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Research article

DNA barcoding reveals mislabeling and commercial fraud in the marketing of fillets of the genus *Brachyplatystoma* Bleeker, 1862, the Amazonian freshwater catfishes economically important in Brazil

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ABSTRACT

The substitution and mislabeling is facilitated by the processing of fish products. We employed a DNA barcoding to authenticate fillets labeled as "dourada" (*Brachyplatystoma rousseauxii*), and "piramutaba" (*Brachyplatystoma vaillantii*) marketed in the Brazil. A 615 bp of the Cytochrome oxidase subunit I (COI) was sequenced from 305 fillets and subsequently identified to species level by querying public databases and sequences of reference species. The results revealed a global mean substitution rate of 17%. The highest substitution rate was detected in "dourada" (26%), the most valuable species, followed by "piramutaba" (9%). The most cases of substitutions were by species of lower commercial value, suggesting fraud aimed at increased profits. Therefore, we suggest the improvement of food-labeling regulation, continued inspection, as well as the adoption of the DNA barcode for the molecular authentication of processed fish to prevent substitution of these products in Brazil.

1. Introduction

Fish is an important source of nutrients, which is widely accepted by consumers, with the record-high worldwide per capita consumption reaching 20.5 kg in 2018 (FAO, 2020). Although live, fresh and chilled fish are the most preferred for the human consumption (FAO, 2020), processed fish products have been gaining increasing space in consumer markets worldwide due to factors such as their longer durability, more practical preparation, and the reduction of the microbial load (Magnussen et al., 2008). However, processing eliminates the morphological characteristics necessary for species identification, making these products vulnerable to substitution, whether accidental or intentional (Brito et al., 2015; Helgoe et al., 2020; Xiong et al., 2018).

Accidental substitutions may occur when the species are morphologically similar, and may thus be misidentified (Ardura et al., 2010; Gordoa et al., 2017) or due to the ambiguities of their common names (Delpiani et al., 2020; Staffen et al., 2017).

The intentional substitutions typically involve the marketing of fish products derived from species of lower value, or that are poorly accepted by consumers, labeled as species of higher commercial value (Brito et al., 2015; Delpiani et al., 2020; Giovos et al., 2020; Helgoe et al., 2020). One other practice is the mislabeling of species that are under some restriction, due to overexploitation, exceeded quotas or endangered status (Christiansen et al., 2018; Delpiani et al., 2020). In both cases, the aim is to increase profitability.

Substitutions are undesirable and can cause economic and ecological impacts, and may affect food safety (Delpiani et al., 2020; Guardone et al., 2017; Kappel and Schröder, 2016; Xiong et al., 2018). Given these problems, the Brazil has initiated measures to reduce fraud in the processed fish sector, such as Normative Instruction No. 29/2015 published by the Ministry of Agriculture, Livestock, and Supply (available at: http://pesquisa.in.gov.br/imprensa/jsp/visualiza/index.jsp?jornal=1&data=24/09/2015&pagina=3), correlating the common and scientific names of the principal target species by the Brazilian fishery sector, which should be used to label the products inspected by the Ministry. However, this list contains several ambiguities, with different species being linked to same common names, as in the case of the genus *Brachyplatystoma* Bleeker, 1862, the target taxon of the present study. In addition to these questions, the decree NI 29/2015 only requires the inclusion of the common name of the species on the labels, with exception to Salmonidae and Gadidae where the scientific name is necessary. The Ministry has also published a manual identifying the main commercial fish species harvested in Brazil, including a morphological diagnosis of the entire fish and the characteristics of the species musculature, in order to prevent the substitution of whole fish and processed products (Brasil, 2016). However, the diagnosis of processed products is

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difficult for non-specialists, even with the assistance of manuals, and the effective implementation of such regulatory mechanisms is limited, especially in the case of processed fish products. This situation can only be rectified through the adoption of forensic genetics tools for the identification of traded species.

In this context, the DNA barcode is highly sensitive for the reliable identification of vertebrate species, including fish, and has been widely used to evaluate the authenticity of the labeling of processed fish products (Hebert et al., 2003a; Delpiani et al., 2020; Helgoe et al., 2020). In Brazil, several studies using DNA barcode have revealed species substitution in fish products marketed all over the country (Brito et al., 2015; Barbosa et al., 2020; Carvalho et al., 2011, 2015, 2017; Staffen et al., 2017; Veneza et al., 2018; Gomes et al., 2019).

Therefore, given the efficiency of the DNA barcode for species identification, the present study applied this molecular tool for the evaluation of the occurrence of species substitution in the trade of Amazon catfish fillets. The target species were *Brachyplatystoma vaillantii* Valenciennes, 1840 and *Brachyplatystoma rousseauxii* Castelnau, 1855, which are sold in Brazil under the common names "piramutaba" and "dourada", respectively. These species are freshwater catfish of the family Pimelodidae, endemic to the Neotropical region, where they are an important fishery resource, especially in Brazil (Nelson et al., 2016).

In Brazil, the fishery statistics are discontinuous, temporal, and spatially limited and the country has not reported official catches data by species since 2011. Therefore, while no recent data on fishery production are available, the published fishery statistics indicate that these catfish are among the principal freshwater fish species traded in Brazil, with a total catch of 191,631 tons (t) being landed between 2007 and 2011, mainly in the North region, where more than half of which (122,461 t) was *B. vaillantii*, followed by the *B. rousseauxii* (69,171 t) (IBAMA, 2007; MPA, 2012a; MPA, 2012b). In the large supermarket chains of Pará, in the Brazilian Amazon, these species are sold primarily as whole fish or fillets, with fillets being sold at a higher price. Based on *in loco* observations, the "dourada" is sold at the highest mean price, i.e., BRL 29.49 per kg, with the "piramutaba" being sold at a mean price of BRL 21.05 per kg.

The species of the genus *Brachyplatystoma* are similar in their morphology, and may often be given the same common name, as in the case of *B. rousseauxii* and *Brachyplatystoma flavicans* Castelnau, 1855 (which has recently been reclassified as *Zungaro zungaro* Humboldt, 1821), which are both known popularly as "dourada". These taxa are morphologically similar, with subtle differences in the shape of the head, which is nearly rounded in cross section in *Z. zungaro* while is somewhat flattened dorsally in *Brachyplatystoma* spp., and interorbital space, which is nearly 8–10 times larger than eyes in *Z. zungaro* and less than 8 times larger than eyes in *Brachyplatystoma* spp. (Marceniuk et al., 2017), which may hinder the correct identification of species. Phylogenetically, *Zungaro* and *Brachyplatystoma* are members of the Sorubimines clade together with *Sorubim* Cuvier, 1829, *Sorubimichthys* Bleeker, 1862, *Pseudoplatystoma* Bleeker, 1862, *Hemisorubim* Bleeker, 1862, *Platynematichthys* Bleeker, 1858, *Platysilurus* Haseman, 1911, *Platystomatichthys* Bleeker, 1862 and *Hypophthalmus* Cuvier, 1829, however the phylogenetic interrelationships within the Sorubimines are poorly resolved (Lundberg et al., 2011). Additionally, the fillets of these species are extremely difficult to differentiate, and there are considerable differences in their prices. These factors obviously facilitate the substitution of the products derived from these species, whether accidentally or intentionally. In this context, the present study aimed to evaluate the authenticity of catfish fillets marketed as "dourada" and "piramutaba" in Brazil using DNA barcode, which is an effective molecular tool for the evaluation of mislabeling.

