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DNA-based species identification of shark finning seizures in Southwest Atlantic: implications for wildlife trade surveillance and law enforcement

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Abstract

Sharks developed life history traits that make them susceptible to overfishing. This is, in turn, a risk for extinction, and several species are affected. The high price of shark fins in the international trade has triggered the widespread capture of sharks at unsustainable levels, prompting illegal and unethical practices, such as finning. To address these concerns, the present study aimed to identify species composition using molecular techniques based on DNA barcoding and DNA polymorphism on samples taken from illegal shark fin seizures conducted by the Federal Environmental Agency of Brazil. A species-specific DNA-based identification from three finning seizures in Brazil found at least 20 species from 747 shark fins, some of which were identified as endangered and protected under Brazilian legislation, while others were representative of restricted catches, according to Appendix II of CITES. In the seizure from Belém, 338 fins were identified as belonging to at least 19 different species, while in the seizure from Natal 211, fins belonging to at least 8 different species were identified. Furthermore, 198 fins from Cananéia were identified through PCR-Multiplex as belonging to *Isurus oxyrinchus*. These results raise concerns about the environmental and socioeconomic effects of finning on developing countries. Furthermore, this study represents the first finning evaluation from Brazil in the Southwest Atlantic, highlighting the importance of developing policies aimed toward restricting and regulating the shark trade and detecting IUU fisheries and illegal trade of endangered species, mainly in developing countries, where fisheries management, surveillance, and species-specific fisheries catch data are often sporatic.

Keywords Genetic identification · Elasmobranchs · DNA barcoding · Fisheries management · Threatened species · Conservation

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Introduction

Sharks usually play a major role in marine environments, and large species act mostly as food-web top-predators (Heithaus et al. 2012; Ferretti et al. 2010). Elasmobranchs are naturally more vulnerable to extinction than bony fishes (Myers and Worm 2005) because some species are "k-strategists" characterized by slow growth, late sexual maturity, high longevity, and low fertility, all characteristics which make them particularly susceptible to anthropogenic pressures, such as overfishing (García et al. 2008; Musick et al. 2000). Once overfished, shark populations require long recovery periods (Davidson et al. 2016; Stevens et al. 2000). However, notwithstanding their ecological importance, sharks are among the most threatened vertebrates, mainly because of intense fishing pressure (Bradley and Gaines 2014; Dulvy et al. 2014; Hoffmann et al. 2010).

In the past half century, shark fisheries have expanded rapidly worldwide. At first, they were captured by different fishing gear, such as driftnets, gillnets, purse-seine, and trawling nets, and their products were consumed locally. However, longline fisheries that target tuna (*Thunnus* spp.) and swordfish (*Xiphias gladius*) are mostly responsible for shark captures and bycatches (Bonfil 1994; Molina and Cooke 2012; Oliver et al. 2015; Stevens et al. 2000), and now they also supply the demand for shark fins in the international shark fin trade (CITES 2018). According to the Food and Agriculture Organization (FAO) of the United Nations (UN), shark fisheries reached a peak of nearly 880,000 tons in 2003, and, ever since, catches have decreased by 20% (Davidson et al. 2016; Dulvy et al. 2014). Despite this, the reported catches could be highly underestimated, mainly because these statistics have uncertainties with respect to small-scale artisanal catch data (Zeller et al. 2015), and they do not account for by-catches (Zeller and Pauly 2005), illegal catches and discards (Pauly and Zeller 2016), raising concerns about the true magnitude of ecological impact suffered by sharks and rays.

In the South Atlantic, artisanal, industrial and sport fisheries with different types of fishing gear capture several shark species, but land in different fishing ports along the Brazilian coast. For instance, Brazil reported a peak capture of sharks, rays, and chimaeras of 31,259 tons (t) in 1982, but a decrease of 44% in reported captures in 2017 (FAO 2019). As a consequence, different shark species are severely overexploited and now face extinction (e.g., Barreto et al. 2016; Lessa et al. 2016). Even though alarming, the lack of proper policymaking in Brazil's fisheries management is unlikely to improve as a result of insufficient funding. Moreover, the failure to institute science-based regulations just favors legal breaches and increases the risk of extinction (Fiedler et al. 2017). In fact, sharks are caught on a large scale worldwide, and based on an analysis of average shark weight performed in 2000, mortality of 100 million sharks was estimated and almost 97 million in 2010 with a possible range of values between 63 and 273 million sharks per year (Worm et al. 2013). In their study, Clarke et al. (2006a) stated that the biomass of the global fin trade was four times higher than previously reported by the FAO and largely underestimated. Shark fin supplies come from different sources, but mainly from longline fisheries, by-catches on international waters, and exclusive economic zones of developed and developing countries (Barreto et al. 2016; Jaiteh et al. 2016, 2017; Schiller et al. 2015).

