HQ689368	Fillet	*Cynoscion virescens* (99.58)	*Cynoscion virescens* (99.58)	*Perciformes* sp. BOLD:AAJ3285 (99)	Yes
70	HQ689369	Fillet	*Cynoscion virescens* (99.58)	*Cynoscion virescens* (99.58)	*Perciformes* sp. BOLD:AAJ3285 (99)	Yes
71	HQ689370	Fillet	*Cynoscion virescens* (99.58)	*Cynoscion virescens* (99.58)	*Perciformes* sp. BOLD:AAJ3285 (99)	Yes
72	HQ689371	Fillet	*Cynoscion virescens* (99.16)	*Cynoscion virescens* (99.16)	*Perciformes* sp. BOLD:AAJ3285 (100)	Yes
73	HQ689372	Fillet	*Cynoscion virescens* (99.16)	*Cynoscion virescens* (99.16)	*Perciformes* sp. BOLD:AAJ3285 (100)	Yes
74	HQ689373	Fillet	*Cynoscion virescens* (99.16)	*Cynoscion virescens* (99.16)	*Perciformes* sp. BOLD:AAJ3285 (100)	Yes
14	HQ689347	Whole	*Pseudoplatystoma tigrinum* (100)	*Pseudoplatystoma tigrinum* (100)	*Brachyplatystoma filamentosum* (99)	Yes
16	HQ689348	Whole	*Pseudoplatystoma tigrinum* (99.41)	*Pseudoplatystoma tigrinum* (99.41)	*Brachyplatystoma filamentosum* (99)	Yes
17	HQ689349	Whole	*Pseudoplatystoma tigrinum* (98.82)	*Pseudoplatystoma tigrinum* (98.82)	*Brachyplatystoma filamentosum* (98)	Yes
18	HQ689350	Whole	*Pseudoplatystoma tigrinum* (100)	*Pseudoplatystoma tigrinum* (100)	*Brachyplatystoma filamentosum* (100)	Yes
20	HQ689351	Whole	*Pseudoplatystoma tigrinum* (100)	*Pseudoplatystoma tigrinum* (100)	*Brachyplatystoma filamentosum* (99)	Yes
21	HQ689352	Whole	*Pseudoplatystoma tigrinum* (100)	*Pseudoplatystoma tigrinum* (100)	*Brachyplatystoma filamentosum* (100)	Yes
78	HQ689323	Whole	*Pseudoplatystoma tigrinum* (99.62)	*Pseudoplatystoma tigrinum* (99.62)	*Brachyplatystoma filamentosum* (99)	Yes
79	HQ689336	Whole	*Pseudoplatystoma tigrinum* (100)	*Pseudoplatystoma tigrinum* (100)	*Brachyplatystoma filamentosum* (100)	Yes
80	HQ689337	Whole	*Pseudoplatystoma tigrinum* (100)	*Pseudoplatystoma tigrinum* (100)	*Brachyplatystoma filamentosum* (100)	Yes
81	HQ689338	Whole	*Pseudoplatystoma tigrinum* (100)	*Pseudoplatystoma tigrinum* (100)	*Brachyplatystoma filamentosum* (100)	Yes
82	HQ689339	Whole	*Pseudoplatystoma tigrinum* (100)	*Pseudoplatystoma tigrinum* (100)	*Brachyplatystoma filamentosum* (100)	Yes
83	HQ689340	Whole	*Pseudoplatystoma tigrinum* (100)	*Pseudoplatystoma tigrinum* (100)	*Brachyplatystoma filamentosum* (100)	Yes
84	HQ689341	Whole	*Pseudoplatystoma tigrinum* (100)	*Pseudoplatystoma tigrinum* (100)	*Brachyplatystoma filamentosum* (100)	Yes
85	HQ689342	Whole	*Pseudoplatystoma tigrinum* (100)	*Pseudoplatystoma tigrinum* (100)	*Brachyplatystoma filamentosum* (100)	Yes
86	HQ689343	Whole	*Pseudoplatystoma tigrinum* (100)	*Pseudoplatystoma tigrinum* (100)	*Brachyplatystoma filamentosum* (100)	Yes