2. Materials and methods

2.1. Sample collection

A total of 305 fillets were collected from the 81 randomly-selected batches (labeled B1–B81), between May 2016 and July 2017. Bimonthly collections were performed in five large supermarket chains in the Brazilian state of Pará, located in the towns of Belém, Ananindeua and Castanhal. The fillets were obtained whenever new batches were available, making sure not to sample repeated batches along the study period, and the packages containing between 1 and 9 fillets. The samples were collected in Pará due to its high production and marketing of the target species, and the selected cities comprise the supermarket chains that sell these species as processed products. The samples consisted of fillets packaged by the supplier companies and, some of which were repacked by the supermarkets and, in these cases, the name of the supply company was indicated on the label.

The samples were stored on ice and taken to the Laboratory of Fish Microbiology of the Institute of Coastal Studies of the Federal University of Pará in Bragança, Brazil. In the laboratory, a sample of muscle tissue was removed from the innermost region of each fillet to avoid possible cross-contamination and then stored in absolute ethanol.

To identify the fillets, we used a reference database obtained from three samples of each species, *B. vaillantii,* and *B. rousseauxii*, and also included samples of *B. filamentosum* Lichtenstein, 1819, other important commercial catfish of the genera. All the whole fish were collected by the artisanal fishery in the Pará state, being *B. rousseauxii* collected in the Amazon River (2°24′S, 54°42′W), and *B. vaillantii* collected in the Amazon estuary (0°15′N, 48°25′W). The fishes were identified based on the specialized literature (Santos et al., 1984; Barthem and Goulding, 1997). The species *B. vaillantii* is morphologically characterized by having a dense body, slightly compressed, terminal mouth with two overlapping dentigerous plates, wide and flattened maxillary barbels, adipose fin larger than anal fin, dark gray color, being lighter in the ventral region (Santos et al., 1984; Marceniuk et al., 2017). The species *B. rousseauxii* differs from the other *Brachyplatystoma* species by having small maxillary barbels, silver head, and clear body with golden highlights (Barthem and Goulding, 1997). The species *B. filamentosum* has a body round, flattened head, small eyes on the top of the head, subinferior mouth with upper jaw surpassing to lower jaw, round and long maxillary barbels, and adipose fin shorter than anal fin. The juveniles of *B. filamentosum* have light body with several dark and rounded spots, whereas adult specimens have dark gray color in the dorsal region and light belly without the spots (Santos et al., 1984; Marceniuk et al., 2017).

The tissue samples were stored in the Laboratory of Fish Microbiology at the Institute of Coastal Studies of the Federal University of Pará. The voucher specimens were deposited in the ichthyological collection of the Museu Paraense Emilio Goeldi (MPEG) (Numbers: MPEG 38938 - 38943).

2.2. DNA extraction, PCR conditions, and DNA sequencing

The total DNA was obtained using the Wizard Genomic DNA Purification kit (Promega, USA), following the manufacturer's instructions for muscle tissue. The total DNA was quantified in a Nanodrop 2000 spectrophotometer (Thermo Scientific).

The barcode fragment of the COI gene was amplified by Polymerase Chain Reaction (PCR) using the primers FishF1 and FishR1 (Ward et al., 2005). The PCR reactions were performed in a final volume of 15 μL containing 2 μL of dNTPs (1.25 mM), 1.25 μL of 10x buffer, 0.7 μL of $MgCl_2$ (50 mM), 0.2 μL of each primer (50 ng/μL), 1.0 μL of the total genomic DNA (100 ng/μL), 0.2 μL (5U/μL) of Taq DNA Polymerase, and pure water to complete the final reaction volume. The PCRs were run under the following amplification conditions: initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 20 s, hybridization at 56 °C for 20 s, extension at 72 °C for 30 s, and then a final extension at 72 °C for 7 min. The quality of the PCR reactions was evaluated in an ultraviolet light transilluminator following electrophoresis in 1% agarose gel stained with GelRed (Biotium, USA).

All positive PCRs were cycle-sequenced with the primers used in the COI amplification, using the Big Dye v. 3.1 terminator kit (Applied

Biosystems), following the manufacturer's instructions. The samples were electrophoresed in an ABI 3500XL sequencer (Applied Biosystems).

2.3. Data analysis

The DNA sequences of the fillets and samples from the reference database were edited and aligned using Bioedit 7.0.9.0 (Hall, 1999), and the haplotypes identified were used in the subsequent analyses.

The fillet sequences were compared to those of the reference database and then with the public databases of the Barcoding of Life Database (BOLD: http://www.barcodinglife.org) and GenBank (http://www.ncbi.nlm.nih.gov), using the BLASTn search tool (Altschul et al., 1990). In all cases, the criterion of identification at species level was that adopted by BOLD, which considers that individuals of the same species have a maximum genetic divergence of 2%. Each species identified was attributed the scientific and common names defined in the decree NI 29/2015, and where more than one common name is assigned to a species, we adopted only the name(s) used in the Northern region of Brazil.

The haplotypes of the fillets, reference species, BOLD and GenBank sequences were aligned in Bioedit and the Fasta file was used in MEGA 10 (Kumar et al., 2018) to calculate the within- and between-taxon genetic divergence, based on the uncorrected *p* distances, and to generate the Maximum Likelihood cladogram using GTR + I + G model of nucleotide substitution selected by jModeltest 2.1.10 (Darriba et al., 2012; Guindon and Gascuel, 2003). The cladogram was generated to represent the clusters of the species identified in the fillets, using *Chanus chanus* and *Gonorynchus abbreviatus* as outgroups, members of the Order Gonorynchiformes, which was considered the common evolutionary ancestor of the taxa identified in this study in the phylogenetic review of bony fish (Betancur-R et al., 2017). The statistical support of the branches was evaluated through 1,000 bootstrap pseudoreplicates. The tree was visualized and edited in FigTree 1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/).

