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Research article

DNA barcoding reveals fraud in commercial common snook (*Centropomus undecimalis*) products in Santa Marta, Colombia

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ABSTRACT

The common snook *Centropomus undecimalis* is one of the main commercial fish species in the Caribbean region, including Colombia, where its populations have drastically decreased due to overfishing and environmental degradation. Thus, there is a market imbalance between the availability of snook products and their demand by consumers, which creates an opening for fraudulent actions such as species substitutions. Legislation in Colombia (and most Caribbean countries) lacks effective tools for the easy and rapid detection of frauds. Furthermore, there are very few studies published in scientific journals addressing this issue, of which none include *C. undecimalis* as the target species. Therefore, in order to investigate the existence of mislabeling in common snook products in Santa Marta, the present study analysed 44 frozen snook fillets from the five commercial brands available in the city. Moreover, 15 fresh snook fillets from six of the main fish markets were also analysed. To discover the frequency of possible frauds in labeling, samplings were carried out in July, September and November of 2019. Sample analyses involved the identification of each fillet at species level through molecular barcodes (16S-rRNA and COI), whose sequences were verified using BLAST and BOLD, and corroborated by a phylogenetic analysis. As a result, an astonishing 98% of the supermarkets fillets were found to be fraudulent, contrasting with a single case registered in the fish shop samples. The species used to substitute snook include the Pacific bearded brotula *Brotula clarkae* (38 samples), the Nile perch *Lates niloticus* (4 samples) and the acoupa weakfish *Cynoscion acoupa* (1 sample). Based on these results, there is a high rate of fraudulent labeling in the marketing of common snook in the city of Santa Marta, which calls for urgent actions to be taken by the corresponding authorities.

1. Introduction

The high rates of exploitation of most commercial marine fish species have led not only to a decrease in their stocks, but also to an imbalance between the demand and the availability of these products in the market (FAO, 2016). This disparity, among other causes, has led to a pronounced incidence of fraud in the labeling of marine fish products, as has been reported in several studies (Cunha et al., 2015; Xiong et al., 2016; Pardo et al., 2018; Horreo et al., 2019; de Carvalho et al., 2020; Peterson et al., 2021). In Colombia, fish commercialization is controlled by the National Institute for the Vigilance of Drugs and Food (INVIMA), which establishes the use of taxonomic keys (macroscopic characteristics of the whole fish) as the identification protocol for commercial species (Salinas et al., 2014). This regulation presents two problems: the first being related to the incidence of fish identification errors due to physical similarities among some species and the second, to the transformation of the fish into different products (frozen fillets, breaded, precooked, etc.), which do not allow the use of the established identification protocol. In addition to the economic impact, mislabeling of fish products is an illegal practice that affects the conservation and sustainable exploitation of marine species as it alters the accuracy of catch monitoring and market activity; furthermore, it creates a scenario of distrust in which consumers are left wondering about the authenticity and safety of fish products (Rasmussen and Morrissey, 2011).

In some countries such as the USA, fish product authenticity is verified through simple, fast and highly sensitive methods such as DNA

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barcodes, which are short fragments of genes that allow the reliable identification of species (Teletchea, 2009). In general, DNA barcodes are based on the amplification of DNA fragments belonging to the mitochondrial genome (i.e. such as mitochondrial cytochrome oxidase c subunit I or COI, and 16S ribosomal RNA or 16S rRNA), that are conserved at the species level and can be amplified in most food products. Importantly, this includes processed foods, even though DNA extraction might be problematic due to degradation (Hebert et al., 2003; Ogden, 2008; Ferrito et al., 2016). In this context, numerous studies have detected frauds in the commercialization of fish products in almost all countries where research has been carried out using the barcode technique. For example, in two studies in the USA and Canada, 91% and 77% of the studied snapper samples were found to be mislabeled (Khaksar et al., 2015; Hu et al., 2018), in China 85.7% of species substitution was found when analysing the sablefish *Anoplopoma fimbria* (Xiong et al., 2016). Moreover, in Brazil a rate of 77% fraud was reported in the commercialization of croaker fish (Sciaenidae) (de Brito et al., 2015), and in Spain, Finland, Germany and Iceland, 50% of the analysed seafood samples were found to be sold under the wrong name (Pardo et al., 2018). In Colombia, only two studies have been published on the subject: firstly, Salinas et al. (2014) reported a drastic change in the catfish species sold as "capaz" (Pimelodidae family), as overfishing has led to the introduction of smaller, lesser known species, whose capture involves the killing of river dolphins and caimans to be used as bait. Secondly, Castaño (2015) reported 40% of fish mislabeling in the capital city Bogotá, finding that one out of three fillets sold as common snook belonged to the basa catfish (*Pangasius* sp). Despite the importance of the results obtained in the latter study, it was published as a newspaper article and has not had the expected impact on the authorities, national policies, nor an increase in research along this line. Therefore, formal investigation is needed to explore the possibility of fraudulent fish substitutions throughout Colombia.

The common snook *Centropomus undecimalis* has a wide distribution along the coastline of the western Atlantic Ocean (Riede, 2004). It belongs to the Centropomidae family, being the largest species within its genus ($TL_{máx}$: 140 cm, $TW_{máx}$: 24.3 kg) (Chávez, 1963). In Colombia, it is actively captured by artisanal fisheries due to its commercial importance and high market prices, which range between 5 and 12 USD kg^{-1} (Álvarez-Lajonchère, 2003). Historical data from common snook landings in Santa Marta show a drastic decrease in populations, ranging from 640 t reported in the 1970s to 28 t in 2014 (Morales, 1975; Grijalba--Bendeck et al., 2017). At present, only 10.9% of common snook catches belong to fish whose total length is greater than the length at first maturity (46.2 cm) (Duarte et al., 2019). Furthermore, in 2019 national commercialization of common snook reached 107 t, however, for the same year, the reported landings were only 62.89 t, with no records of this species being imported from overseas. Taking into account all the above, the aim of this study was to investigate, for the first time in the city of Santa Marta, the possible existence of mislabeling in common snook products using DNA sequencing for species identification.