Table I – *continued*

Sample number	Genbank accession number	Type	BOLD full database	BOLD reference database	Genbank	Mislabeled*
87	HQ689344	Whole	*Pseudoplatystoma tigrinum* (99.62)	*Pseudoplatystoma tigrinum* (99.62)	*Brachyplatystoma filamentosum* (99%)	Yes
88	HQ689345	Whole	*Pseudoplatystoma tigrinum* (100)	*Pseudoplatystoma tigrinum* (100)	*Brachyplatystoma filamentosum* (100)	Yes
89	HQ689346	Whole	*Pseudoplatystoma tigrinum* (100)	*Pseudoplatystoma tigrinum* (100)	*Brachyplatystoma filamentosum* (100)	Yes
90	HQ689324	Whole	*Pseudoplatystoma tigrinum* (100)	*Pseudoplatystoma tigrinum* (100)	*Brachyplatystoma filamentosum* (100)	Yes
91	HQ689325	Whole	*Pseudoplatystoma tigrinum* (100)	*Pseudoplatystoma tigrinum* (100)	*Brachyplatystoma filamentosum* (100)	Yes
92	HQ689326	Whole	*Pseudoplatystoma tigrinum* (100)	*Pseudoplatystoma tigrinum* (100)	*Brachyplatystoma filamentosum* (100)	Yes
93	HQ689327	Whole	*Pseudoplatystoma tigrinum* (100)	*Pseudoplatystoma tigrinum* (100)	*Brachyplatystoma filamentosum* (100)	Yes
94	HQ689328	Whole	*Pseudoplatystoma tigrinum* (100)	*Pseudoplatystoma tigrinum* (100)	*Brachyplatystoma filamentosum* (100)	Yes
95	HQ689329	Whole	*Pseudoplatystoma tigrinum* (100)	*Pseudoplatystoma tigrinum* (100)	*Brachyplatystoma filamentosum* (100)	Yes
96	HQ689330	Whole	*Pseudoplatystoma tigrinum* (100)	*Pseudoplatystoma tigrinum* (100)	*Brachyplatystoma filamentosum* (100)	Yes
97	HQ689331	Whole	*Pseudoplatystoma tigrinum* (100)	*Pseudoplatystoma tigrinum* (100)	*Brachyplatystoma filamentosum* (100)	Yes
98	HQ689332	Whole	*Pseudoplatystoma tigrinum* (100)	*Pseudoplatystoma tigrinum* (100)	*Brachyplatystoma filamentosum* (100)	Yes
99	HQ689333	Whole	*Pseudoplatystoma tigrinum* (100)	*Pseudoplatystoma tigrinum* (100)	*Brachyplatystoma filamentosum* (100)	Yes
100	HQ689334	Whole	*Pseudoplatystoma tigrinum* (100)	*Pseudoplatystoma tigrinum* (100)	*Brachyplatystoma filamentosum* (100)	Yes
BS1	HQ689335	Whole	*Pseudoplatystoma corruscans* (100)	*Pseudoplatystoma corruscans* (100)	*Siluriformes* sp. BOLD:AAD0242 (100)	No

Note: Sixty-three commercial samples labeled as *surubim* were obtained from the markets in Belo Horizonte (Brazil). Recovered COI sequences were compared with BOLD Full and Reference databases, as well as with GenBank for identification (identity percentage score in parentheses). GenBank accession numbers for each sample are provided; * Acceptable species follow FishBase nomenclature.