According to Dulvy et al. (2014), a worldwide decline in shark populations is likely associated with the international fin trade. The high demand for shark fin soup in the Asian market triggered widespread shark catches for fins and encouraged finning, an illicit act of removing fins from sharks and discarding the animals back into the ocean, sometimes still alive. This unethical and illegal act has led to unsustainable fishing pressure on these k-strategist species, and many are currently threatened with extinction (Clarke et al. 2006b; de Mitcheson et al. 2018; Dulvy et al. 2014, 2017). The highest incomes from the shark fin trade originate mainly from illegal, unreported, and unregulated (IUU) fisheries, which lack species-specific identification (Dulvy et al. 2014; Stevens et al. 2000) and are often omitted in fisheries catch reports (Pauly and Zeller 2016). As a result of fisheries mismanagement in Brazil, little is known about the shark fin trade. In general, however, we do know that shark meat is exported, but also consumed locally, while fins are mostly traded to Asia and mostly obtained through IUU fisheries. Furthermore, since 1998, Brazilian authorities have dismantled several local finning operations, and it was documented that Brazil is, indeed, a major supplier for the international fin trade (Barreto et al. 2017). Consequently, protected shark species are captured and traded without any regulation or documentation (Fields et al. 2018; Steinke et al. 2017). Nevertheless, field identification of processed fisheries products is, in most cases, unreliable. Dried shark fins are typically missing key diagnostic characteristics, making morphological species identification challenging, or even impossible (Fields et al. 2018), compromising surveillance and law enforcement.

To overcome this obstacle, DNA barcoding (Hebert et al. 2003) is a taxonomic method that employs standardized genetic markers in an organism's DNA to identify it as belonging to a particular species. This makes it possible to identify unknown DNA samples to a registered species based on comparison to a reference library. Consequently, over the last few years, several studies have aimed to create a Chondrichthyes DNA database and tools for their identification in order to evaluate their biodiversity (Mendonça et al. 2009, 2010; Ribeiro et al. 2012; Ward et al. 2005, 2008, 2009; Wong et al. 2009; Cerutti-Pereyra et al. 2012). Moreover, this technique has been applied for shark fin species identification worldwide (Fields et al. 2015, 2018; Holmes et al. 2009; Liu et al. 2013; Sembiring et al. 2015; Shivji et al. 2002; Steinke et al. 2017). However, a growing number of studies have been dedicated to the application of these genetic approaches for law enforcement (Chapman et al. 2009; Barbuto et al. 2010; De-Franco et al. 2012; Domingues et al. 2013; Steinke et al. 2017; Bunholi et al. 2018; Feitosa et al. 2018; Ferrette et al. 2019). Therefore, the present study aims to apply DNA-based techniques, such as DNA barcoding and PCR-multiplex, to identify the species composition of shark fins from three finning seizures conducted by the Federal Environmental Agency in Brazil totaling more than 13 tons of seized fins. These results highlight the importance of conservation policies that aim to restrict and regulate the shark fin trade, as well as increase knowledge about finning and IUU fisheries in the southwestern Atlantic Ocean.

Materials and methods

Sampling

Seized shark fins were collected from three different surveillance operations conducted by the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA), a federal agency under the Brazilian Ministry of Environment. In the first seizure, in the city of Belém in Pará state, northern Brazil, 8 tons of dried fins were seized from a finning recidivist Brazilian company exporting to China, and a total of 400 dried fins were randomly sampled. In the second seizure, more than 5 tons of dried fins from two foreign fishing companies, one Spanish and the other Chinese, both accused of finning in the city of Natal, Rio Grande do Norte state, northeastern Brazil, were randomly sampled, totaling 300

