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Mislabeling, illegal capture, and commercialization of Atlantic goliath grouper (*Epinephelus itajara*) on the Brazilian coast using DNA barcoding

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The Atlantic goliath grouper *Epinephelus itajara* is the largest grouper species in the Atlantic Ocean. Despite the conservation status being Vulnerable (VU) globally and Critically Endangered (CR) in Brazil, the species continues to face threats from overfishing throughout its range. We used DNA barcoding employing the mitochondrial Cytochrome c oxidase subunit I (COI) gene to identify the illegal commercialization of *E. itajara* in fish markets from the northern (NC) and southern (SC) Brazilian coasts. Sampling was conducted in fish markets and aimed to confirm the identification of mischaracterized fish sold as *E. itajara* in the NC, as well as identifying fish fillets sold as the Dusky grouper in the SC. DNA barcoding allowed the unambiguous identification of 22 (84.6%) of the 26 analyzed fish market samples. Both sampled areas had confirmation of *E. itajara* illegal commercialization and from the 22 analyzed samples, 17 (77.3%) were confirmed to be *E. itajara*. Here we report two crimes, the illegal sale of *E. itajara* and commercial fraud by species substitution. This study has highlighted that the existing legislation that protects *E. itajara* in Brazilian waters requires the adoption of better public policies for the conservation of the species.

Keywords: COI, Endangered species, Illegal fisheries, Molecular identification, Species substitution.

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O mero do Atlântico *Epinephelus itajara* é a maior espécie de garoupa no Oceano Atlântico. Apesar do seu status de conservação Vulnerável (VU) globalmente e Criticamente em Perigo (CR) no Brasil, a espécie continua enfrentando ameaças da sobrepesca em toda a sua área de distribuição. Utilizamos o sequenciamento de DNA por código de barras, empregando o gene mitocondrial da Citocromo c oxidase subunidade I (COI), para identificar a comercialização ilegal de *E. itajara* em mercados de peixe nas costas norte (CN) e sul (CS) do Brasil. A coleta de amostras foi realizada em mercados de peixe e teve como objetivo confirmar a identificação de peixes descaracterizados e vendidos como *E. itajara* na CN, bem como identificar filés de peixe vendidos como garoupa verdadeira na CS. O sequenciamento de DNA por código de barras permitiu a identificação inequívoca de 22 (84,6%) das 26 amostras de mercado de peixe analisadas. Ambas as áreas amostradas tiveram confirmação da comercialização ilegal de *E. itajara*, e das 22 amostras analisadas, 17 (77,3%) foram confirmadas como *E. itajara*. Nós relatamos aqui dois crimes, a venda ilegal de *E. itajara* e a fraude comercial por substituição de espécies. Este estudo ressaltou que a legislação existente que protege *E. itajara* em águas brasileiras requer a adoção de melhores políticas públicas para a conservação da espécie.

Palavras-chave: COI, Espécies ameaçadas, Identificação molecular, Pesca ilegal, Substituição de espécies.

INTRODUCTION

Epinephelus itajara (Lichtenstein, 1822), also known as the Atlantic goliath grouper, is the largest grouper species in the Atlantic Ocean and inhabits marine and estuarine habitats (Koenig *et al*., 2007). The species can live up to 37 years (Sadovy, Eklund, 1999) and reach 2.5 m (total length, TL) in size and 320 kg (Heemstra, Randall, 1993). Currently, *E. itajara* is classified globally as Vulnerable (VU) according to the International Union for Conservation of Nature – IUCN (Bertoncini *et al*., 2018). However, in Brazilian waters, the species remains classified as Critically Endangered (CR) (ICMBio, 2018).

The Atlantic goliath grouper was the first marine fish species to receive a specific fishing ban in Brazil (Hostim-Silva *et al*., 2005) and has been fully protected in Brazilian jurisdictional waters for over 20 years. The first ordinance was set in place in 2002 (Ordinance N° 121/2002 – IBAMA, 2002) and protected the species for five years, after that, the ordinance was renewed in 2007 (Ordinance 42/2007 – IBAMA, 2007), in 2012 (Ordinance 13/2012 – MMA/MPA, 2012), and last in 2015 (Ordinance 13/2015 – MMA/MPA, 2015) protecting the species until 2023 when the species conservation status will be reevaluated.