Subsequently, the relative frequency of the species identified and the total substitution rates of the fillets of each of the study species were plotted in MS Excel 2013. The substitution rates were calculated for the batches packaged by both the supplier companies and the supermarkets. Then, the Chi-square test was performed in MS Excel 2013, to assess whether there were significant differences between the product's origin and the replacement rates.

3. Results

A COI fragment of 615 base pairs was sequenced from 305 fillets, of which 42% (N = 128) were packaged at source by the supplier companies and 58% (N = 177) were repacked by the supermarkets. Overall, 160 of these fillets were labeled as *B. vaillantii* "piramutaba" (38 batches), and 145 as *B. rousseauxii* "dourada" (43 batches) (Table 1). Nine samples of reference species were also sequenced, and the uncorrected *p* mean of intraspecific divergence were invariably low ranging from zero in *B. rousseauxii* to 0.4% in *B. vaillantii*. In the interspecific comparisons the divergence ranged from 2.1% between *B. rousseauxii* and *B. filamentosum* to 8.3% between *B. vaillantii* and the other two species. No insertions, deletions or stop codons were observed in any of the sequences obtained here, indicating that they correspond to functional segments of the COI gene. The haplotypes from fillets and reference species sequences generated in this study were deposited at GenBank under accession numbers MT551748 – MT551783.

A total of 27 haplotypes were identified, which were named H1 to H27, and used in all subsequent analyses. An additional seven COI sequences obtained from the reference species samples were included in the database, i.e., three specimens each of *B. vaillantii* and *B. filamentosum*, and one specimen of *B. rousseauxii* (as all three sequenced samples were 100% similar, then only one was included here). We also included 10 sequences obtained from GenBank and BOLD, which returned a similarity of at least 99.6% in comparison with the fillets sequenced in the present study. Therefore, based on the distance matrix and the Maximum Likelihood cladogram, we identified the sequences of the 305 fillets analyzed in the present study as belonging to five species: *B. vaillantii* (Laulao catfish, piramutaba), *B. rousseauxii* (Gilded catfish, dourada), *Colossoma macropomum* Cuvier, 1816 (Blackfin pacu, tambaqui), *Macrodon ancylodon* Bloch and Schneider, 1801 (King weakfish, pescada gó), and *Genyatremus luteus* Bloch, 1790 (Torroto grunt, peixe pedra) (Table 1; Figure 1).

Overall, 83% of the 305 fillets analyzed (N = 252) were labeled correctly, while 17% (N = 53) had been replaced by a distinct species from that described on the label, and all of the mislabeled fillets had been repackaged by the supermarkets. The Chi-square test revealed that there is a significant difference between the proportion of replaced samples in supermarket and in supplier companies ($\chi^2 = 46.39$; df = 1; $p < 0.001$).

The highest substitution rate was observed in the fillets labeled as "dourada" (26%, N = 38/145), the most valuable fish, followed by the "piramutaba" (9%, N = 15/160) (Figure 2 a and b). Of the 145 samples of "dourada" (*B. rousseauxii*), 74% were labeled correctly, while 26% of fillets had been substituted, primarily (25% of the samples) by *B. vaillantii*, and the other 1% by *G. luteus* (Figure 2a). Overall, 30% (N = 43) of these fillets were packaged by the supplier companies and 70% (N = 102) were repackaged by the supermarkets.

The vast majority (91%, N = 145) of the 160 fillets marketed as "piramutaba" (*B. vaillantii*) were labeled correctly, while 4% had been substituted by *M. ancylodon*, 3% by *B. rousseauxii* and 2% by *C. macropomum* (Figure 2b). Just over half (53%, N = 85) of the fillets were packaged by the supplier companies, and 47% (N = 75) were repackaged by the supermarkets.

When the data are considered by batch, 20% (N = 16) of the 81 batches were mislabeled, and in all cases the fillets identified by the DNA barcode did not correspond to the species described on the label (Table 1). The 12 batches labeled as "dourada" were mostly substituted by *B. vaillantii* (B10, B18, B19, B25, B30, B31, B58, B67, B70, B72, B75, B77) with one case of substitution by *G. luteus* (B81). The three "piramutaba" batches were substituted by *M. ancylodon* (B14), *C. macropomum* (B66) and *B. rousseauxii* (B68) (Table 1).

4. Discussion

While consumers appreciate the convenience of processed fish products, the elimination of the species morphological characteristics facilitates the mislabeling of these products (Brito et al., 2015; Christiansen et al., 2018; Giovos et al., 2020; Staffen et al., 2017). These substitutions have become a worldwide concern, given their potential impacts in the economic, ecological or public health spheres (Christiansen et al., 2018; Guardone et al., 2017; Xiong et al., 2018).

The present study confirmed the efficiency of DNA barcoding for the identification of fish species, with 100% of the fillets being identified at the species level. The general substitution rate recorded in the present study was 17%. The fillets analyzed were derived from five species representing four fish families, the Pimelodidae, Haemulidae, Sciaenidae, and Serrasalmidae. This was one of the lesser substitution rates detected for processed fish in Brazil, where previous studies have recorded rates between 16% and 80%. For example, Carvalho et al. (2011) recorded a substitution rate of 80% in "surubim" catfish (*Pseudoplatystoma* spp. Bleeker, 1862), while Brito et al. (2015) found that more than 70% of croakers (*Cynoscion leiarchus* Cuvier, 1830 and *Plagioscion squamosissimus* Heckel, 1840) fillets had been substituted, Leonardo et al. (2016) recorded a 40% rate in sardine (*Sardinella aurita* Valenciennes, 1847, *Sardina pilchardus* Walbaum, 1792, *Sardinops sagax* Jenyns, 1842, *Sardinops caeruleus* Jenyns, 1842), Carvalho et al. (2017) recorded 41% in cod (*Gadus macrocephalus* Tilesius, 1810, *Gadus morhua* Linnaeus, 1758, *Gadus ogac* Richardson, 1836, and *Boreogadus saida* Lepechin, 1774) fillets, and Barbosa et al. (2020) registered a rate of 45.4% in pescada amarela (*Cynoscion acoupa* Lacepède, 1802) fillets. Slightly lower rates

Table 1. Summary of the molecular identification of the fillets labeled as "piramutaba" (*B. vaillantii*) and "dourada" (*B. rousseauxii*). The identification was based on the reference species, BOLD (using the Species Level database) and GenBank BLAST search engines. N indicates the sample size per batch, H refers to haplotypes identified in the batches, * the absence of reference species in reference database, and in bold cases of substitution. The accession numbers correspond to the sequences that the query sequences were matched.