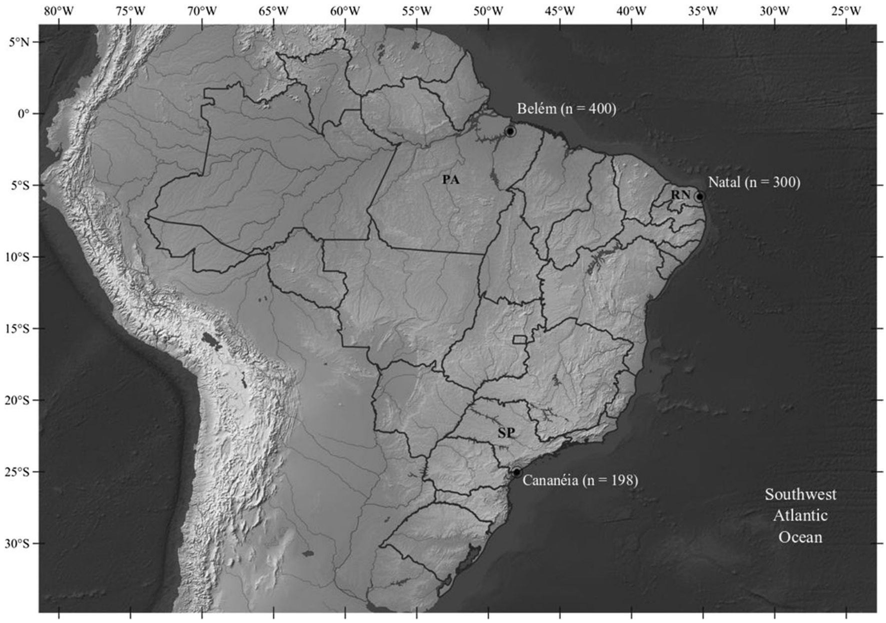

Fig. 1 Finning seizure locations and sampling sizes in the cities of Belém in Pará (PA) state, Natal in Rio Grande do Norte (RN) state and Cananéia in São Paulo (SP) state in Brazil in the Southwest Atlantic Ocean

fins. During the third seizure, 198 fresh fin tissues were sampled from a Brazilian pelagic longline fishing company during landings in the city of Cananéia, São Paulo, southeastern Brazil, and this company was also accused of finning (Fig. 1). All sampled tissue fragments were stored in 100% ethyl alcohol at − 20 °C and were supplied by IBAMA.

DNA-based species identification

Genomic DNA from fins was extracted using the NucleoSpin® Tissue kit (MACHEREY–NAGEL GmbH and Co. KG). Samples from the first two seizures were identified by amplifying cytochrome *c* oxidase subunit I (COI), a mitochondrial DNA (mtDNA) gene. Polymerase chain reaction (PCR) was carried out according to the parameters and specifications of Platinum® *Taq* DNA Polymerase (Invitrogen™) using two primers (Forward: FishF1: 5′-TCA ACC AAC CAC AAA GAC ATG GGC AC-3′ and Reverse: FishR1: 5′-TAG AGT TCT GGG TGG CCA AAG AAT CA-3′) (Ward et al. 2005). For sequencing reaction, PCR products were submitted to an enzymatic purification protocol using the ExoSAP-IT™ PCR Clean-Up kit (Applied Biosystems™). The sequencing protocols followed the procedures of the BigDye™ Terminator v3.1 Sequencing Kit (Applied Biosystems™), and sequences were generated on the ABI 3130 Genetic Analyzer (Applied Biosystems™). In addition, shark fin seizures from Cananéia came exclusively from pelagic longline fisheries. Accordingly, a PCR-multiplex developed by Shivji et al. (2002) for species-specific pelagic shark identification was used. This system was applied to differentiate DNA sequences from among different pelagic shark species using the nuclear ribosomal internal transcribed spacer (ITS) 2 locus. Such method combines six pelagic shark primers (*P. glauca, Isurus oxyrinchus, I. paucus, Lamna nasus, Carcharhinus falciformis*, and *C. obscurus*) with two universal primers in an eight-primer single tube reaction. This PCR-multiplex was performed by changing the annealing temperature to 65 °C to provide a diagnostic-sized amplicon for each shark species. The reaction effectiveness was analyzed on a 1.5% agarose gel under UV light.

Samples from Natal and Belém seizures were identified using two methods: (i) a sequence similarity-based identification and (ii) a tree-based identification (van Velzen et al. 2012). First, a sequence similarity comparison was undertaken to match the closest sequence on the public library databases, such as the Barcode of Life Data (BOLD) system, version 4 (http://v4.boldsystems.org/) (Ratnasingham and Hebert 2007) and GenBank® (https://www.ncbi.nlm.nih.gov/genbank/) (Benson et al. 2017), using similarity scores (e.g., BLAST, https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Altschul et al. 1990) and assigning the species label of the closest reference to the query. Second, a tree-based strategy used a classic phylogenetic method (Ronquist and Huelsenbeck 2003) to assign unidentified barcodes to species based on their clusters in the tree, attempting to estimate the phylogeny between the reference barcodes and the query sequence. The query was assigned to the species within which it was clustered.