2. Materials and methods

2.1. Sampling

A total of 59 snook fillet samples were collected from large supermarket chains and fish markets in Santa Marta, Colombia. In the case of supermarkets, all commercial brands available in the city were sampled, which constitutes samples coming from five different seafood suppliers. On the other hand, six fish markets were selected due to their size and importance as suppliers for smaller shops. Samples were randomly collected in July, September, and November 2019 to avoid repeated batches during the study. Each month, three fillets of each commercial brand and one fillet per fish shop were purchased. However, due to product unavailability in November, one branded sample was missing from the supermarkets, and three fish markets did not have snook fillets to sell. Once acquired, the fillets were taken to the Molecular Biology Laboratory of the Universidad del Magdalena (Santa Marta), where a 1 g sample of tissue was taken from each fillet, labeled, placed in 1.5 mL eppendorf tubes with 90% ethanol, and stored at -20 °C until processing.

2.2. DNA extraction

This procedure was carried out using the Isolate II kit (Genomic DNA, Bioline®, UK), following the manufacturer's protocol. Verification of DNA extraction and its quality was checked using a 1% agarose gel stained with a GelRed (Biotium, USA).

2.3. Amplification and sequencing

The fragments of the mitochondrial genes, 16S-rRNA (600bp) and COI (650bp), were amplified in a thermocycler (Eppendorf Mastercycler® Pro, USA) by conventional PCR and using the primers 16Sar (5′ CGCCTGTTTATCAAAAACAT 3′) and 16Sbr (5′ CCGGTCTGAACTCA GATCACGT3′) from Palumbi (1996) for the 16S gene; and the primers FishF2 (5′ TCGACTAATCATAAAGATATCGGCAC 3′) and FishR2 (5′ACT TCAGGGTGACCGAAGAATCAGAA 3′) from Ward et al. (2005) for the COI gene.

For the amplification of both fragments, each PCR reaction was conducted in 25 μL volumes containing: 0.1 U of Taq Polymerase (0.5 μL of Taq Polymerase 5 U; abm, USA), 2.0 mM $MgCl_2$ (1.0 μL of $MgCl_2$ at 50 mM; abm, USA), 1X of Buffer PCR (2.5 μL of Buffer PCR at 10X; Bioline, USA), 0.4 mM of DNTP's (1.0 μL of dNTP's to 10 mM; Bioline, USA), 0.4 mM of each primer (1 μL of each primer at 10 mM) and 2 μL of DNA. The reaction conditions for PCR included an initial denaturation temperature of 94 °C for two minutes, followed by 35 cycles with the following parameters: 30 s at 94 °C, 45 s at 56 °C and one minute at 72 °C, followed by a final extension step at 72 °C for seven minutes. PCR products were verified by electrophoresis on 2% agarose gels stained with GelRed (Biotium, USA). Following the amplification, the samples were purified using the Isolate II kit (PCR and Gel Kit, Bioline, USA), following the manufacturer's protocol. The purified products were sequenced in both directions at SSiGMol (Universidad Nacional de Colombia, Bogotá).

2.4. Data analysis

Sequences were manually edited using BioEdit (v. 7.0.9.0) (Hall, 1999) and MEGA-X (Kumar et al., 2018). COI sequences were subsequently verified using the nBLAST tool in NCBI GenBank (https://blast.ncbi.nlm.nih.gov/) and the search engine in the Barcode of Life Database (BOLD, https://www.boldsystems.org/) using the option "Species Level Barcode Record"; whereas 16S rRNA sequences were queried only in nBLAST. Following Hebert et al. (2003), a similarity cutoff of ≥97% was used for species level identification for sequences submitted to both GenBank and BOLD databases. Subsequently, an alignment of all the sequences obtained was carried out to establish possible relationships and similarity between them and reference sequences downloaded from GenBank. The validation was performed for both genes (16S-rRNA and COI) using distance analyses, such as Neighbor Joining (NJ) using the K2P model (Mega-X), as well as a Bayesian inference (IB) and Maximum Likelihood (ML) analyses using MrBayes v.3.2.2 and RAxML v.8.0.24 programs, respectively. For the NJ and ML analysis, the Bootstrap (BP) algorithm was implemented with 1000 repetitions. As for the Bayesian analysis, 10^7 generations were carried out, sampling the trees every 100 generations and making an initial 25% exclusion of the trees built. Then the total substitution rates and the relative frequency of frauds were calculated for each source of fish products (supermarkets and fish markets) and month; subsequently, the Chi-square test was performed in order to assess the existence of significant differences or temporal trends (MS Excel, 2013).

3. Results and discussion

3.1. Amplification and sequencing of 16S rRNA and COI genes

All samples were successfully amplified and sequenced with both COI and 16S genes. Sequences were submitted to GenBank under accession numbers MT787423-MT787462. Only one sample did not sequence properly with the 16S gene (sample no. 3, Table 2), however, the sample information was accurately identified by COI. Thus, our results indicate, as suggested by previous studies, and further discussed below, that the inclusion of more than one marker is beneficial as it ensures the correct identification of species, and that both COI and 16S genes are reliable markers to be used separately or in combination for barcoding and traceability of fish studies (Cawthorn et al., 2012a, 2012b; Teletchea, 2009; Kochzius et al., 2010; Almerón-Souza et al., 2018; Hossain et al., 2019).