Sample information			Molecular identification of species							Common Name	Substitution
Batch	N	H	Description on the label/species	Reference species/Similarity (%)	Accession number	GenBank/Similarity (%)	Accession number	BOLD/Similarity (%)	Accession number		
B1	3	H1	Piramutaba (*B. vaillantii*)	*B. vaillantii*/99.8	MT551751	*B. vaillantii*/100	KT952409	*B. vaillantii*/100	ACZ9033	Piramutaba	NO
B2	9	H1-H4	Piramutaba (*B. vaillantii*)	*B. vaillantii*/≥99.8	MT551751	*B. vaillantii*/≥99.7	KT952409	*B. vaillantii*/≥99.6	ACZ9033	Piramutaba	NO
B3	5	H18	Dourada (*B. rousseauxii*)	*B. rousseauxii*/100	MT551748	*B. rousseauxii*/100	KT952407	*B. rousseauxii*/100	AAW7699	Dourada	NO
B4	3	H1, H4	Piramutaba (*B. vaillantii*)	*B. vaillantii*/≥99.8	MT551751	*B. vaillantii*/≥99.8	KT952409	*B. vaillantii*/≥99.8	ACZ9033	Piramutaba	NO
B5	3	H1, H15	Piramutaba (*B. vaillantii*)	*B. vaillantii*/99.8	MT551751	*B. vaillantii*/≥99.7	KT952409	*B. vaillantii*/≥99.8	ACZ9033	Piramutaba	NO
B6	2	H1, H4	Piramutaba (*B. vaillantii*)	*B. vaillantii*/≥99.8	MT551751	*B. vaillantii*/≥99.8	KT952409	*B. vaillantii*/≥99.8	ACZ9033	Piramutaba	NO
B7	6	H1, H4, H5	Piramutaba (*B. vaillantii*)	*B. vaillantii*/≥99.7	MT551751	*B. vaillantii*/≥99.8	KT952409	*B. vaillantii*/≥99.8	ACZ9033	Piramutaba	NO
B8	6	H1, H3, H4, H6	Piramutaba (*B. vaillantii*)	*B. vaillantii*/≥99.7	MT551751	*B. vaillantii*/≥99.8	KT952409	*B. vaillantii*/≥99.6	ACZ9033	Piramutaba	NO
B9	3	H3, H4	Piramutaba (*B. vaillantii*)	*B. vaillantii*/≥99.8	MT551751	*B. vaillantii*/≥99.8	KT952409	*B. vaillantii*/≥99.6	ACZ9033	Piramutaba	NO
B10	**2**	**H1, H14**	**Dourada (*B. rousseauxii*)**	***B. vaillantii*/99.8**	**MT551751**	***B. vaillantii*/≥99.7**	**KT952409**	***B. vaillantii*/≥99.8**	**ACZ9033**	**Piramutaba**	**YES**
B11	7	H18, H23	Dourada (*B. rousseauxii*)	*B. rousseauxii*/≥99.8	MT551748	*B. rousseauxii*/≥99.8	KT952407	*B. rousseauxii*/≥99.8	AAW7699	Dourada	NO
B12	4	H1, H3, H4, H7	Piramutaba (*B. vaillantii*)	*B. vaillantii*/≥99.7	MT551751	*B. vaillantii*/≥99.8	KT952409	*B. vaillantii*/≥99.6	ACZ9033	Piramutaba	NO
B13	6	H1, H3, H8, H9	Piramutaba (*B. vaillantii*)	*B. vaillantii*/≥99.7	MT551751	*B. vaillantii*/≥99.8	KT952409	*B. vaillantii*/≥99.6	ACZ9033	Piramutaba	NO
B14	**7**	**H24**	**Piramutaba (*B. vaillantii*)**	*	*	***M. ancylodon*/100**	**KP331678**	***M. ancylodon*/100**	**AAE8381**	**Pescada gó**	**YES**
B15	2	H18	Dourada (*B. rousseauxii*)	*B. rousseauxii*/100	MT551748	*B. rousseauxii*/100	KT952407	*B. rousseauxii*/100	AAW7699	Dourada	NO
B16	2	H1, H3	Piramutaba (*B. vaillantii*)	*B. vaillantii*/99.8	MT551751	*B. vaillantii*/100	KT952409	*B. vaillantii*/≥99.6	ACZ9033	Piramutaba	NO
B17	2	H1	Piramutaba (*B. vaillantii*)	*B. vaillantii*/99.8	MT551751	*B. vaillantii*/100	KT952409	*B. vaillantii*/100	ACZ9033	Piramutaba	NO
B18	**3**	**H1**	**Dourada (*B. rousseauxii*)**	***B. vaillantii*/99.8**	**MT551751**	***B. vaillantii*/100**	**KT952409**	***B. vaillantii*/100**	**ACZ9033**	**Piramutaba**	**YES**
B19	**5**	**H1, H3, H4**	**Dourada (*B. rousseauxii*)**	***B. vaillantii*/≥99.8**	**MT551751**	***B. vaillantii*/≥99.8**	**KT952409**	***B. vaillantii*/≥99.6**	ACZ9033	**Piramutaba**	**YES**