Following this, we performed the two phylogenetic methods with the generated sequences coupled with the highest similarity scores for species sequences downloaded from Barcode Index Numbers (BINs) (Ratnasingham and Hebert 2013) in BOLD v4, as described in Online Resource 1. The sequence of Collared Carpetshark *Parascyllium_collare* (FMVIC281-08) was used as outgroup. All sequences were aligned using MUSCLE (multiple sequence comparison by log-expectation) algorithms (Edgar 2004) in Geneious R11.1.5 (Kearse et al. 2012).

The COI matrix was tested for nucleotide substitution saturation (Xia et al. 2003) with DAMBE6 (Xia 2017) and showed no significant saturation. The best-fit nucleotide substitution model, HKY+I+G ($pinv=0.6$, $\alpha=1.65$), was evaluated by the Bayesian Information Criterion (BIC) and Decision Theory (DT) performance-based selection with jModelTest2 on XSEDE (Darriba et al. 2012; Towns et al. 2014) through CIPRES Science Gateway v3.3 (Miller et al. 2010). A Maximum-Likelihood (ML) phylogenetic tree was constructed by PhyML v 3.3 (Guindon and Gascuel 2003; Guindon et al. 2010) implemented in Geneious R11.1.5. A BioNJ (Gascuel 1997) tree was used as the starting tree to define the strategy of the tree topology search as the best of Nearest Neighbor Interchanges (NNI) and Subtree Pruning and Regrafting (SPR) with 1000 non-parametric bootstrap replicates. The PhyML tree was set as the initial tree of Bayesian inference performed in BEAST v1.10.4 (Suchard et al. 2018), adopting an unregulated relaxed lognormal clock (Drummond et al. 2006) and the Yule speciation process (Gernhard 2008; Yule 1925) as priors. The posterior probability of parameters was estimated using Markov chain Monte Carlo (MCMC) with 500 million generations and 10% of burn-in. The model convergence, effective sample size (ESS) and 95% of highest posterior density (HPD) intervals were all calculated by TRACER, v1.7.1 (Rambaut et al. 2018). TreeAnnotator, v1.10.4, summarized the information of a sample of trees produced by BEAST onto a single Highest Log Clade Credibility Tree. FigTree, v1.4.4 (Rambaut 2012) was used to view the phylogenies.

Results

A total of 747 shark fins were sequenced and identified at the lowest taxonomic level, using 651 base pairs (bp) of the COI gene and PCR-Multiplex. Two orders were identified (Carcharhiniformes and Lamniformes), four families (Carcharhinidae, Sphyrnidae, Alopiidae, and Lamnidae) and at least 20 shark species (Table 1). The seizure from Belém was the most sampled and biodiverse, with a total of 338 shark fins identified, and at least 19

Table 1 Species identified by DNA barcoding

Family	Common name	Species	n	%	IUCN	ICMBio	CITES
Carcharhinidae	Blacknose shark	*Carcharhinus acronotus*	35	4.69	NT	NT	
	Copper shark	*Carcharhinus brachyurus*	1	0.13	NT	DD	
	Silky shark	*Carcharhinus falciformis*	35	4.69	VU	NT	II
	Bull shark	*Carcharhinus leucas*	7	0.94	NT	NT	
	Blacktip shark	*Carcharhinus limbatus*	2	0.27	NT	NT	
	Dusky/galapagos sharks	*C. obscurus/C. galapagensis*	4	0.54	VU/NT	EN/CR	
	Caribbean reef shark	*Carcharhinus perezi*	10	1.34	NT	VU	
	Small tail shark	*Carcharhinus porosus*	98	13.12	DD	CR	
	Night shark	*Carcharhinus signatus*	2	0.27	VU	VU	
	Tiger shark	*Galeocerdo cuvier*	4	0.54	NT	NT	
	Blue shark	*Prionace glauca*	252	33.73	NT	NT	
	Brazilian sharpnose shark	*Rhizoprionodon lalandii*	3	0.40	DD	NT	
	Caribbean sharpnose shark	*Rhizoprionodon porosus*	31	4.15	LC	DD	
Sphyrnidae	Scalloped hammerhead shark	*Sphyrna lewini*	5	0.67	EN	CR	II
	Great hammerhead shark	*Sphyrna mokarran*	3	0.40	EN	EN	II
	Bonnethead shark	*Sphyrna tiburo*	11	1.47	LC	CR	
	Smalleye hammerhead shark	*Sphyrna tudes*	16	2.14	VU	CR	
Alopiidae	Bigeye thresher shark	*Alopias superciliosus*	5	0.67	VU	VU	II
Lamnidae	Shortfin mako shark	*Isurus oxyrinchus*	207	27.71	VU	NT	II
	Longfin mako shark	*Isurus paucus*	16	2.14	VU	DD	II
			747	100			