3.2. Species identification of supermarket samples

All samples were able to be compared with reference sequences available at genus and species level in GenBank (COI and 16S) and BOLD (COI). This process allowed us to establish that from all samples acquired in the supermarkets, only one was authentic, as it genetically belonged to *C. undecimalis* (sample No. 18, Table 1). In turn, it was evident that the practice of substitution was widely present in the observed commercial

Table 1. Molecular sequence comparison (COI and 16S-rRNA) of 44 samples of common snook fillets purchased from supermarkets in the city of Santa Marta, Colombia.

Sample	Species labeled	Accession number COI	COI BLAST identity %	BOLD identity %	COI Species identified	Accession number 16S	16 BLAST identity %	16S Species identified	Fraud
1	Common snook	MT787429	99%	100%	*Brotula clarkae*	MT584428	92%	*Ammodytes americanus*	YES
2	Common snook	MT787430	99%	99%	*B. clarkae*	MT584429	93%	*A. americanus*	YES
3	Common snook	MT787431	99%	100%	*B. clarkae*	MT584430	93%	*A. americanus*	YES
4	Common snook	MT787432	99%	99%	*B. clarkae*	MT584431	91%	*A. americanus*	YES
5	Common snook	MT787433	99%	99%	*B. clarkae*	MT584432	91%	*A. americanus*	YES
6	Common snook	MT787423	99%	99%	*B. clarkae*	MT584433	91%	*A. americanus*	YES
7	Common snook	MT787434	99%	92%	*B. clarkae*	MT584434	92%	*A. americanus*	YES
8	Common snook	MT787435	99%	97%	*B. clarkae*	MT584435	90%	*A. americanus*	YES
9	Common snook	MT787428	99%	99%	*B. clarkae*	MT584436	91%	*A. americanus*	YES
10	Common snook	MT787436	99%	99%	*B. clarkae*	MT584437	91%	*A. americanus*	YES
11	Common snook	MT787438	99%	100%	*B. clarkae*	MT584440	91%	*A. americanus*	YES
12	Common snook	MT787439	99%	100%	*B. clarkae*	MT584441	91%	*A. americanus*	YES
13	Common snook	MT787426	99%	99%	*B. clarkae*	MT584442	91%	*A. americanus*	YES
14	Common snook	MT787427	99%	98%	*B. clarkae*	MT584438	91%	*A. americanus*	YES
15	Common snook	MT787437	99%	99%	*B. clarkae*	MT584439	91%	*A. americanus*	YES
16	Common snook	MT787448	99%	99%	*B. clarkae*	MT584453	91%	*A. americanus*	YES
17	Common snook	MT787449	99%	99%	*B. clarkae*	MT584454	91%	*A. americanus*	YES
18	Common snook	MT775814	99%	99%	*Centropomus undecimalis*	MT584475	91%	*Centropomus undecimalis*	NO
19	Common snook	MT787450	99%	100%	*B. clarkae*	MT584455	91%	*A. americanus*	YES
20	Common snook	MT787451	99%	99%	*B. clarkae*	MT584456	91%	*A. americanus*	YES
21	Common snook	MT787452	99%	100%	*B. clarkae*	MT584457	91%	*A. americanus*	YES
22	Common snook	MT787453	99%	100%	*B. clarkae*	MT584458	91%	*A. americanus*	YES
23	Common snook	MT787454	99%	99%	*B. clarkae*	MT584459	91%	*A. americanus*	YES
24	Common snook	MT787455	99%	100%	*B. clarkae*	MT584460	91%	*A. americanus*	YES
25	Common snook	MT787424	99%	100%	*B. clarkae*	MT584461	91%	*A. americanus*	YES
26	Common snook	MT787457	99%	99%	*B. clarkae*	MT584463	91%	*A. americanus*	YES
27	Common snook	MT787462	99%	97%	*B. clarkae*	MT584464	91%	*A. americanus*	YES
28	Common snook	MT787458	99%	99%	*B. clarkae*	MT584465	91%	*A. americanus*	YES
29	Common snook	MT787456	99%	100%	*B. clarkae*	MT584462	91%	*A. americanus*	YES
30	Common snook	MT773642	100%	100%	*Cynoscion acoupa*	MT584481	91%	*Cysnocion acoupa*	YES
31	Common snook	MT787459	99%	99%	*B. clarkae*	MT584443	91%	*A. americanus*	YES
32	Common snook	MT787440	99%	99%	*B. clarkae*	MT584444	91%	*A. americanus*	YES
33	Common snook	MT787425	99%	99%	*B. clarkae*	MT584445	91%	*A. americanus*	YES
34	Common snook	MT787460	99%	99%	*B. clarkae*	MT584446	91%	*A. americanus*	YES
35	Common snook	MT772007	98%	98%	*Lates niloticus*	MT584482	91%	*Lates niloticus*	YES
36	Common snook	MT787441	99%	100%	*B. clarkae*	MT584447	91%	*A. americanus*	YES
37	Common snook	MT772008	99%	99%	*L. niloticus*	MT584483	99%	*L. niloticus*	YES
38	Common snook	MT787442	99%	99%	*B. clarkae*	MT584448	91%	*A. americanus*	YES
39	Common snook	MT787443	99%	100%	*B. clarkae*	MT584449	91%	*A. americanus*	YES
40	Common snook	MT787445	99%	96%	*B. clarkae*	MT584451	91%	*A. americanus*	YES
41	Common snook	MT772009	93%	99%	*L. niloticus*	MT584485	99%	*L. niloticus*	YES
42	Common snook	MT787446	99%	100%	*B. clarkae*	MT584452	91%	*A. americanus*	YES
43	Common snook	MT772006	99%	99%	*L. niloticus*	MT584484	99%	*L. niloticus*	YES
44	Common snook	MT787444	99%	100%	*B. clarkae*	MT584450	91%	*A. americanus*	YES

brands that offered snook fillets in the city and that are distributed by all the supermarkets included in the study.