This iconic species suffered a severe population decline due to increasing fishing pressure along its geographical distribution areas, potentially increased by a vulnerability associated with its biological and behavioral characteristics. Similar to other Epinephelidae, *E. itajara* is known as slow-growing, long-lived, late maturing, and forming spawning aggregations (Sadovy, Eklund, 1999; Koenig *et al*., 2007),

which added to the loss and/or fragmentation of habitats and the effects caused by the contamination of these environments (Koenig *et al*., 2011) make *E. itajara* particularly vulnerable to overfishing (Giglio *et al*., 2018).

Despite being protected in Brazilian waters, the species is still being harvested and marketed along the Brazilian Coast, especially in Northern Brazil (Silva-Oliveira *et al*., 2008; Giglio *et al*., 2014; Pereira *et al*., 2016; Matos *et al*., 2021). The biggest problem encountered in complying with the legislation is the difficulty of enforcement since the distribution area of the species along the Brazilian coast is more than 7,000 km. Besides the extensive territory and insufficient enforcement (Pereira *et al*., 2016, 2020), the Northern Brazilian Coast faces additional challenges, such as difficult access for enforcement agencies, resulting in an inadequate inspection process (Matos *et al*., 2021; Oliveira *et al*., 2021).

Once you find markets that commercialize endangered fish, another difficulty is in identifying the species, as most of the time fish are mischaracterized (*e.g*., filet, cut shape, without skin, and others), making it challenging for law enforcement to inspect and identify the illegal capture and commercialization of *E. itajara* and other threatened fish species (ICMBio, 2018). To solve this problem in the identification and commercialization of endangered species, molecular techniques started to be used (Bartlett, Davidson, 1991; Holmes *et al*., 2009; Damasceno *et al*., 2016; Sharrad *et al*., 2023). In this context, accurate species identification by applying molecular techniques represents an important tool for monitoring and inspecting fisheries (Oliveira *et al*., 2021). These include the use of DNA barcoding which has proven to be an important instrument and plays a key role in identifying species accurately (Hebert *et al*., 2003; Damasceno *et al*., 2016) at different life stages and even incomplete specimens and mischaracterized individuals (Basheer *et al*., 2014; Feitosa *et al*., 2018; Matos *et al*., 2021). This methodology has also proved to be helpful in identifying mislabeled fish products (Calegari *et al*., 2020) and illegal catches of protected species (Almerón-Souza *et al*., 2018; Matos *et al*., 2021). The DNA barcoding technique is a fast, safe, and robust output based on the Cytochrome c oxidase subunit I (COI) gene and has been extensively used for the identification of marine fish at the species level worldwide (Ward *et al*., 2005; Damasceno *et al*., 2016; Fadli *et al*., 2021; Vences *et al*., 2022).

Given the Atlantic goliath grouper status in Brazil, the need to preserve its stocks, and the difficulty of illegal fishing control (even after more than 20 years of the fishing ban prohibiting its capture), the present study used DNA barcoding, a valid tool to demonstrate mislabeling and illegal fishing, to analyze samples collected at fish markets in the northern and southern Brazilian coasts to identify the illegal commercialization of the species.

MATERIAL AND METHODS

Sample collection. To test if the morphological and molecular identification of the species would match and to see how the analyzed samples would group in the tree clusters with the sequences downloaded from GenBank® we collected samples from whole morphologically identified individuals (n = 2) that dyed from being trapped at an oyster farming in the municipality of Curuçá in the State of Pará, these samples

were used as control and were not used in the analyses. Sampling was conducted in fish markets on the northern (NC) and southern (SC) Brazilian coasts (Fig. 1). In the NC samples were collected in Pará from April to July 2019 (Bragança n = 20). In the SC samples were collected in São Paulo in April 2022 (Cananéia n = 1) and in Paraná from February to March 2022 (Paranaguá n = 1 and Curitiba n = 4).