DNA barcode analysis of whole and filleted fishes commercialized as surubim

The careful inspection of the results presented in Table I revealed a higher rate of substitution within species from genera other than *Pseudoplatystoma* among fishes sold as fillets rather than whole fish. To better evaluate this difference, a neighbor-joining tree using the K2P evolutionary model was built for each group (fillets and whole fishes) separately (Figure 1a,c). The overall K2P distance for the whole fish group (Figure 1a) was 1.1%, and the divergence of commercial samples from the standard barcode sequence of *P. corruscans* varied from 0 to 8.4%. The overall K2P distance for the filleted fish group (Figure 1c) was higher (15.5%), and the divergence of commercial samples from the standard barcode sequence of *P. corruscans* varied widely from 5.9 to 24.8%. Within whole fish only two clades were detected (99% bootstrap support value), one representing the Amazonian species *Pseudoplatystoma tigrinus* and the other representing *P. corruscans* clade, with only one sample grouping together with our standard barcode sequence for *P. corruscans* (Table I and Figure 1a). When considering fillets, at least seven distinct clades with moderate to high-bootstrap value (99–68%) were recovered, characterizing at least seven different species (Figure 1), with not even one haplotype belonging to *P. corruscans* (Table I and Figure 1c). The pie charts (Figure 1b,d) show more explicit graphical representations of the percentage of samples identified as *P. corruscans* within the whole fish and fillet categories. When considering the whole fish group (Figure 1b), one sample was identified as belonging to *P. corruscans*, whereas the remaining 97% of this group was identified as *P. tigrinum* (100% similarity with this Amazonian species). In contrast, only 42% of the filleted samples (Figure 1d) belonged to *Pseudoplatystoma* sp., whereas 58% of them had higher similarity with other genera (i.e. *Brachyplatystoma*, *Netuma,* and *Genidens*).

Discussion

The application of COI sequences in forensics has already been investigated for reproducibility, heteroplasmy, mixed DNA samples, chemical treatments, environmental conditions, and other factors showing consistent results in which a great range of reference data exist (Dawnay et al. 2007). Several examples have already highlighted the potential of such molecular tools to flag the mislabeling of fish and seafood products (e.g. DeSalle and Birstein 1996; Marko et al. 2004; Kyle and Wilson 2007; Jerome et al. 2008; Rasmussen and Morrissey 2008; Wong and Hanner 2008; Yancy et al. 2008; Barbuto et al. 2010; Filonzi et al. 2010; Lowenstein et al. 2010), identifying tuna sushi samples analyzed for mercury contamination