When analysing the COI gene, in 90% of the cases of fraud found in supermarkets, the databases reported the Pacific bearded brotula *Brotula clarkae* (nBLAST and BOLD) as the substitute species with a similarity percentage of over 97% (Figure 1A). Results from the 16S gene confirmed that the samples do not correspond to *C. undecimalis*, not even to the genus *Centropomus* and the closest similarity reported by the genetic databases for the 16S was with the American sand spearfish *Ammodytes americanus*. However, this candidate is unlikely to be a substitute in this case, firstly due to the low percentage of similarity obtained (max. 93.2%), secondly because of the species' distribution (northwestern coast of North America) and thirdly due to its maximum length (23.5 cm), which would make it difficult to fulfill the size of common snook fillets (Nizinski et al., 1990). Thus, the incongruence in the results between the COI gene and the 16S gene might be due to the lack of 16S reference sequences for the *Brotula* genus in GenBank at the time when the present study performed the BLAST search (09/2020).

Taking the above into consideration, we obtained samples from *B. clarkae*, as it is one of the most commonly caught fish on the Colombian Pacific coast (Duarte et al., 2019), and proceeded to compare the former sequences obtained in the supermarkets samples with the newly generated sequences for both regions (COI and 16S-rRNA). The new comparisons showed a perfect alignment with our supermarket samples and the *B. clarkae* sequences generated by this study (MT787461 and MT787447T787423 – COI/MT584426 and MT584427 – 16S). It is possible that the imbalance between the high availability of *B. clarkae* in the landings and its low demand in the market has generated the opportunity for its use as a *C. undecimalis* substitute. As mentioned above, the Pacific bearded brotula is caught by artisanal fisheries on the Pacific coast where part of the landings is consumed locally, while the rest appears to be taken to major cities where it might be relabeled as common snook and sent to the main supermarkets around the country. Indeed, between July and December of 2019, the Colombian Fisheries Statistics Service (SEPEC, 2019) reported 531.2 t of *B. clarkae* captured on the Pacific coast; however, only 65.66 t were registered as commercialized in that same period. Even taking into the account the impossibility of recording data from all the markets in the country, this number represents only 12.36% of the landings of *B. clarkae*. In contrast, for the same year SEPEC reported landings of 62.89 t of common snook and interestingly, 107 t of this species were registered as being commercialized in that period. Although it is clear that a more detailed study must be performed in order to further quantify the imbalance between captures and commercialized products of these or any other species, the reported numbers further indicate the use of *B. clarkae* as the substitute of common snook and, perhaps, other marine fish species.

Other substitutions found in supermarket samples when analysing both molecular barcodes were those related to the acoupa weakfish *Cynoscion acoupa* (sample N° 30) and the Nile perch *Lates niloticus* (samples N° 39 and 36), for which the similarity percentages were equal to or greater than 99% (Figure 2). In the case of the acoupa weakfish, this species is commonly fished on the Colombian Caribbean coast (Duarte et al., 2019) and its commercial importance is not as relevant as the common snook; therefore, it may be being used as the replacement in the markets. Regarding the Nile perch, this case is of special interest since it is an African freshwater species, and the Colombian National Authority for Aquaculture and Fisheries (AUNAP) does not have any record of it as being introduced in Colombia for aquaculture practices. Therefore, it is probable that its arrival in the country and subsequent use as a substitute species is occurring through the importation of perch fillets (TradeAtlas, 2020), taking advantage of trade agreements between countries that are, for example, part of the Pacific Alliance (SICE, 2019). The presence of this species in the country is of concern as it might also be used in the fraudulent substitution of other marine fish. Moreover, if perch products originated from local fish farms, the risk of the species' introduction into natural environments is high as it is one of the most aggressive fish in terms of colonizing new niches, thus becoming a threat to local species (Goudswaard et al., 2002).

The high incidence of fraud found in supermarkets in the present study shows the severity of this practice in the city of Santa Marta. In order to meet the demand for common snook, the use of species that are not commonly commercialized in the country, or at least not in the larger cities, is more acute than expected. In Colombia, fish consumption has doubled in the last 20 years and it is thought that this number will double again in the next four years (AUNAP, 2019). However, contrary to what was reported by Castaño (2015), and the initial approach of the present study, the substitutions found in the city of Santa Marta were not made with the basa *Pangasius* sp. Importantly, the substitute species found by the present study (Pacific bearded brotula and acoupa weakfish) do not necessarily imply a fraud with fillets of a lower nutritional quality; however, there are economic and organoleptic differences, which are often masked due to the freezing and thawing of products (Bland et al., 2018). In the case for the Nile perch, there are many studies that suggest nutritional differences not only between marine and freshwater fish, but also between wild and farmed fish (Alasalvar et al., 2002; Erdem et al., 2009; Bhouri et al., 2010; Ravichandran et al., 2012). Furthermore, in this case, there is a lack of information on the origin of the product and if