Sampling aimed to confirm the identification of fish that was mischaracterized and being sold as *E. itajara* in the NC, as well as identifying fish fillets that were being sold as the Dusky grouper (*Epinephelus marginatus* (Lowe, 1834)), in the SC. Samples were fixed in 96% ethanol placed in a 1 mL microcentrifuge tube and stored at -20 °C at the Laboratório de Genética e Conservação Animal at the Universidade Federal do Espírito Santo (UFES) for further processing.

DNA extraction, primer, and PCR assay. Approximately ~25 mg of each tissue sample was used for total genomic DNA extraction using the DNeasy Blood & Tissue Kit (Qiagen, Brazil). Extracted DNA samples were stored at -20 °C until further amplification processing was implemented.

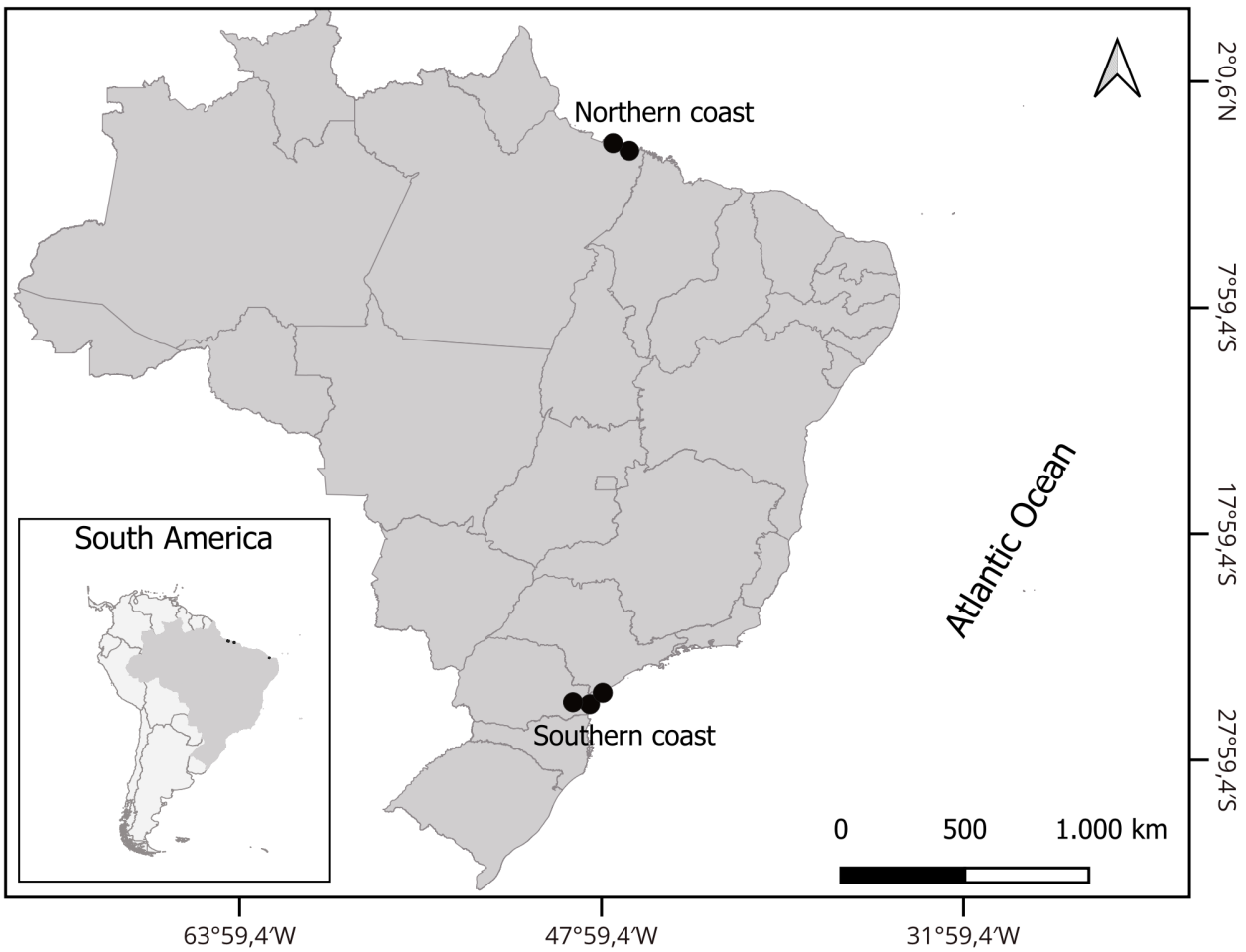

FIGURE 1 | Distribution of sampling sites in the northern (Pará State: Bragança n = 20 and Curuçá n = 2) and southern (São Paulo: Cananéia n = 1; Paraná: Paranaguá n = 1 and Curitiba n = 4) Brazilian coasts.

The Cytochrome c oxidase subunit I (COI) gene was amplified by PCR using the universal primer set FishF1 5'TCAACCAACCACAAAGACATTGGCAC3' and FishR2 5'ACTTCAGGGTGACCGAAGAATCAGAA3' (Ward *et al*., 2005). The amplifications were performed in 20 µl mixture reactions containing 10X buffer; 3.125 mM of MgCl2 (50 mM); 0.125 µM of each primer (10 mM), 0.05 µM of each dNTP (10 mM); 0.625 U of Taq polymerase and 20 ng of DNA template. Thermocycling conditions consisted of an initial denaturation at 95 °C for 2 min followed by 35 cycles of denaturation at 94 °C for 30 s; annealing at 54 °C for 30 s; and elongation at 72 °C for 1 min; followed by a final 72 °C extension for 10 min.

Gel electrophoresis, staining, and DNA sequencing. The amplified fragments were separated and visualized on a 1% agarose gel. Three µL of PCR product stained with bromophenol blue and 2 µL of GelRed™ were loaded onto 1% agarose gel, along with a 100 bp DNA ladder, and electrophoresed to assess the quality of amplicons. The gel was visualized and photo-documented using a transilluminator. The remaining PCR product was purified using 1.8 µL of ExoSap-IT enzyme (USB Corporation), and the purified PCR products were sent to bidirectional sequencing using Big Dye chemistry with capillary electrophoresis in an ABI 3730xl DNA Analyzer (Seoul, KR).