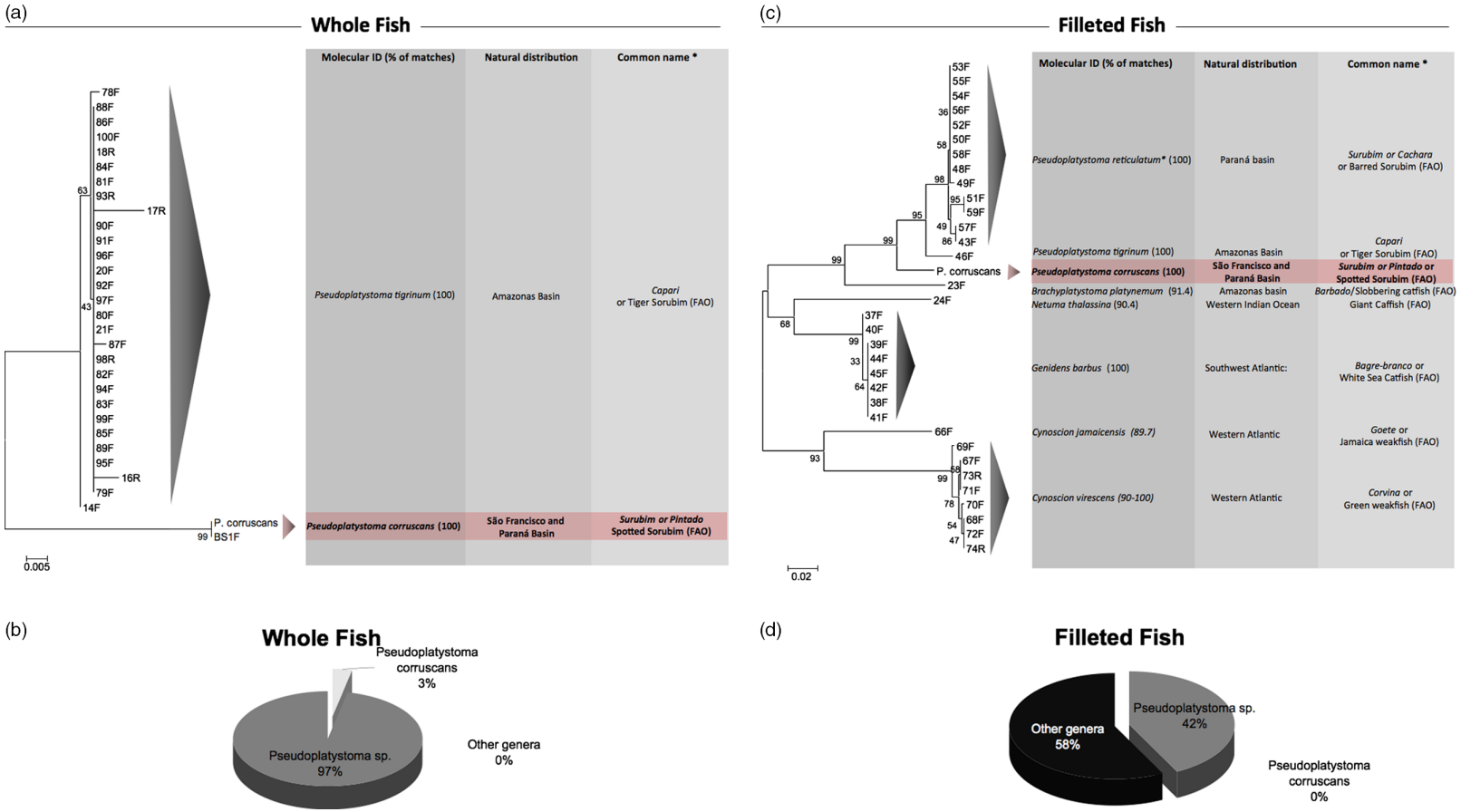

Figure 1. Distance analysis of whole and filleted fishes commercialized as *surubim*. Neighbor-joining trees of K2P distances were generated among *surubim* samples commercialized as (a) whole and (c) filleted fishes. Common names in Portuguese and English are shown according to FishBase. Graphical representation of the percentage of *surubim* samples commercialized as (b) whole or (d) filleted that belong to *P. corruscans* species, to the genus *Pseudoplatystoma* (except for *P. corruscans*), or to other genera.

Table II. Common names and price per kilogram of commercialized fish mislabeled as *surubim*.

Common name	Type	Price/kg (US$)
Surubim or *Pintado*	Whole	11.56
Surubim or *Pintado*	Fillets	14.59
Bagre	Fillets	8.16
Corvina	Fillets	5.63

(Lowenstein et al. 2010) plus the identification of smoked fish products (Smith et al. 2008). In Brazil, Ardura et al. (2010) utilizing two mtDNA genes showed that at least seven distinct species have been sold under the common name "Acará", making the real estimation of exploitation rates impossible.

In this study, we identified commercial samples labeled as *surubim* through the comparisons of COI mtDNA sequences using the BLAST engine to search GenBank. In addition, the BOLD identification engine was employed to search barcode records within BOLD, as well as using standard *P. corruscans* barcode sequences (Carvalho et al. 2011), Strikingly, analysis of *surubim* fillets revealed a 58% rate of substitution by fishes from the species that do not belong to the *Pseudoplatystoma* genus. These include marine species (*G. barbus* and *C. virescens*) that were identified with a great overall match (99–100%) as being the preferred substitutive species (Table I). Other recovered haplotypes showed poorer matches and were only tentatively assigned to the species level (*N. thalassina* and *Brachyplatystoma platynemum*; Table I). Within whole fish, only *P. tigrinum* was detected. Nonetheless, if we consider the identification via FishBase, which lists only *P. reticulatum* and *P. corruscans* as valid names for *surubim*, selling *P. tigrinum* as *surubim* would also qualify as mislabeling.

The high rate of substitution of this freshwater species could be due to the fact that the vernacular name *surubim* is well known within the Brazilian market. Therefore, by using this label traders might be able to sell their product for a better price. This becomes clear as we compare the market prices of the fishes sold as *surubim* identified in this study (Table II). For instance, species labeled *bagres*, a Brazilian vernacular name for a less known catfish group, are sold at a 70% lower price than fishes under the *surubim* label. Therefore, we have strong evidence that intentional mislabeling of cheaper fish products is a more frequent phenomenon mainly within processed fish (Figure 1).