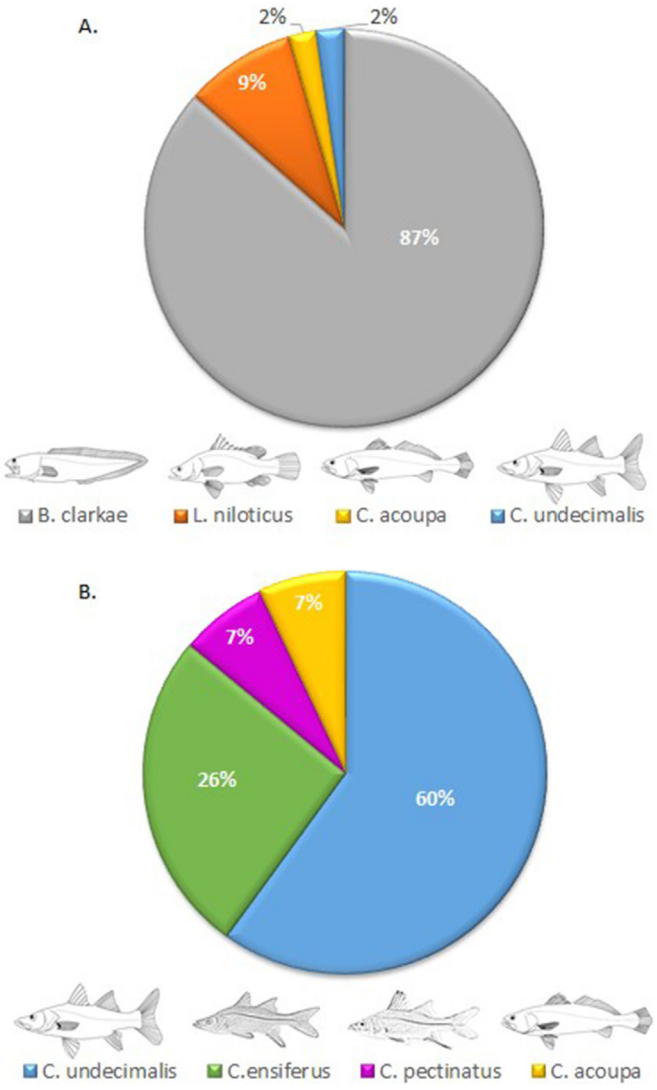

Figure 1. The relative frequency of occurrence of the species identified in fillets marketed as "common snook" sampled in supermarkets (A) and fish markets (B) in Santa Marta (Colombia).

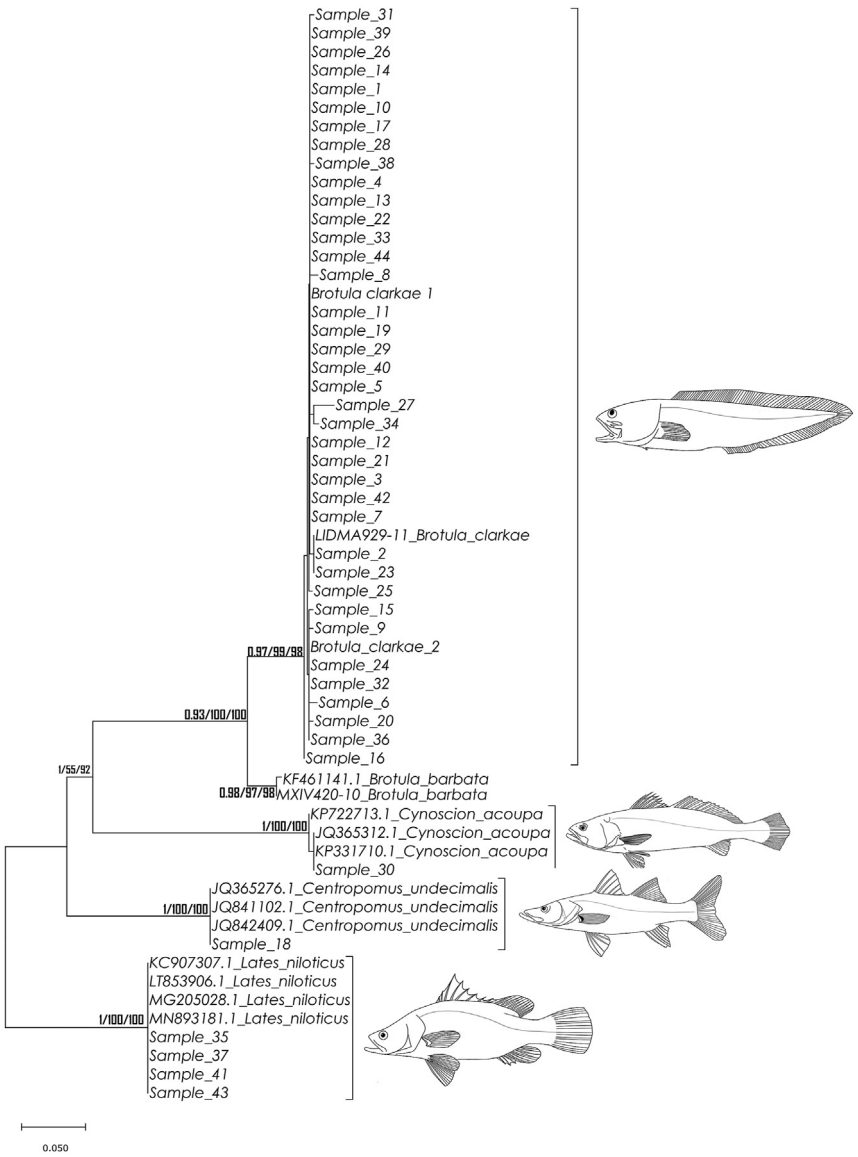

Figure 2. Tree topology determined using IB/NJ/ML from COI sequences of common snook samples obtained from the different local supermarkets in Santa Marta, Colombia, including reference GenBank sequences. The 16S-rRNA analyses recovered the same topology.

Table 2. Molecular sequence comparison study (COI and 16S-rRNA) of 15 samples of common snook fillets obtained in six fish markets in the city of Santa Marta, Colombia.