n, number of identified individuals; %, the percentage of total sampling; IUCN, IUCN Red List species status; ICMBIO, Brazilian Red List species status; CITES (Convention on International Trade in Endangered Species) listed Appendix. CR, Critically Endangered; EN, Endangered; VU, Vulnerable; NT, Near Threatened; LC, Least Concern; DD, Data Deficient

coastal and pelagic species were found. The seizure from Natal was the second in sampling size (n = 211), accounting for at least seven pelagic species (Table 1; Online Resources 2 and 3).

Prionace glauca, *Isurus oxyrinchus,* and *Carcharhinus porosus* were the most abundant identified species, representing 33.73%, 27.71%, and 13.12%, respectively (Table 1). Miscellaneous shark species from Carcharhinidae, Sphyrnidae, Alopiidae, and Lamnidae accounted for 25.43% of the total sampling (Table 1). At least nine identified species are listed in the threatened categories of the IUCN Red List, accounting for almost 40% of all individuals (Fig. 2a; Table 1), whereas 20.61% of the total is also on the Brazilian Red List (Fig. 2b; Table 1). In addition, six identified species (i.e., *C. falciformis*, *Sphyrna*

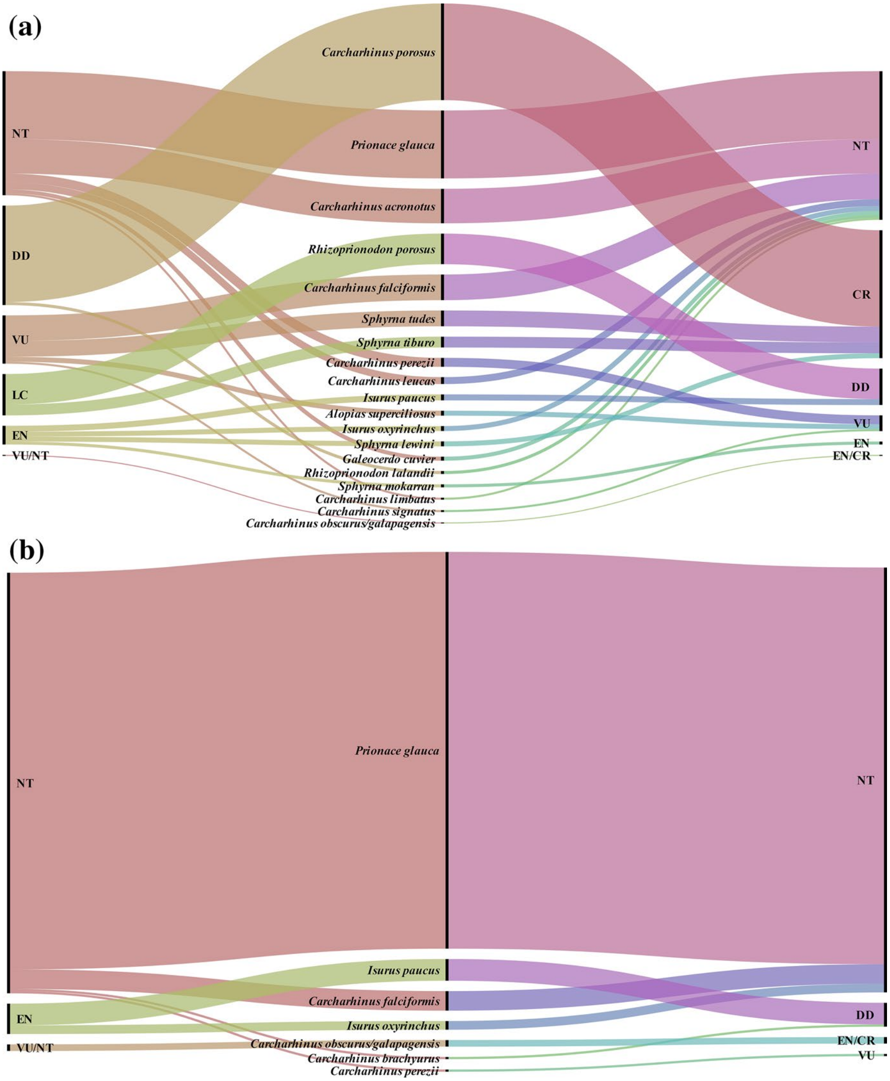

Fig. 2 Alluvial diagrams, built by RAWGraphs (Mauri et al. 2017), represent correlations between the number of identified species and their conservation status according to the IUCN Red List of Threatened Species™ on the left and the Chico Mendes Institute for Biodiversity Conservation (ICMBio) from Brazil on the right. **a** Belém seizure (n = 400). **b** Natal seizure (n = 300). *CR* critically endangered, *EN* endangered, *VU* vulnerable, *NT* near threatened, *LC* least concern, *DD* data deficient

lewini, *S. mokarran*, *Isurus oxyrinchus*, *I. paucus* and *Alopias superciliosus*) are currently listed in Appendix II of CITES.

All sequences matched BOLD and GenBank® reference libraries with high similarity (> 99%). Most identified species clustered into high-support clades with DNA Barcode BIN sequences in the phylogenetic tree, evidencing success in genetic identification, except *C. obscurus* and *C. galapagensis* that could not be differentiated by only a single-locus approach and thus clustered within the same clade (Fig. 3). All sequences from the Belém (accession nos. MH911009–MH911346) and Natal (accession nos. MH719774–MH719984) seizures were deposited in GenBank®. In the third evaluated finning seizure in Cananéia, using the PCR-Multiplex approach, all shark fins (n = 198) were identified as belonging to only a single species, *I. oxyrinchus* (Fig. 2; Online Resource 4), according to the amplicon size pattern determined by gel electrophoresis (Fig. 4).