One limitation of DNA barcode analysis is the fact that, by using a mitochondrial gene, only the matrilineal lineage is examined. This limits the interpretation of results when hybridization within species is common, as is the case for the genus *Pseudoplatystoma*. Hybrids between *P. corruscans* males and *P. reticulatum* females have been reported (Bignotto et al. 2009), figuring as the most common hybrid catfish produced on Brazilian aquaculture farms in Brazil (Carvalho et al. 2008). Therefore, we were expecting a higher representation of *P. reticulatum* haplotypes among the commercial samples analyzed in this study. Surprisingly, most haplotypes recovered belong to Amazonian species. Whether these species represent a new type of hybrids is a subject of future research. Further analysis using nuclear markers is recommended for the identification of different types of commercial hybrids. That said, recovered haplotypes from distinct genera (even from marine species, e.g. *Genidens* and *Cynoscion*) are unlikely to be due to hybridization, reinforcing our hypothesis of intentional mislabeling of lower value species as *surubim*.

Conclusions

In this study, we have clearly shown the occurrence of substitutions of the freshwater catfish *surubim* by other species of lower commercial value, including marine species. If we consider FishBase, only the species *P. corruscans* and *P. reticulatum* have the common name *surubim* valid. Therefore, close to 80% of the fish sold in the surveyed markets, Brazilian markets of Belo Horizonte city are mislabeled. This figure is higher than those reported for North American seafood (25%; Wong and Hanner 2008) and Italian fish products (32%; Filonzi et al. 2010). The high rates of substitution of *P. corruscans* by other species could also be an indication that its wild stocks have not been coupled with market growth. In fact, overexploitation of *P. corruscans* might explain, in part, why it has been substituted by morphologically similar species when commercialized as whole fish, and in some cases (e.g. fillets) substituted by quite dissimilar species (Figure 1). The establishment of conservation strategies and the normalization of vernacular names for native commercially important Brazilian fishes, together with the molecular inspection of fish products, have the potential to form an important tool for the preservation of the Brazilian fish fauna and protect consumers from mislabeled products.

We strongly recommend the establishment of a valid list of commercial and Latin names for the fishes commercialized in Brazil. Such a reference list would make possible for State Fish and Game Departments to be able to regulate and detect fraud, substitution, and the commercialization of threatened species. In addition, customs services will have the ability to regulate and inspect imported/exported items, for the purpose of taxation and to protect the consumer from misguidance. Such a list is currently in use together with Barcoding analysis to detect market substitution in North American seafood (Wong and Hanner 2008).

Acknowledgments

The authors are grateful to José Vanderval Melo Junior and Arno Soares Seerig for helping with the

collection of fish to all personnel of the Laboratory of Animal Genetics at UFMG Veterinary College for their assistance and to Shannon Loughnan and Sven Becker for their comments on the manuscript. This study was supported by CNPq/FAPEMIG (INCT 573899/2008-8) and Instituto Estadual de Florestas. D.C.C. is also grateful to CNPq for the PDJ fellowship (150420/2009-9) and CAPES postdoctoral fellowship (#4095-09-0).

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

Ardura A, Linde AR, Moreira JC, Garcia-Vazquez E. 2010. DNA barcoding for conservation and management of amazonian commercial fish. Biol Conserv 143:1438–1443.

Baker CS, Lento GM, Cipriano F, Palumbi SR. 2000. Predicted decline of protected whales based on molecular genetic monitoring of Japanese and korean markets. Proc R Soc Lond B Biol Sci 267:1191–1199.

Barbuto M, Galimberti A, Ferri E, Labra M, Malandra R, Galli P, Casiraghi M. 2010. DNA barcoding reveals fraudulent substitutions in shark seafood products: The italian case of "Palombo" (*Mustelus* spp.). Food Res Int 43:376–381.

Bignotto TS, Prioli AJ, Prioli SMAP, Maniglia TC, Boni TA, Lucio LC, Gomes RA, Oliveira VN, Prioli AV, Júlio HF, Jr, Prioli LM. 2009. Genetic divergence between *Pseudoplatystoma corruscans* and *Pseudoplatystoma reticulatum* (Siluriformes: Pimelodidae) in the parana river basin. Braz J Biol 69:681–689.