Sample	Species labeled	Accession number COI	COI BLAST identity %	COI Species identified	Accession number 16S	16 BLAST identity %	16S Species identified	Fraud
1	Common snook	MT775599	85%	*Centropomus* sp	MT584466	99%	*Centropomus ensiferus*	NO
2	Common snook	MT775817	99%	*C. undecimalis*	MT584471	100%	*C. undecimalis*	NO
3	Common snook	MT775600	81%	*Centropomus* sp	-	-	-	NO
4	Common snook	MT773641	97%	*C. acoupa*	MT584480	100%	*C. acoupa*	YES
5	Common snook	MT775816	99%	*C. undecimalis*	MT584472	99%	*C. undecimalis*	NO
6	Common snook	MT775815	98%	*C. undecimalis*	MT584473	99%	*C. undecimalis*	NO
7	Common snook	MT775598	87%	*Centropomus* sp	MT584468	100%	*C. ensiferus*	NO
8	Common snook	MT775819	100%	*C. undecimalis*	MT584476	99%	*C. undecimalis*	NO
9	Common snook	MT775821	98%	*C. undecimalis*	MT584477	100%	*C. undecimalis*	NO
10	Common snook	MT775822	99%	*C. undecimalis*	MT584478	100%	*C. undecimalis*	NO
11	Common snook	-	86%	*C. ensiferus*	MT584469	100%	*C. ensiferus*	NO
12	Common snook	MT775820	99%	*C. undecimalis*	MT584479	100%	*C. undecimalis*	NO
13	Common snook	MT775597	86%	*Centropomus armatus*	MT584467	100%	*C. ensiferus*	NO
14	Common snook	MT775596	88%	*Centropomus* sp	MT584470	100%	*Centropomus pectinatus*	NO
15	Common snook	MT775818	99%	*C. undecimalis*	MT584474	100%	*C.undecimalis*	NO

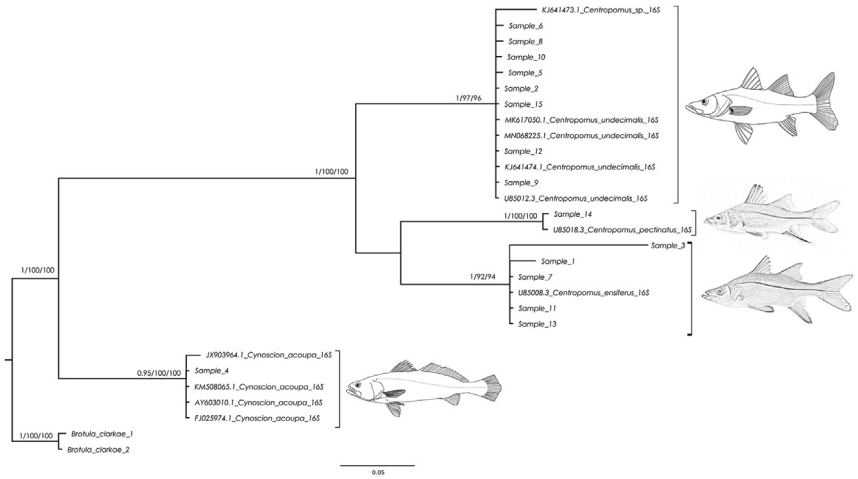

Figure 3. Tree topology determined using IB/NJ/ML from 16S-rRNA gene sequences of common snook samples obtained from the different local fish markets in Santa Marta, Colombia, including reference GenBank sequences. The COI analyses recovered the same topology.

the health and safety controls for imported frozen products are being fulfilled.

3.3. Species identification of fish market samples

In contrast to the results obtained in the supermarkets, the examined fish markets samples indicated a very low occurrence of fraud by substitution, as there was only one case in 15 analysed samples, with the acoupa weakfish being the species used to substitute the common snook (Figure 1B). As shown in Table 2 and Figure 3, the 16S gene was more accurate than the COI to identify species within the *Centropomus* genus due to the lack of *Centropomus* sequences for the COI in the databases. These results evidence the commercialization of other species of the genus *Centropomus*, such as *C. ensiferus* and *C. pectinatus*, which are common throughout Colombia's coasts (Chávez, 1963) and exploited by artisanal fishing (Duarte et al., 2019). These cases do not represent fraud as such, since they are all marketed under the name of common snook, which includes these species. Moreover, due to their small size (common TL: 25 cm and 40 cm for *C. ensiferus* and *C. pectinatus* respectively) (Cervigón et al., 1992), they are not used for filleting and thus are not sold by commercial brands in supermarkets. They are, nevertheless, sold in fish markets, where customers can choose the size or weight of the product.

When using both COI and 16S genes as barcodes for fish identification, we found that the former performed better as 16S similarity values were generally well below the set identification threshold of ≥97% and did not provide any additional information or identifications that were not achieved through COI sequencing. This is probably due to an underrepresentation of reference 16S rRNA sequences from local/regional fish species in GenBank, in comparison to those available for COI. On the other hand, the 16S gene was informative when analysing the fish shops samples, as it was more accurate for identifying species within the *Centropomus* genus. This highlights the benefit of including more than one marker in this type of studies.

When comparing the substitution rates found in the branded fillets obtained from supermarkets (98%) with those found in the fish markets (7%), it is evident that the mislabeling of common snook is a practice widely performed in the former as all commercial brands were found to be selling other species under this name. This contrasts with the findings registered in the latter, as substitution with an unrelated species (*C. acoupa*) was only found on a single occasion. Indeed, the Chi-square test shows that there is a significant difference between the incidence of frauds observed in these businesses ($X2 = 48.92$; $df = 1$; $p < 0.001$). On the other hand, the number of mislabeling cases observed throughout the sampling months did not show a particular trend as no significant differences were found in supermarkets ($X2 = 1.98$; $df = 2$; $p < 0.001$) or fish markets ($X2 = 1.98$; $df = 2$; $p < 0.001$). Thus, in branded products, the substitution of common snook might not only be a widely performed activity but also one which follows a frequent and constant pattern of incidence.