Molecular data analysis. All molecular data analyses were performed with the software MEGA X (Kumar *et al*., 2018). Forward and reverse sequences were aligned using MUSCLE with the default settings, manually edited, and translated into protein to ensure accurate alignment and detection of stop codons, if present. The sequences' similarity was compared with the sequences available in the Barcode of Life Online Database (BOLD) (http://www.boldsystems.org/) (Ratnasingham, Hebert, 2007) and the Basic Local Alignment Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) from GenBank® (Benson *et al*., 2017). A threshold of > 98 % identity was set for all sequences during identification (Hebert *et al*., 2003; Wainwright *et al*., 2018).

The Neighbor-Joining (NJ) methodology is the standard method adopted for phylogenetic inference in DNA barcoding studies (Hebert *et al*., 2003), and one of the reasons it is used in DNA barcoding studies is because of the capability of analyzing large species assemblages at once (Kumar, Gadadkar, 2000). Therefore, the evolutionary history was inferred by generating an NJ tree (Saitou, Nei, 1987) with 10,000 bootstrap pseudoreplications (Felsenstein, 1985). The evolutionary distances were computed using the Kimura 2-parameter (K2P) substitution model (Kimura, 1980), the most commonly used and standard model in DNA barcoding studies.

To construct the phylogenetic NJ tree, we added COI sequences available on GenBank to make sure that the specific taxa identified from the samples collected in this study would separate into specific clusters. The downloaded sequences were: *E. itajara* (family Epinephelidae; KF836456); *E. marginatus* (family Epinephelidae; KC500686); *Hyporthodus niveatus* (Valenciennes, 1828) (family Epinephelidae; KF836478 and KF836483); *Mycteroperca bonaci* (Poey, 1860) (family Epinephelidae; JQ841289 and KF836486); *Conodon nobilis* (Linnaeus, 1758) (family Haemulidae; JQ365304); and *Conodon serrifer* Jordan & Gilbert, 1882 (family Haemulidae; JQ741172 and JQ741174). The confidence of the branches was verified by Bootstrap (10.000 repetitions; Felsenstein, 1985). *Centropomus undecimalis* (Bloch, 1792) (family Centropomidae; JQ365276) sequence was used as an outgroup to root the tree.