B20	3	H1, H4	Piramutaba (*B. vaillantii*)	*B. vaillantii*/≥99.8	MT551751	*B. vaillantii*/≥99.8	KT952409	*B. vaillantii*/≥99.8	ACZ9033	Piramutaba	NO
B21	2	H1, H3	Piramutaba (*B. vaillantii*)	*B. vaillantii*/99.8	MT551751	*B. vaillantii*/100	KT952409	*B. vaillantii*/≥99.6	ACZ9033	Piramutaba	NO
B22	2	H1, H4	Piramutaba (*B. vaillantii*)	*B. vaillantii*/≥99.8	MT551751	*B. vaillantii*/≥99.8	KT952409	*B. vaillantii*/≥99.8	ACZ9033	Piramutaba	NO
B23	6	H1, H3	Piramutaba (*B. vaillantii*)	*B. vaillantii*/≥99.8	MT551751	*B. vaillantii*/≥99.8	KT952409	*B. vaillantii*/≥99.6	ACZ9033	Piramutaba	NO
B24	6	H18	Dourada (*B. rousseauxii*)	*B. rousseauxii*/100	MT551748	*B. rousseauxii*/100	KT952407	*B. rousseauxii*/100	AAW7699	Dourada	NO
B25	**1**	**H1**	**Dourada (*B. rousseauxii*)**	***B. vaillantii*/99.8**	**MT551751**	***B. vaillantii*/100**	**KT952409**	***B. vaillantii*/100**	**ACZ9033**	**Piramutaba**	**YES**
B26	2	H18	Dourada (*B. rousseauxii*)	*B. rousseauxii*/100	MT551748	*B. rousseauxii*/100	KT952407	*B. rousseauxii*/100	AAW7699	Dourada	NO
B27	5	H1, H3	Piramutaba (*B. vaillantii*)	*B. vaillantii*/≥99.8	MT551751	*B. vaillantii*/≥99.8	KT952409	*B. vaillantii*/≥99.6	ACZ9033	Piramutaba	NO
B28	2	H1	Piramutaba (*B. vaillantii*)	*B. vaillantii*/99.8	MT551751	*B. vaillantii*/100	KT952409	*B. vaillantii*/100	ACZ9033	Piramutaba	NO
B29	6	H1, H4	Piramutaba (*B. vaillantii*)	*B. vaillantii*/≥99.8	MT551751	*B. vaillantii*/≥99.8	KT952409	*B. vaillantii*/≥99.8	ACZ9033	Piramutaba	NO
B30	**6**	**H1, H4**	**Dourada (*B. rousseauxii*)**	***B. vaillantii*/≥99.8**	**MT551751**	***B. vaillantii*/≥99.8**	**KT952409**	***B. vaillantii*/≥99.8**	**ACZ9033**	**Piramutaba**	**YES**
B31	**2**	**H1, H12**	**Dourada (*B. rousseauxii*)**	***B. vaillantii*/≥99.7**	**MT551751**	***B. vaillantii*/≥99.8**	**KT952409**	***B. vaillantii*/≥99.8**	**ACZ9033**	**Piramutaba**	**YES**
B32	2	H1	Piramutaba (*B. vaillantii*)	*B. vaillantii*/99.8	MT551751	*B. vaillantii*/100	KT952409	*B. vaillantii*/100	ACZ9033	Piramutaba	NO
B33	1	H18	Dourada (*B. rousseauxii*)	*B. rousseauxii*/100	MT551748	*B. rousseauxii*/100	KT952407	*B. rousseauxii*/100	AAW7699	Dourada	NO
B34	8	H1, H4	Piramutaba (*B. vaillantii*)	*B. vaillantii*/≥99.8	MT551751	*B. vaillantii*/≥99.8	KT952409	*B. vaillantii*/≥99.8	ACZ9033	Piramutaba	NO
B35	5	H1, H4, H10, H16	Piramutaba (*B. vaillantii*)	*B. vaillantii*/≥99.7	MT551751	*B. vaillantii*/≥99.8	KT952409	*B. vaillantii*/≥99.8	ACZ9033	Piramutaba	NO
B36	4	H1, H3, H11	Piramutaba (*B. vaillantii*)	*B. vaillantii*/≥99.8	MT551751	*B. vaillantii*/≥99.7	KT952409	*B. vaillantii*/≥99.7	ACZ9033	Piramutaba	NO
B37	5	H18, H19	Dourada (*B. rousseauxii*)	*B. rousseauxii*/≥99.8	MT551748	*B. rousseauxii*/≥99.8	KT952407	*B. rousseauxii*/≥99.8	AAW7699	Dourada	NO
B38	6	H1-H4	Piramutaba (*B. vaillantii*)	*B. vaillantii*/≥99.8	MT551751	*B. vaillantii*/≥99.7	KT952409	*B. vaillantii*/≥99.6	ACZ9033	Piramutaba	NO
B39	7	H1, H4, H10	Piramutaba (*B. vaillantii*)	*B. vaillantii*/≥99.7	MT551751	*B. vaillantii*/≥99.8	KT952409	*B. vaillantii*/≥99.8	ACZ9033	Piramutaba	NO
B40	2	H18	Dourada (*B. rousseauxii*)	*B. rousseauxii*/100	MT551748	*B. rousseauxii*/100	KT952407	*B. rousseauxii*/100	AAW7699	Dourada	NO