4. Conclusions

The results obtained in the present study indicate a high rate of common snook substitution found in frozen fillets sold in supermarkets in Santa Marta which calls for urgent measures to be taken. Moreover, the high incidence of frauds found in the three observed months indicates that this is not an isolated situation but rather a repetitive procedure performed by the commercial fish industry with the common snook. The corresponding authority (National Institute for Food and Drug Surveillance - INVIMA) must urgently consider the use of molecular techniques as a quick and practical tool in the authentication of fish, thus ensuring compliance with the health and safety standards of the products and, of course, compliance with consumers' rights. To this end, more studies referring to the substitution or mislabeling of fishery products, including genetic validation tools, should be performed. For the time being, fish markets offer a more reliable source of fish for consumers, at least regarding the common snook.

Declarations

Author contribution statement

Edison Lea-Charris: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Lyda R. Castro: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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References

Alasalvar, C., Taylor, K.D.A., Zubcov, E., Shahidi, F., Alexis, M., 2002. Differentiation of cultured and wild sea bass (*Dicentrarchus labrax*): total lipid content, fatty acid and trace mineral composition. Food Chem. 79 (2), 145–150.

Almerón-Souza, F., Sperb, C., Castilho, C.L., Figueiredo, P.I.C.C., Gonçalves, L.T., Machado, R., Oliveira, L.R., Valiati, V.H., Fagundes, N.J.R., 2018. Molecular identification of shark meat from local markets in Southern Brazil based on DNA barcoding: evidence for mislabeling and trade of endangered species. Front. Genet. 9 (APR).

Álvarez-Lajonchère, L., 2003. El cultivo de róbalos. In: Universidad Nacional de Colombia (Ed.), Memorias del IV Seminario Internacional de Acuicultura, first ed. Universidad Nacional de Colombia, p. 25.

AUNAP - National Authority for Aquaculture and Fisheries, 2019. El mercado nacional de pescado es de 350.000 toneladas. AUNAP Press Bulletin, 11th April, 2019. https://www.portafolio.co/economia/pescado-en-colombia-panorama-del-sector-pesquero-en-el-pais-528367.

Bhouri, M., Bouhlel, I., Chouba, L., Hammami, M., Cafsi, M. El, Chaouch, A., 2010. Total lipid content , fatty acid and mineral compositions of muscles and liver in wild and farmed sea bass (Dicentrarchus labrax). Afr. J. Food Sci. 4 (8), 522–530 (August).

Bland, J.M., Bett-Garber, K.L., Li, C.H., Brashear, S.S., Lea, J.M., Bechtel, P.J., 2018. Comparison of sensory and instrumental methods for the analysis of texture of cooked individually quick frozen and fresh-frozen catfish fillets. Food Sci. Nutr.

Castaño, A., 2015. El fraude en la comercialización de los pescados, ¿gato por liebre o basa por róbalo? Semana Sostenible, 24th May, 2015. https://www.semana.com/fraude-comercializacion-pescado-gato-liebre-basa-robalo/33081/.

Cawthorn, D.M., Steinman, H.A., Witthuhn, R.C., 2012a. Evaluation of the 16S and 12S rRNA genes as universal markers for the identification of commercial fish species in South Africa. Gene 491 (1), 40–48.

Cawthorn, D.M., Steinman, H.A., Witthuhn, R.C., 2012b. DNA barcoding reveals a high incidence of fish species misrepresentation and substitution on the South African market. Food Res. Int. 46 (1), 30–40.

Cervigón, F., Cipriani, R., Fischer, W., Garibaldi, L., Hendrickx, M., Lemus, A.J., Márquez, R., Poutiers, J.M., Robaina, G., Rodriguez, B., 1992. FAO Identification Sheets for Fisheries Species. Field Guide for Commercial marine and Estuarine Species of the Septentrional Coast of South America. FAO, Rome, p. 513.

Chávez, H., 1963. Contribución al conocimiento de la biología de los robalos, Chucumite y Constantino (*Centropomus* spp.) del Estado de Veracruz. Ciencia 22 (5), 141–161.

Cunha, H.A., Da Silva, V.M.F., Santos, T.E.C., Moreira, S.M., Do Carmo, N.A.S., Solé-Cava, A.M., 2015. When you get what you haven't paid for: molecular identification of "douradinha" fish fillets can help end the illegal use of river dolphins as bait in Brazil. J. Hered.

de Brito, M.A., Schneider, H., Sampaio, I., Santos, S., 2015. DNA barcoding reveals high substitution rate and mislabeling in croaker fillets (Sciaenidae) marketed in Brazil: the case of "pescada branca" (*Cynoscion leiarchus* and *Plagioscion squamosissimus*). Food Res. Int. 70, 40–46.

de Carvalho, S.C., Sampaio, I., Santos, S., 2020. 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. Heliyon 6 (9), e04888.

Duarte, L.O., De la Hoz, -M.J., Manjarres, L.M., 2019. Análisis de los desembarcos pesqueros artesanales registrados en las cuencas y litorales de Colombia (julio-diciembre de 2018). Autoridad Nacional de Acuicultura y Pesca (AUNAP), Bogotá, p. 52.

Erdem, M.E., Baki, B., Samsun, S., 2009. Fatty acid and amino acid compositions of cultured and wild sea bass (*Dicentrarchus labrax* L., 1758) from different regions in Turkey. J. Anim. Vet. Adv. 8 (10), 1959–1963.

FAO, 2016. El estado mundial de la pesca y la acuicultura. FAO, Rome, p. 42, 2016.

Ferrito, V., Bertolino, V., Pappalardo, A.M., 2016. White fish authentication by COIBar-RFLP: toward a common strategy for the rapid identification of species in convenience seafood. Food Contr. 70, 137–170.

Goudswaard, P.C., Witte, F., Katunzi, E.F.B., 2002. The tilapiine fish stock of Lake Victoria before and after the Nile perch upsurge. J. Fish. Biol. 60 (4), 838–856.