RESULTS

The control samples collected from the whole individuals morphologically identified as *E. itajara* in the municipality of Curuçá in the State of Pará were confirmed by DNA barcoding as *E. itajara* (Tab. 1). The results of the BLAST and BOLD searches showed that from the 26 sequences from samples collected at fish markets, 22 were identified at the species level (Tab. 1), while four sequences from the municipality of Bragança (NC) were under the threshold identity, due to sequence quality, and were not used in further analyses. From the 16 analyzed samples of fish being sold as *E. itajara* in the State of Pará, only one was not *E. itajara* and was confirmed to be *Conodon nobilis*. The sample collected in the State of São Paulo that was being sold as Dusky grouper was *E. itajara*, while from the five samples that were being sold as Dusky grouper (n = 5) in the State of Paraná, one was confirmed to be *E. itajara* and the other four samples were confirmed to be the Dusky grouper *Epinephelus marginatus* (Tab. 1). Therefore, from the 22 analyzed samples collected in fish markets during our study, 17 (77.3%) belonged to *E. itajara*.

TABLE 1 | Samples collected in the northern (Pará State: Bragança Fish Market n = 16 and Curuçá n = 2) and southern (São Paulo: Cananéia Fish Market n = 1; Paraná: Paranaguá Fish Market n = 1 and Curitiba Fish Market n = 4) Brazilian coasts. Sample information (ID), sequence size (bp), sample collection location (Location), sold as (Sold), species identification (Species), and results for identity percentage (% Identity) from the Basic Local Alignment Tool – BLAST (GenBank) and the Barcode of Life Online Database (BOLD). *Control samples from whole individuals morphologically identified as Atlantic goliath grouper.

ID	bp	Location	Sold	Species	% Identity	
					Genbank	Bold
E.itaPA01	699	Bragança	Atlantic goliath grouper	*Epinephelus itajara*	100	100
E.itaPA03	635	Bragança	Atlantic goliath grouper	*Epinephelus itajara*	100	100
E.itaPA05	686	Bragança	Atlantic goliath grouper	*Epinephelus itajara*	100	100
E.itaPA08	682	Bragança	Atlantic goliath grouper	*Epinephelus itajara*	100	100
E.itaPA09	667	Bragança	Atlantic goliath grouper	*Epinephelus itajara*	100	100
E.itaPA12	685	Bragança	Atlantic goliath grouper	*Epinephelus itajara*	100	100
E.itaPA15	676	Bragança	Atlantic goliath grouper	*Epinephelus itajara*	99.69	99.69
E.itaPA17	681	Bragança	Atlantic goliath grouper	*Epinephelus itajara*	100	100
E.itaPA19*	646	Curuçá	Atlantic goliath grouper	*Epinephelus itajara*	100	100
E.itaPA20*	707	Curuçá	Atlantic goliath grouper	*Epinephelus itajara*	100	100
E.itaPA22	681	Bragança	Atlantic goliath grouper	*Epinephelus itajara*	100	100
E.itaPA23	644	Bragança	Atlantic goliath grouper	*Conodon nobilis*	100	100
E.itaPA24	679	Bragança	Atlantic goliath grouper	*Epinephelus itajara*	99.84	99.84
E.itaPA33	819	Bragança	Atlantic goliath grouper	*Epinephelus itajara*	100	100
E.itaPA34	646	Bragança	Atlantic goliath grouper	*Epinephelus itajara*	100	100
E.itaPA35	668	Bragança	Atlantic goliath grouper	*Epinephelus itajara*	100	100
E.itaPA36	646	Bragança	Atlantic goliath grouper	*Epinephelus itajara*	100	100
E.itaPA41	676	Bragança	Atlantic goliath grouper	*Epinephelus itajara*	100	100
MOPE844	643	Curitiba	Dusky grouper	*Epinephelus marginatus*	100	100
MOPE897	650	Paranaguá	Dusky grouper	*Epinephelus itajara*	100	100
MOPE952	643	Curitiba	Dusky grouper	*Epinephelus marginatus*	100	100
MOPE953	643	Curitiba	Dusky grouper	*Epinephelus marginatus*	99.85	100
MOPE1006	643	Curitiba	Dusky grouper	*Epinephelus marginatus*	100	100
MOPE1007	653	Cananéia	Dusky grouper	*Epinephelus itajara*	100	100