Discussion

This evaluation is the first genetic identification of shark finning seizures from Brazil in the Southwest Atlantic. Two of the biggest finning seizures in Brazil were sampled, resulting in multimillion-dollar penalties against the law-breaking companies (Barreto et al. 2017). In Belém, at least 19 shark species were identified, a quantity of pelagic species equal to that of coastal species, a statistic indicating that fins came from different fishing modalities and gear. On the other hand, in Natal and Cananéia, mainly pelagic species were identified, indicating that they were caught mostly by a longline fishing fleet on the high seas.

Our results are similar to other evaluations from finning, such as those of Steinke et al. (2017), who also identified 20 shark species from dried fins and gill plates obtained in Canada, China, and Sri Lanka. Despite high species diversity, according to Fields et al. (2018), the contemporary shark fin trade is dominated by only 8 species or complexes, likely comprising more than 1%: *P. glauca*, *C. falciformis*, *S. lewini* and *S. zygaena*, *Carcharhinus* spp., *C. brevipina*, *C. leucas*, and *Isurus* spp., as evidenced also by our results. The most abundant identified shark species were *P. glauca*, *I. oxyrinchus*, and *C. porosus*, accounting altogether for almost 75% of all individuals (Table 1). Although, *P. glauca* is not considered overfished in the Atlantic (ICCAT 2015), Indian (IOTC 2017), or Pacific (ISC 2017) Oceans, it is the most captured shark worldwide by high seas fisheries (Coelho et al. 2018; Oliver et al. 2015) and also the species supplying the international shark fin trade (Clarke et al. 2006a, b; Fields et al. 2018). *Isurus oxyrinchus* was the second most frequently identified species. This species is also the second most frequently found in the Hong Kong fin trade (Fields et al. 2018), and it is currently overfished in the North Atlantic. The International Commission for the Conservation of Atlantic Tuna (ICCAT) recommends a catch-quota reduction, while in the South Atlantic, the stocks could be overfished, but owing to assessment uncertainties, ICCAT recommends that catch-quota should not be raised (de Bruyn 2017). The species is currently globally assessed as “Endangered” (EN) (Rigby et al. 2019) since its weighted global population trend estimated a high probability of 50–79% reduction over three generation lengths (72–75 years) and slow stock rebuilding, even with prohibited capture (Sims et al. 2018).