(*continued on next page*)

Table 1 (*continued*)

Sample information			Molecular identification of species							Common Name	Substitution
Batch	N	H	Description on the label/species	Reference species/Similarity (%)	Accession number	GenBank/Similarity (%)	Accession number	BOLD/Similarity (%)	Accession number		
B41	6	H1, H3	Piramutaba (*B. vaillantii*)	*B. vaillantii*/≥99.8	MT551751	*B. vaillantii*/≥99.8	KT952409	*B. vaillantii*/≥99.6	ACZ9033	Piramutaba	NO
B42	4	H18	Dourada (*B. rousseauxii*)	*B. rousseauxii*/100	MT551748	*B. rousseauxii*/100	KT952407	*B. rousseauxii*/100	AAW7699	Dourada	NO
B43	6	H18	Dourada (*B. rousseauxii*)	*B. rousseauxii*/100	MT551748	*B. rousseauxii*/100	KT952407	*B. rousseauxii*/100	AAW7699	Dourada	NO
B44	2	H18	Dourada (*B. rousseauxii*)	*B. rousseauxii*/100	MT551748	*B. rousseauxii*/100	KT952407	*B. rousseauxii*/100	AAW7699	Dourada	NO
B45	4	H1-H4	Piramutaba (*B. vaillantii*)	*B. vaillantii*/≥99.8	MT551751	*B. vaillantii*/≥99.7	KT952409	*B. vaillantii*/≥99.6	ACZ9033	Piramutaba	NO
B46	2	H18	Dourada (*B. rousseauxii*)	*B. rousseauxii*/100	MT551748	*B. rousseauxii*/100	KT952407	*B. rousseauxii*/100	AAW7699	Dourada	NO
B47	5	H18, H23	Dourada (*B. rousseauxii*)	*B. rousseauxii*//≥99.8	MT551748	*B. rousseauxii*//≥99.8	KT952407	*B. rousseauxii*//≥99.8	AAW7699	Dourada	NO
B48	4	H18	Dourada (*B. rousseauxii*)	*B. rousseauxii*/100	MT551748	*B. rousseauxii*/100	KT952407	*B. rousseauxii*/100	AAW7699	Dourada	NO
B49	4	H18	Dourada (*B. rousseauxii*)	*B. rousseauxii*/100	MT551748	*B. rousseauxii*/100	KT952407	*B. rousseauxii*/100	AAW7699	Dourada	NO
B50	4	H18, H19	Dourada (*B. rousseauxii*)	*B. rousseauxii*//≥99.8	MT551748	*B. rousseauxii*//≥99.8	KT952407	*B. rousseauxii*//≥99.8	AAW7699	Dourada	NO
B51	3	H1, H3	Piramutaba (*B. vaillantii*)	*B. vaillantii*/≥99.8	MT551751	*B. vaillantii*/≥99.8	KT952409	*B. vaillantii*/≥99.6	ACZ9033	Piramutaba	NO
B52	3	H18	Dourada (*B. rousseauxii*)	*B. rousseauxii*/100	MT551748	*B. rousseauxii*/100	KT952407	*B. rousseauxii*/100	AAW7699	Dourada	NO
B53	2	H1	Piramutaba (*B. vaillantii*)	*B. vaillantii*/99.8	MT551751	*B. vaillantii*/100	KT952409	*B. vaillantii*/100	ACZ9033	Piramutaba	NO
B54	2	H18	Dourada (*B. rousseauxii*)	*B. rousseauxii*/100	MT551748	*B. rousseauxii*/100	KT952407	*B. rousseauxii*/100	AAW7699	Dourada	NO
B55	7	H18, H23	Dourada (*B. rousseauxii*)	*B. rousseauxii*//≥99.8	MT551748	*B. rousseauxii*//≥99.8	KT952407	*B. rousseauxii*//≥99.8	AAW7699	Dourada	NO
B56	2	H18	Dourada (*B. rousseauxii*)	*B. rousseauxii*/100	MT551748	*B. rousseauxii*/100	KT952407	*B. rousseauxii*/100	AAW7699	Dourada	NO
B57	4	H18	Dourada (*B. rousseauxii*)	*B. rousseauxii*/100	MT551748	*B. rousseauxii*/100	KT952407	*B. rousseauxii*/100	AAW7699	Dourada	NO
B58	**1**	**H1**	**Dourada (*B. rousseauxii*)**	***B. vaillantii*/99.8**	**MT551751**	***B. vaillantii*/100**	**KT952409**	***B. vaillantii*/100**	**ACZ9033**	**Piramutaba**	**YES**
B59	4	H18	Dourada (*B. rousseauxii*)	*B. rousseauxii*/100	MT551748	*B. rousseauxii*/100	KT952407	*B. rousseauxii*/100	AAW7699	Dourada	NO
B60	2	H18	Dourada (*B. rousseauxii*)	*B. rousseauxii*/100	MT551748	*B. rousseauxii*/100	KT952407	*B. rousseauxii*/100	AAW7699	Dourada	NO
B61	4	H18, H20	Dourada (*B. rousseauxii*)	*B. rousseauxii*//≥99.8	MT551748	*B. rousseauxii*//≥99.8	KT952407	*B. rousseauxii*//≥99.8	AAW7699	Dourada	NO
B62	9	H1, H3, H4	Piramutaba (*B. vaillantii*)	*B. vaillantii*/≥99.8	MT551751	*B. vaillantii*/≥99.8	KT952409	*B. vaillantii*/≥99.6	ACZ9033	Piramutaba	NO
B63	2	H4, H10	Piramutaba (*B. vaillantii*)	*B. vaillantii*/≥99.7	MT551751	*B. vaillantii*/≥99.8	KT952409	*B. vaillantii*/≥99.8	ACZ9033	Piramutaba	NO
B64	2	H18	Dourada (*B. rousseauxii*)	*B. rousseauxii*/100	MT551748	*B. rousseauxii*/100	KT952407	*B. rousseauxii*/100	AAW7699	Dourada	NO
B65	7	H18, H21, H22	Dourada (*B. rousseauxii*)	*B. rousseauxii*//≥99.8	MT551748	*B. rousseauxii*//≥99.8	KT952407	*B. rousseauxii*//≥99.8	AAW7699	Dourada	NO
B66	**3**	**H27**	**Piramutaba (*B. vaillantii*)**	*	*	***C. macropomum*/100**	**JN032695**	***C. macropomum*/100**	**AAR9990**	**Tambaqui**	**YES**
B67	**4**	**H1**	**Dourada (*B. rousseauxii*)**	***B. vaillantii*/99.8**	**MT551751**	***B. vaillantii*/100**	**KT952409**	***B. vaillantii*/100**	**ACZ9033**	**Piramutaba**	**YES**
B68	**5**	**H18, H23**	**Piramutaba (*B. vaillantii*)**	***B. rousseauxii*/≥99.8**	**MT551748**	***B. rousseauxii*/≥99.8**	**KT952407**	***B. rousseauxii*/≥99.8**	**AAW7699**	**Dourada**	**YES**
B69	2	H18	Dourada (*B. rousseauxii*)	*B. rousseauxii*/100	MT551748	*B. rousseauxii*/100	KT952407	*B. rousseauxii*/100	AAW7699	Dourada	NO
B70	**3**	**H1, H4**	**Dourada (*B. rousseauxii*)**	***B. vaillantii*/≥99.8**	**MT551751**	***B. vaillantii*/≥99.8**	**KT952409**	***B. vaillantii*/≥99.8**	**ACZ9033**	**Piramutaba**	**YES**
B71	3	H1, H4, H10	Piramutaba (*B. vaillantii*)	*B. vaillantii*/≥99.7	MT551751	*B. vaillantii*/≥99.8	KT952409	*B. vaillantii*/≥99.8	ACZ9033	Piramutaba	NO
B72	**2**	**H1, H17**	**Dourada (*B. rousseauxii*)**	***B. vaillantii*/≥99.7**	**MT551751**	***B. vaillantii*/≥99.8**	**KT952409**	***B. vaillantii*/≥99.7**	**ACZ9033**	**Piramutaba**	**YES**
B73	2	H18, H23	Dourada (*B. rousseauxii*)	*B. rousseauxii*/≥99.8	MT551748	*B. rousseauxii*/≥99.8	KT952407	*B. rousseauxii*/≥99.8	AAW7699	Dourada	NO
B74	3	H1, H3	Piramutaba (*B. vaillantii*)	*B. vaillantii*/≥99.8	MT551751	*B. vaillantii*/≥99.8	KT952409	*B. vaillantii*/≥99.6	ACZ9033	Piramutaba	NO
B75	**3**	**H1**	**Dourada (*B. rousseauxii*)**	***B. vaillantii*/99.8**	**MT551751**	***B. vaillantii*/100**	**KT952409**	***B. vaillantii*/100**	**ACZ9033**	**Piramutaba**	**YES**
B76	2	H18	Dourada (*B. rousseauxii*)	*B. rousseauxii*/100	MT551748	*B. rousseauxii*/100	KT952407	*B. rousseauxii*/100	AAW7699	Dourada	NO
B77	**4**	**H1, H4**	**Dourada (*B. rousseauxii*)**	***B. vaillantii*/≥99.8**	**MT551751**	***B. vaillantii*/≥99.8**	**KT952409**	***B. vaillantii*/≥99.8**	**ACZ9033**	**Piramutaba**	**YES**
B78	4	H1	Piramutaba (*B. vaillantii*)	*B. vaillantii*/99.8	MT551751	*B. vaillantii*/100	KT952409	*B. vaillantii*/100	ACZ9033	Piramutaba	NO
B79	3	H18, H19	Dourada (*B. rousseauxii*)	*B. rousseauxii*/≥99.8	MT551748	*B. rousseauxii*/≥99.8	KT952407	*B. rousseauxii*/≥99.8	AAW7699	Dourada	NO
B80	2	H1	Piramutaba (*B. vaillantii*)	*B. vaillantii*/99.8	MT551751	*B. vaillantii*/100	KT952409	*B. vaillantii*/100	ACZ9033	Piramutaba	NO
B81	**2**	**H25, H26**	**Dourada (*B. rousseauxii*)**	*	*	***G. luteus*/≥99.8**	**HQ676760**	***G. luteus*/≥99.8**	**ACC0598**	**Peixe Pedra**	**YES**
Total	**305**										