Stop codons were not detected and the read lengths varied from 635 to 819 bp. For *E. itajara* and *E. marginatus*, the average nucleotide composition was G = 18.42%, C = 27.30%, A = 24.04%, and T = 30.24%, 486 conserved sites, 158 variable sites, 80 parsimony informative sites and 78 singletons (Tab. 2). The mean GC content was 45.72 ± 1.03% (Tab. 2) and decreased from the first codon position down to the third codon position. For *Conodon nobilis* the average nucleotide composition was G = 20.2%, C = 30.7%, A = 21.1%, and T = 28.0%, while the GC content was 50.9%, and decreased from the first to the second codon position and increased from the second to the third position.

Thirty-four congruent sequences, 24 from this study (22 from fish markets and two used as control) and 10 downloaded from GenBank were used for phylogenetic analysis and the NJ tree showed consistent clusters of conspecific sequences. All clusters were monophyletic and inter-genera relationships between *E. itajara* and *E. marginatus* and *C. nobilis* and *Conodon serrifer* were well resolved with high bootstrap values (Fig. 2), showing the efficiency of COI sequences to provide species-level resolution.

TABLE 2 | Summary statistics for the nucleotide frequency distribution of COI sequences of samples collected in the northern and southern Brazilian coasts for *Epinephelus itajara* and *E. marginatus*.

	Min	Max	Mean	SE
G %	17.9	18.6	18.42	0.2000
C %	27.2	27.8	27.30	0.1098
A %	23.8	25.0	24.04	0.3340
T %	29.3	30.4	30.24	0.2141
GC %	45.2	45.8	45.72	1.0316
GC % Codon Pos 1	57.2	58.1	57.34	0.3200
GC % Codon Pos 2	43.3	43.5	43.31	0.040
GC % Codon Pos 3	34.1	36.9	36.51	2.8974