Grijalba-Bendeck, M., Leal-Flórez, J., Bolaños-Cubillos, N., Acero, A., 2017. *Centropomus undecimalis* (Bloch, 1792) 222-225. In: Chasqui, V.L., Polanco, A., Acero, F.A., Mejía-Falla, P.P.A., Navia, A., Zapata, L.A., Caldas, J.P. (Eds.), Libro rojo de peces marinos de Colombia. Instituto de Investigaciones Marinas y Costeras José Benito Vives de Andréis - Invemar, p. 552.

Hall, T.A., 1999. BIOEDIT: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser.

Hebert, P.D.N., Cywinska, A., Ball, S.L., DeWaard, J.R., 2003. Biological identifications through DNA barcodes. Proc. Biol. Sci. 270 (1512), 313–321.

Horreo, J.L., Fitze, P.S., Jiménez-Valverde, A., Noriega, J.A., Pelaez, M.L., 2019. Amplification of 16S rDNA reveals important fish mislabeling in Madrid restaurants. Food Contr. 96, 146–150.

Hossain, M.A.M., Uddin, S.M.K., Chowdhury, Z.Z., Sultana, S., Johan, M.R., Rohman, A., Erwanto, Y., Ali, M.E., 2019. Universal mitochondrial 16s rRNA biomarker for mini-barcode to identify fish species in Malaysian fish products. Food Addit. Contam. Part A Chem. Anal. Contr. Expo. Risk Assess. 36 (4), 493–506.

Hu, Y., Huang, S.Y., Hanner, R., Levin, J., Lu, X., 2018. Study of fish products in Metro Vancouver using DNA barcoding methods reveals fraudulent labeling. Food Contr. 94, 38–47.

Khaksar, R., Carlson, T., Schaffner, D.W., Ghorashi, M., Best, D., Jandhyala, S., Traverso, J., Amini, S., 2015. Unmasking seafood mislabeling in U.S. markets: DNA barcoding as a unique technology for food authentication and quality control. Food Contr. 56, 71–76.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., et al., 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35 (6), 1547–1549.

Kochzius, M., Seidel, C., Antoniou, A., Botla, S.K., Campo, D., Cariani, A., Vazquez, E.G., Hauschild, J., Hervet, C., Hjörleifsdottir, S., Hreggvidsson, G., Kappel, K., Landi, M., Magoulas, A., Marteinsson, V., Nölte, M., Planes, S., Tinti, F., Turan, C., Blohm, D., 2010. Identifying fishes through DNA barcodes and microarrays. PloS One 5 (9), 1–15.

Morales, J., 1975. Estudio biologico pesquero del robalo (*Centropomus undecimalis* Bloch, 1792) en la Cienaga grande de Santa Marta. Universidad Jorge Tadeo Lozano, Bogotá, p. 70. BSC Thesis.

Nizinski, M., Collette, B., Washington, B., 1990. Separation of two species of sand lances, *Ammodytes americanus* and *A. dubius*, in the western North Atlantic. Fish. Bull. 88, 241–255.

Ogden, R., 2008. Fisheries forensics: the use of DNA tools for improving compliance, traceability and enforcement in the fishing industry. Fish Fish. 9 (4), 462–472.

Palumbi, S.R., 1996. Nucleic acids II: the polymerase chain reaction. In: Hillis, D.M., Moritz, C., Mable, B.K. (Eds.), Molecular Systematics, pp. 205–247. Sinauer, Sunderland.

Pardo, M.Á., Jiménez, E., Viðarsson, J.R., Ólafsson, K., Ólafsdóttir, G., Daníelsdóttir, A.K., Pérez-Villareal, B., 2018. DNA barcoding revealing mislabeling of seafood in European mass caterings. Food Contr. 92, 7–16.

Peterson, A.M., McBride, G.E., Jhita, S.K., Hellberg, R.S., 2021. An investigation into country of origin labeling, species authentication and short weighting of commercially sold frozen fish fillets. Heliyon 7 (4), e06713.

Rasmussen, Hellberg R.S., Morrissey, M.T., 2011. Advances in DNA-based techniques for the detection of seafood species substitution on the commercial market. J. Lab. Autom. 16 (4), 308–321.

Ravichandran, S., Sharmila Joseph, F.R., Kanagalakshmi, R., Ramya, M.S., 2012. Variation in nutritive composition of two commercially important marine fin fishes. Int. J. Zool. Res. 8 (1), 43–51.

Riede, K., 2004. Global register of migratory species - from global to regional scales. In: Final Report of the R&D-Projekt 808 05 081. Federal Agency for Nature Conservation, Bonn, p. 330.

Salinas, C., Cubillos, J.C., Gómez, R., Trujillo, F., Caballero, S., 2014. "Pig in a poke (gato por liebre)": the "mota" (*Calophysus macropterus*) fishery, molecular evidence of commercialization in Colombia and toxicological analyses. EcoHealth 11, 197–206.

SEPEC - Servicio Estadistico Pesquero Colombiano, 2019. December. http://sepec.aunap.gov.co/.

SICE, 2019. SICE – foreign trade information system. Trade agreements. Colombia and Panama free trade agreement. Anexo 2B.

Teletchea, F., 2009. Molecular identification methods of fish species: reassessment and possible applications. Rev. Fish Biol. Fish. 19, 265–293.

TradeAtlas, 2020. Trade Atlas. Importer Search Engine. Colombia. https://www.tradeatlas.com/en.

Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R., Hebert, P.D.N., 2005. DNA barcoding Australia's fish species. Phil. Trans. Biol. Sci. 360, 1847–1857.

Xiong, X., Guardone, L., Cornax, M.J., Tinacci, L., Guidi, A., Gianfaldoni, D., Armani, A., 2016. DNA barcoding reveals substitution of Sablefish (*Anoplopoma fimbria*) with Patagonian and Antarctic Toothfish (*Dissostichus eleginoides* and *Dissostichus mawsoni*) in online market in China: how mislabeling opens door to IUU fishing. Food Contr. 70, 380–391.