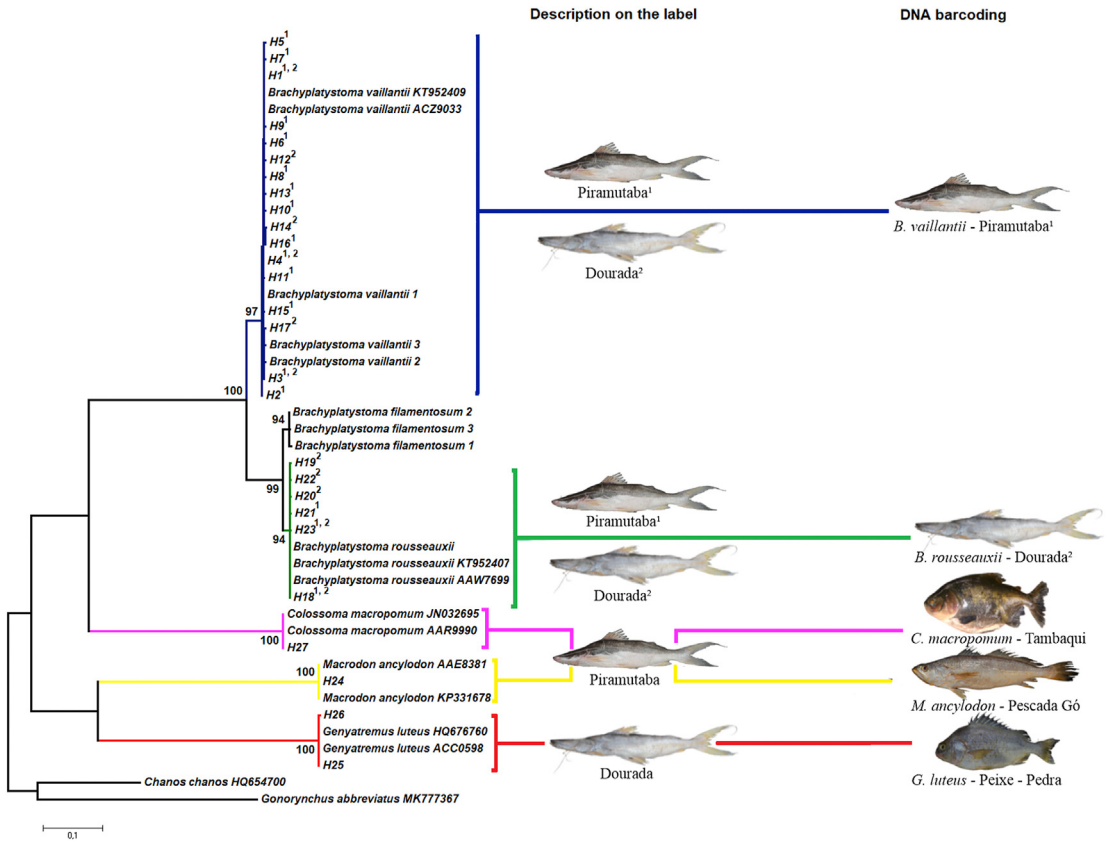

Figure 1. Cladogram of Maximum Likelihood, based on the GTR + I + G model, showing the grouping of the sequences of fillets marketed as "piramutaba" (*Brachyplatystoma vaillantii*), and "dourada" (*Brachyplatystoma rousseauxii*) and sequences from the reference species database, GenBank and BOLD. The numbers "1" and "2" superscript in the haplotypes correspond to the species labeled as *B. vaillantii* (piramutaba) and *B. rousseauxii* (dourada), respectively. The superscript "1,2" indicates that this haplotype was present in the batches labeled as "piramutaba" and "dourada". The species *Chanus chanus* and *Gonorynchus abbreviatus* compose the outgroup. The values shown in each node are the bootstrap probabilities, based on 1,000 pseudoreplicates. The scale in the bottom refers to the scale bar distance.

were recorded in other studies, such as 26% for a range of species sold in fishmongers and 30% for those sold in restaurants (Staffen et al., 2017), while Carvalho et al. (2015) registered a rate of 24% for pink cusk-eel (*Genypterus blacodes* Forster, 1801), flounder (*Atheresthes stomias* Jordan and Gilbert, 1880) and cod (*G. morhua*), Veneza et al. (2018) found 22% in red snapper (*Lutjanus purpureus* Poey, 1866), and Gomes et al. (2019) registered 16% in "gurijuba" (*Sciades parkeri* Traill, 1832). The substitution rate recorded in the present study was nevertheless similar to that documented in an analysis of teleost fish (18%) marketed in South Africa (Cawthorn et al., 2015), and much higher than those recorded in the United Kingdom (5.66%) by Helyar et al. (2014) and Australia (0%) by Lamendin et al. (2015). The lowest substitution rates registered in Europe and Australia were due to an efficient and rigorous legislation of food-labeling, especially with emphasis on the standardization and traceability of fish products, as well as effective inspection (Helyar et al., 2014; Lamendin et al., 2015). Therefore, the Brazilian government agencies could adopt similar measures, improving the NI 29/2015 to avoid ambiguities in commercial nomenclature, and creating stricter law that allow the regulation of labeling, adopting effective inspection