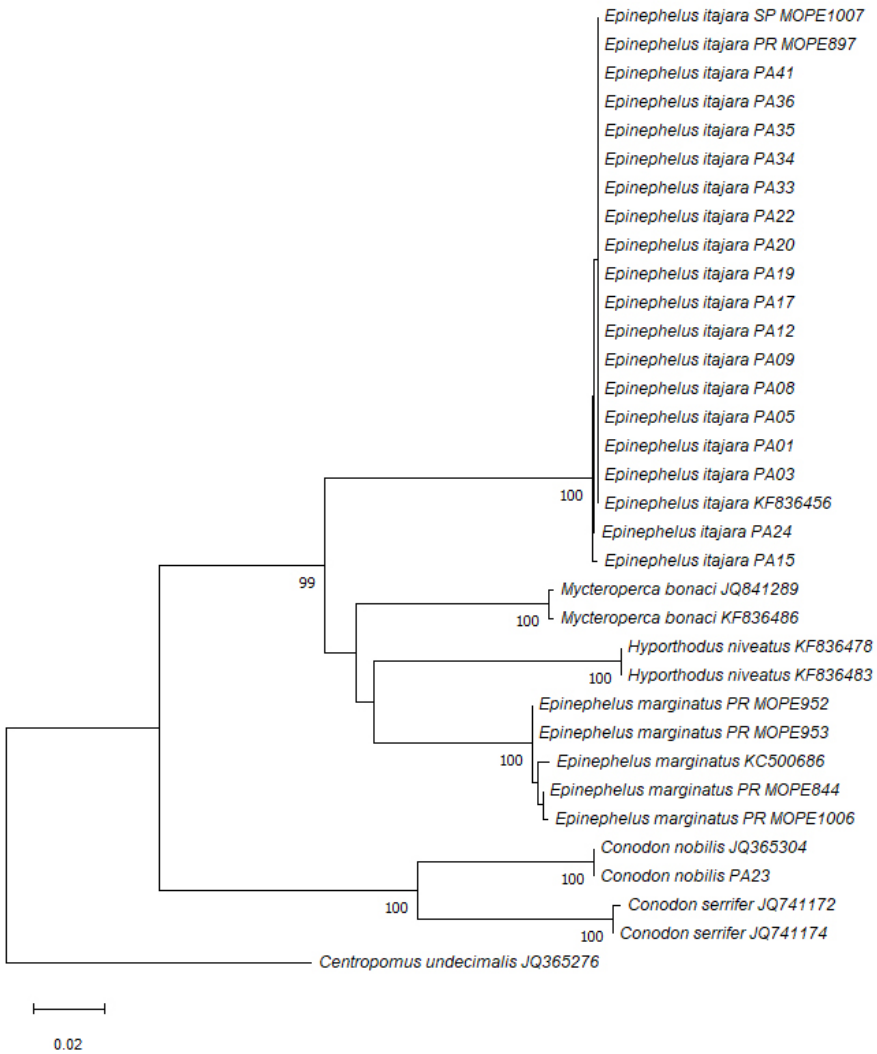

FIGURE 2 | Phylogram based on cytochrome oxidase I (COI) gene amplification using the Neighbor-Joining method. The evolutionary distances were computed using the Kimura 2-parameter model with 10,000 repetitions for samples collected in the northern (Pará State: Bragança Fish Market n = 16 and Curuçá n = 2) and southern (São Paulo: Cananéia Fish Market n = 1; Paraná: Paranaguá Fish Market n = 1 and Curitiba Fish Market n = 4) Brazilian coasts. Bootstrap values are shown next to the branches. The sequences with the accession numbers were downloaded from GenBank.

DISCUSSION

Our study reveals that besides being protected in Brazil, *E. itajara* was commercialized in the sampled areas. These illegal fishing practices have been causing a huge loss for Atlantic goliath grouper recovery for two main reasons: first, the capture of younger specimens in estuarine areas negatively affects the recruitment of juveniles for the adult phase, and second, offshore fishing efforts typically concentrate on the largest and oldest individuals, principally at spawning aggregation sites, because they are more valuable economically.

Groupers form one of the most commercially valuable fish groups globally (Fadli *et al*., 2021), for they are highly admired due to their delicate, desirable taste and flavor (Alcantara, Yambot, 2014). Studies have shown that the fisheries directed to *E. itajara* coincide with spawning aggregations in austral summer, which take place from December to March (Giglio *et al*., 2014, 2016; Bueno *et al*., 2016; Matos *et al*., 2021), and illegally captured fish are mischaracterized (head, fins, and skin are removed) for commercialization (Silva-Oliveira *et al*, 2008; Pereira *et al*., 2020), however, during our study Atlantic goliath grouper samples were also collected from April to July, showing that the fishing pressure is not limited to the spawning aggregation period.

Previous studies have identified fish mislabeling for endangered elasmobranch species in the State of Pará (Palmeira *et al*., 2013; Wosnick *et al*., 2023). In our study, although fish were mischaracterized in the State of Pará, sellers would discretely inform customers that the fish being sold was *E. itajara.* The reality of the State of Pará is different from the states of São Paulo and Paraná, where fish is often mischaracterized and sold as other species, for example, the Dusky grouper *E. marginatus*. In this case, two crimes are being committed, the illegal sale of *E. itajara* and commercial fraud by the substitution of one species for another (Matos *et al*., 2021). The current study surveyed two areas along the Brazilian coast and 77.3% of the samples analyzed were confirmed as *E. itajara*, showing that besides being protected for over 20 years on the Brazilian coast, *E. itajara* is still being captured and commercialized, a practice that hampers conservation efforts, such as the initiatives proposed by Meros do Brasil Project (merosdobrasil.org).