Moreover, ecological risk assessment based on biological productivity and susceptibility, as recently adopted by the ICCAT for data-poor fisheries, has demonstrated that *P. glauca* and *I. oxyrinchus* are among the most vulnerable shark stocks from longline fisheries in the Atlantic Ocean (Cortés et al. 2015). Most fishing of pelagic sharks in the South Atlantic Ocean occurs on the high seas, mostly as by-catch by longline fisheries targeting tunas (Scombridae) and billfishes (Xiphiidae and Istiophoridae), and they are retained on board for their fins (Bonfil 1994). Over the last 40 years, few data were available for catch rates, relative abundances, and distributions of the captured species. Most information was from small-scale longline fleets operating off Brazil and Uruguay where sharks historically have been landed and sold in local markets (Hazin et al. 2008). Atlantic high seas longline fisheries are responsible for about 25% of reported global shark catches (Clarke

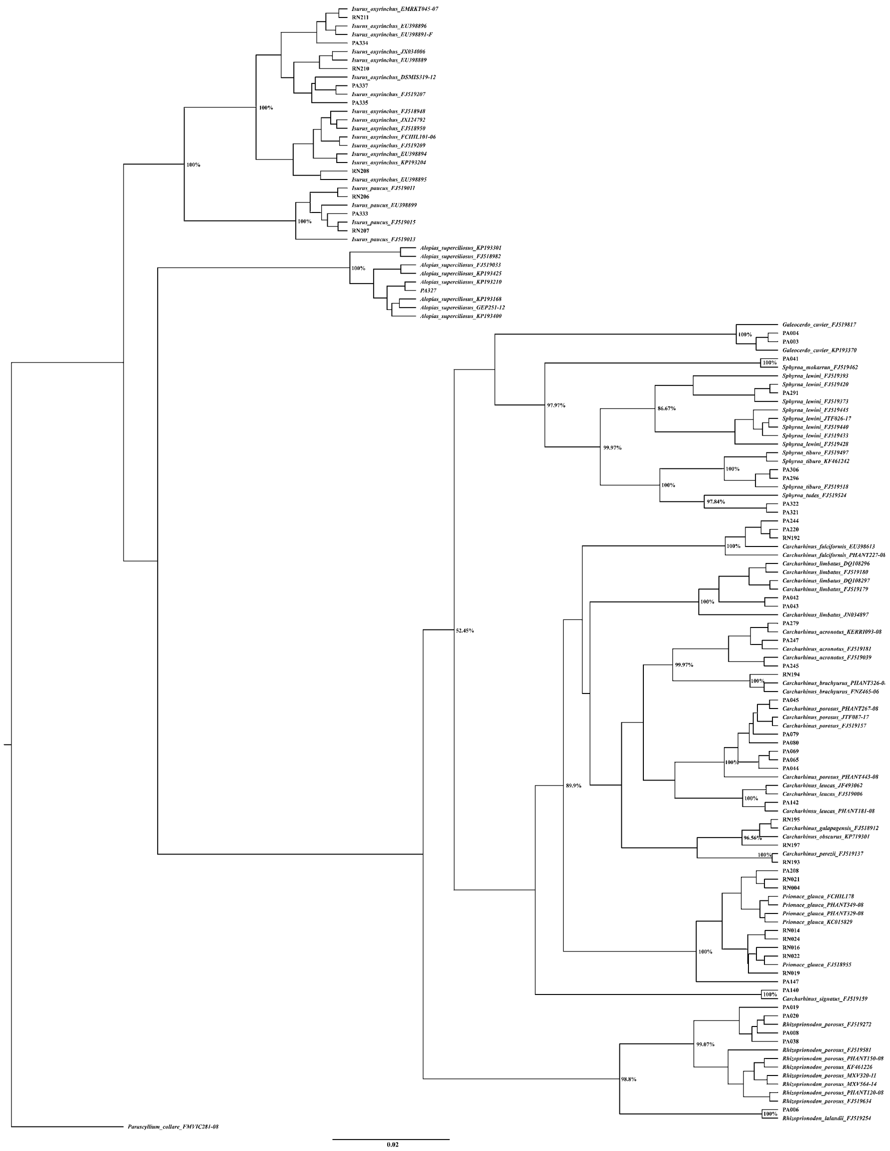

Fig. 3 Bayesian phylogenetic inference tree of cytochrome *c* oxidase subunit I (COI) using 132 haplotypes with 652 base pairs of sequences from BOLD, GenBank®, and from this study. Node values indicate the posterior probability of trees using an evolutionary model based on prior probabilities, producing the plausible phylogenetic tree for the given data. PA and RN codes comprise the sequenced individuals from this study