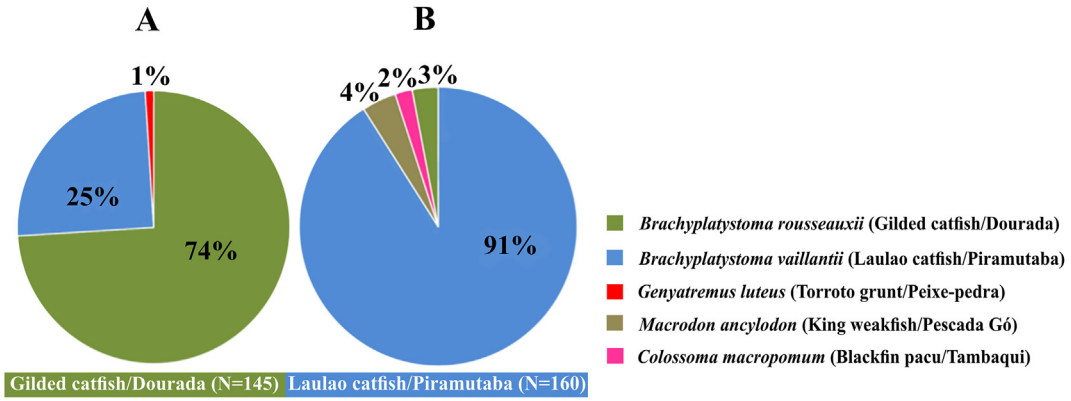

Figure 2. The relative frequency of occurrence of the species identified in fillets marketed in the large supermarket chains of Pará, in the Brazilian Amazon. (A) Fillets labeled as "dourada" (*Brachyplatystoma rousseauxii*) and (B) fillets labeled as "piramutaba" (*Brachyplatystoma vaillantii*). The colors are standardized for each species and correspond to the taxa described in the legend.

mechanisms, as well as, applying sanctions to prevent substitution in fishery market.

In the present study, the highest substitution rate (26%) was recorded in the case of the fillets labeled as "dourada" (*B. rousseauxii*), followed by the "piramutaba" (*B. vaillantii*) (9%). In these products, substitutions were only found in the batches repacked by the supermarkets, being evidenced a significant difference between the substitution rate and the product's origin. Therefore, it could be indicating that the processing companies are more cautious about labeling their fish products, presumably because they are more subject to inspection by government agencies. In fact, in Brazil only supplier companies are target of inspection by the Ministry of Agriculture, Livestock, and Supply, which may corroborate our hypothesis.

The relatively higher substitution rate recorded in the fillets labeled as "dourada" (26%) may be associated with the commercial value and popularity of the fish, which allows traders to sell the products of this species for higher prices. The samples were mainly replaced by *B. vaillantii,* while only two samples were *G. luteus*. Substitutions by *B. vaillantii* may have been accidental, given that the two species are members of the same genus, and their fillets are difficult to distinguish, although *B. vaillantii* fillets have a mean price of only BRL 21.05/kg, in comparison with BRL 29.49/kg for the "dourada". By contrast, *G. luteus* is a scaly fish, with a considerably different morphology from "dourada". Furthermore, *G. luteus* is found in marine environments, while the "dourada" is a freshwater fish, which means that *G. luteus* could not be part of the bycatch of the "dourada" fishery. Therefore, while it is almost impossible to identify a species from the examination of the fillet, which would facilitate accidental substitutions, it is evident that all the recorded substitutions of "dourada" involved species of either a lower commercial value or the product of different fisheries, which indicates that these substitutions were intentional, rather than accidental, with the aim of increasing profits, to the detriment of the consumer.

The "piramutaba" showed a lower substitution rate, with 9% of its fillets being replaced by the *M. ancylodon*, *B. rousseauxii*, and *C. macropomum*. The *B. rousseauxii* and *C. macropomum* are freshwater fish, so they may be captured with the "piramutaba", but the *M. ancylodon* is a marine fish, being not part of the bycatch of "piramutaba" fishery. In addition, *M. ancylodon* and *C. macropomum* are scaly fish morphologically distinct from the "piramutaba", which is a scaleless catfish, and, while *B. rousseauxii* is a congener of the *B. vaillantii*, the external morphology of the adults is easily distinguishable. Therefore, it would seem reasonable to conclude that the substitution of "piramutaba" by *M. ancylodon* was intentional, given not only their morphological and habitat differences, but also the fact that the mean price of weakfish fillets is only BRL 14.00/kg, in contrast with BRL 21.05/kg for *B. vaillantii*. This would constitute commercial fraud aimed at increasing profits. On the other hand, as *B. rousseauxii* and the *C. macropomum* are more expensive fish (BRL 29.49/kg and BRL 33.90/kg, respectively) than the "piramutaba" (BRL 21.05/kg), it would seem unlikely that these substitutions would have been intentional, given that they represent a loss for the supermarket. Even so, there is an ethical aspect, given that any such substitution would harm consumer confidence. While, in general, ethical considerations are overlooked in comparison with economic, ecological, and public health concerns, they cannot be neglected altogether.

Another important question that needs to be considered here is the nutritional value of the different species. The protein content of the "piramutaba" is higher than that of *B. rousseauxii* (Corrêa et al., 2016). The lipid content of the "piramutaba" is also lower than that of the *C. macropomum* (Corrêa et al., 2016; Petenuci et al., 2016), which means that it would be more recommended for a restricted lipid diet. In other words, while the substitution of "piramutaba" fillets by those of "dourada" and *C. macropomum* is unlikely to have been intentional, given the respective differences in prices, the contrasts in their nutritional profiles mean that the consumer is denied an adequate choice, which raises ethical concerns. Leonardo et al. (2016), also found similar results, where most of the substitutes of sardine (*Sardinella* spp.) had a lower nutritional value than the species indicated on the product label.

Beyond the economic and ethical issues, the substitutions also harm the biodiversity, since it masks the real exploitation status of either target or replaced species. Considering the findings of the present study, the exploitation rates of *B. rousseauxii* and *B. vaillantii* have been probably overestimated. On the other hand, there is an underestimation of the exploitation rates of substitute species. This scenario hinders the development of efficient management policies, to mitigate the impact of fishing on biodiversity and ensure the sustainability of fisheries resources (FAO, 2016; Pramod et al., 2014).

5. Conclusions

The DNA barcode proved to be a sensitive and reliable tool for the identification of frozen fillets of *B. vaillantii* and *B. rouseauxii*. The evidence indicates that most of the substitutions were fraud, causing economic and health losses for consumers. Ethical concerns were also identified, given that the mislabeling of the fish products had negative implications for consumer choice. Additionally, it is probable that the substitutions may cause biodiversity concerns, masking the exploitation rates of the fishery resources, preventing the development of management plans to mitigate the impact of the overexploitation of these resources. Given these findings, we would recommend the improvement of food-labeling regulation, the implementation of more effective inspection procedures, and the adoption of the DNA barcode as a molecular tool for the authentication of the processed fish, in order to prevent substitution of these products in Brazil.

Declarations

Author contribution statement

Soraia Costa de Carvalho: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Iracilda Sampaio: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Simoni Santos: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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