Buitrago-Suarez UA, Burr BM. 2007. Taxonomy of the catfish genus *Pseudoplatystoma* Bleeker (Siluriformes: Pimelodidae) with recognition of eight species. Zootaxa 1512:1–38.

Carvalho DC, Seerig AS, Melo DC, Sousa AB, Pimenta D, Oliveira DAA. 2008. Identificação molecular de peixes: O caso do surubim (*Pseudoplatystoma* spp.). Rev Bras Reprod Anim 32: 215–219.

Carvalho DC, Oliveira DAA, Pompeu PS, Leal CG, Oliveira C, Hanner R. 2011. Deep barcode divergence in brazilian freshwater fishes—the case of the São Francisco river basin. Mitochondrial DNA 22(Suppl 1). Epub.

Civera T. 2003. Species identification and safety of fish products. Vet Res Commun 27:481–489.

Cohen NJ, Deeds JR, Wong ES, Hanner RH, Yancy HF, White KD, Thompson TM, Wahl M, Pham TD, Guichard FM, Huh I, Austin C, Dizikes G, Gerber SI. 2009. Public health response to puffer fish (tetrodotoxin) poisoning from mislabeled product. J Food Prot 72:810–817.

Dawnay N, Ogden R, McEwing R, Carvalho GR, Thorpe RS. 2007. Validation of the barcoding gene COI for use in forensic genetic species identification. Forensic Sci Int 173:1–6.

DeSalle R, Birstein VJ. 1996. PCR identification of black caviar. Nature 381:197–198.

Espineira M, Gonzalez-Lavin N, Vieites JM, Santaclara FJ. 2008. Development of a method for the genetic identification of flatfish species on the basis of mitochondrial DNA sequences. J Agric Food Chem 56:8954–8961.

Felsenstein J. 1985. Confidence-limits on phylogenies—an approach using the bootstrap. Evolution 39:783–791.

Filonzi L, Chiesa S, Vaghi M, Nonnis Marzano F. 2010. Molecular barcoding reveals mislabelling of commercial fish products in Italy. Food Res Int 43:1383–1388.

Froese R, Pauly D, Editors. 2011. FishBase. World Wide Web electronic publication. www.fishbase.org, version (02/2011).

Godinho AL, Kynard B, Godinho HP. 2007. Migration and spawning of female surubim (*Pseudoplatystoma corruscans*, Pimelodidae) in the São Francisco river, Brazil. Environ Biol Fish 80:421–433.

Hebert PDN, Gregory TR. 2005. The promise of DNA barcoding for taxonomy. Syst Biol 54:852–859.

Hubert N, Hanner R, Holm E, Mandrak NE, Taylor E, Burridge M, Watkinson D, Dumont P, Curry A, Bentzen P, Zhang J, April J, Bernatchez L. 2008. Identifying canadian freshwater fishes through DNA barcodes. PLoS ONE 3:e2490.

Jerome M, Martinsohn JT, Ortega D, Carreau P, Verrez-Bagnis V, Mouchel O. 2008. Toward fish and seafood traceability: Anchovy species determination in fish products by molecular markers and support through a public domain database. J Agric Food Chem 56:3460–3469.

Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111–120.

Kumar S, Tamura K, Nei M. 2004. MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinform 5:150–163.

Kyle CJ, Wilson CC. 2007. Mitochondrial DNA identification of game and harvested freshwater fish species. Forensic Sci Int 166: 68–76.

Lowenstein JH, Burger J, Jeitner CW, Amato G, Kolokotronis SO, Gochfeld M. 2010. DNA barcodes reveal species-specific mercury levels in tuna sushi that pose a health risk to consumers. Biol Lett 6:692–695.

Manel S, Berthier P, Luikart G. 2002. Detecting wildlife poaching: Identifying the origin of individuals with bayesian assignment tests and multilocus genotypes. Conserv Biol 16:650–659.

Marko PB, Lee SC, Rice AM, Gramling JM, Fitzhenry TM, McAlister JS, Harper GR, Moran AL. 2004. Fisheries: Mislabelling of a depleted reef fish. Nature 430:309–310.