Intense fishing has a negative impact on population maintenance, resulting in severe truncation of the population size and age structure (Hixon *et al*., 2014; Bentes *et al*., 2019), it can also induce evolutionary responses in fish to reproduce at a smaller size to increase reproductive success, which also increases the risk of mortality (Waples, Audzijonyte, 2016; Marshall *et al*., 2019). Although fisheries models have traditionally assumed that many small, young, mature females are reproductively equivalent to fewer big, old, fat, fecund, female, fish, known as BOFFFFs, the reproductive output of larger fish is higher than that of smaller fish, especially in long-lived species with low natural mortality (Hixon *et al*., 2014; Dick *et al*., 2017) such as the Atlantic goliath grouper.

Furthermore, the issue that arises from the illegal commercialization of *E. itajara* is not only one of conservation, but it may also have potential implications for consumer health, due to heavy metal bioaccumulation. Several studies have addressed the high concentration of mercury in Atlantic goliath grouper muscle tissue in the United States (Adams *et al*., 2003; Adams, Sonne, 2013; Malinowski, 2019) and Belize (Evers *et al*., 2009). Mercury is a heavy metal known to be a severe stressor in wildlife, including fish species (Dietz *et al*., 2019) as it is one of the most toxic and persistent heavy metals to all organs and tissues (Adams, Sonne, 2013; Malinowski, 2019), therefore, the regular consumption of Atlantic goliath grouper should raise concerns (Evers *et al*., 2009).

Here we illustrate that DNA barcoding was effective at producing sequences that identified taxonomical units to species-level resolution for the authentication of fish illegally commercialized in local fishing markets on the northern and southern Brazilian coasts. Our dataset computed GC content (or Guanine-cytosine content) mean was 45.92%, being similar to the values reported for groupers from Indonesia (46.28%; Fadli *et al*., 2021), the Philippines (45.16%; Alcantara, Yambot, 2014) and India (45.17%; Sachithanandam *et al*., 2022). The distribution of GC content is essential for assessing

genetic diversity and population health and can be a useful tool for comprehending fundamental processes and can be linked to features associated with species' life history traits (Wu *et al*., 2012; Alcantara, Yambot, 2014), however, environmental elements including genome size, temperature, oxygen need, and habitat can be linked to the mechanism of GC content variation in different species (Wu *et al*., 2012; Alcantara, Yambot, 2014; Sachithanandam *et al*., 2022).

Our results show that the laws that protect the Atlantic goliath grouper in Brazil are not effective in identifying illegal catches (Torres *et al*., 2013), and as a result, the species is still facing fishing pressure and exposure to overexploitation and depletion; especially during spawning aggregation events (Silva-Oliveira *et al*., 2008; Bueno *et al*., 2016; Giglio *et al*., 2017; Pereira *et al*., 2020; Bravo-Calderon *et al*., 2021), an ecological trait that contributes to the vulnerability of the species to fishing. These findings highlight that it is necessary to strengthen enforcement and awareness efforts to ensure compliance with protective regulations.

The inspection difficulties faced by public authorities due to mischaracterized individuals and the large extension of the Brazilian coast present a complex and delicate management challenge for the Atlantic goliath grouper, consequently, conservation efforts and recovery plans that require a multitude of comprehensive actions are required to ensure the species protection. Therefore, considering *E. itajara* vulnerability to extinction (de Mitcheson *et al*., 2012; Bravo-Calderon *et al*., 2021), our results are important to raise awareness of its protection, once consumer awareness is essential for preventing the commercialization of endangered species and protecting biodiversity.

The world is experiencing a global trend of sustainable consumption (Mitchell, 2011; Simeone, Scarpato, 2020), and some consumers may refrain from purchasing threatened species if they are aware of how their decision will affect the ecosystem (Guillen *et al*., 2019), thus, informed consumers might play a significant role in the conservation of endangered species, such as the Atlantic goliath grouper. As a result, it is wise to educate and engage people in conservation initiatives, as this is an important asset for successful management and biodiversity protection.

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