Martinez I, James D, Loréal H. 2005. Application of modern analytical techniques to ensure seafood safety and authenticity. FAO Fisheries Technical Paper. Rome: Food and Agriculture Organization of United Nations.

Moretti VM, Turchini GM, Bellagama F, Caprino F. 2003. Traceability issues in fishery and aquaculture products. Vet Res Commun 27:497–505.

Nelson LA, Wallman JF, Dowton M. 2007. Using COI barcodes to identify forensically and medically important blowflies. Med Vet Entomol 21:44–52.

Pauly D, Christensen V, Guenette S, Pitcher TJ, Sumaila UR, Walters CJ, Watson R, Zeller D. 2002. Towards sustainability in world fisheries. Nature 418:689–695.

Rasmussen RS, Morrissey MT. 2008. DNA-based methods for the identification of commercial fish and seafood species. Compr Rev Food Sci F 7:280–295.

Ratnasingham S, Hebert PDN. 2007. BOLD: The barcode of life data system (www.Barcodinglife.Org). Mol Ecol Notes 7: 355–364.

Rubinoff D. 2006. Utility of mitochondrial DNA barcodes in species conservation. Conserv Biol 20:1026–1033.

Rubinoff D, Cameron S, Will K. 2006. A genomic perspective on the shortcomings of mitochondrial DNA for "barcoding" identification. J Hered 97:581–594.

Saitou N, Nei M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol Biol Evol 4: 406–425.

Sambrook J, Fritsch EF, Maniatis T. 1989. Molecular Cloning: A Laboratory Manual. Cold Springs Harbor, NY, Cold Springs Harbor Laboratory Press. 1659p.

Sevilla RG, Diez A, Noren M, Mouchel O, Jerome M, Verrez-Bagnis V, Van Pelt H, Favre-Krey L, Krey G, Consortium TF, Bautista JM. 2007. Primers and polymerase chain reaction conditions for DNA barcoding teleost fish based on the mitochondrial

cytochrome *b* and nuclear rhodopsin genes. Mol Ecol Notes 7: 730–734.

Smith PJ, Mcveagh SM, Steinke D. 2008. DNA barcoding for the identification of smoked fish products. J Fish Biol 72:464–471.

Teletchea F, Maudet C, Hanni C. 2005. Food and forensic molecular identification: Update and challenges. Trends Biotechnol 23:359–366.

Terol J, Mascarell R, Fernandez-Pedrosa V, Perez-Alonso M. 2002. Statistical validation of the identification of tuna species: Bootstrap analysis of mitochondrial DNA sequences. J Agric Food Chem 50:963–969.

Torrico JP, Hubert N, Desmarais E, Duponchelle F, Rodriguez JN, Montoya-Burgos J, Montoya-Burgos J, Garcia Davila C, Carvajal-Vallejos FM, Grajales AA, Bonhomme F, Renno JF. 2009. Molecular phylogeny of the genus pseudoplatystoma (Bleeker, 1862): Biogeographic and evolutionary implications. Mol Phylogenet Evol 51:588–594.

Valdez-Moreno M, Ivanova NV, Elias-Gutierrez M, Contreras-Balderas S, Hebert PDN. 2009. Probing diversity in freshwater fishes from mexico and guatemala with DNA barcodes. J Fish Biol 74:377–402.

Vinas J, Tudela S. 2009. A validated methodology for genetic identification of tuna species (genus *Thunnus*). PLoS ONE 4: e7606.

Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PDN. 2005. DNA barcoding Australia's fish species. Philos Trans R Soc B 360:1847–1857.

Ward RD, Hanner R, Hebert PDN. 2009. The campaign to DNA barcode all fishes, FISH-BOL. J Fish Biol 74:329–356.

Wong EHK, Hanner RH. 2008. DNA barcoding detects market substitution in north american seafood. Food Res Int 41: 828–837.

Yancy HF, Zemlak TS, Mason JA, Washington JD, Tenge BJ, Nguyen NL, Barnett JD, Savary WE, Hill WE, Moore MM, Fry FS, Randolph SC, Rogers PL, Hebert PD. 2008. Potential use of DNA barcodes in regulatory science: Applications of the regulatory fish encyclopedia. J Food Prot 71:210–217.