2008). Fishing has been historically high in the northern and southern Atlantic, but it intensified after the 1990s (Amorim et al. 1998; Camhi et al. 2008). Simultaneous exploitation in the South Atlantic by multinational fleets may have also resulted in high levels of underreporting, which, when coupled with species migratory patterns, has interfered with

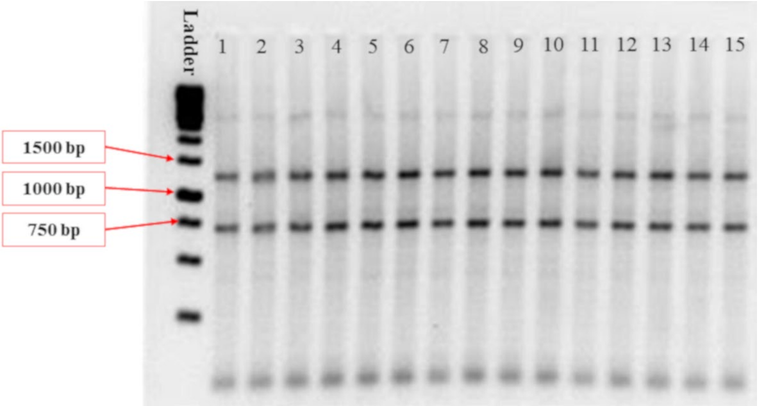

Fig. 4 PCR multiplex electrophoresis agarose gel at 1.5% showing size pattern of fragments identifying fifteen samples as the shortfin mako shark *Isurus oxyrinchus*. Ladder show the size of amplified fragments in base pairs

data collection. Since these fleets changed their target strategies over time owing to market demands, technological advancements, and declines in the abundance of target species (Hazin et al. 2008).

Carcharhinus porosus was the third most commonly identified species. This shark species is a known bycatch of artisanal gillnet fisheries in Brazil's northern region, the center of abundance for this species (Lessa et al. 1999). The increasing fishing pressure, evidence of declines and the lack of management combine to make this species "Critically Endangered" (CR) in Brazil (Lessa et al. 2006). In fact, almost 40% of shark fins belong to species threatened by extinction, and at least a fifth is also protected in Brazil (Table 1; Fig. 2a, b) by Ordinance No. 445 of the Ministry of Environment (MMA), December 17, 2014, which prohibits their capture, transportation, trade, and storage.

Carcharhinus falciformis, *Sphyrna lewini*, *S. mokarran*, and *Alopias superciliosus*, as well as *Isurus oxyrinchus* and *I. paucus* with recent declines from 60–96% worldwide (CITES-CoP18 2019), are also listed in Appendix II of CITES. This includes species not necessarily threatened, but for which trade must be regulated in order to allow their sustainability. Such situation has arisen as a result of inadequate management measures, poor law enforcement and lack of control over the level of international trade, causing declines of over 70% across their range and driving them to extinction in some areas. In other finning evaluations, a high number of CITES-listed and endangered species were also identified (de Mitcheson et al. 2018; Cardeñosa et al. 2018, 2019).

This fact demonstrates the harm that illegal practices, such as finning, causes to the marine environment and shark conservation and management efforts. Even so, the biomass of most large pelagic predators has shown a vigorous decline, and the stock of shark, billfish and tuna cannot support ongoing fishing. Thus, the recovery of these populations, especially for large sharks, toward a sustainable fishery could only be accomplished with a significant reduction in fishing in the South Atlantic (Bornatowski et al. 2018). Otherwise, the removal of large pelagic predators could trigger cascading top-down effects (Pauly et al. 2001; Arancibia and Neira 2005; Sibert et al. 2006; Baeta et al. 2009; Ferretti et al. 2010; Freire and Pauly 2010).

Apart from the identified threatened species, most other species have been classified as non-threatened, such as “Near Threatened” (NT), “Least Concern” (LC) or “Data Deficient” (DD) (Table 1; Fig. 2). According to the IUCN, more than 80% of all Chondrichthyans are in the non-threatened categories, mostly as a result of uncertainty about the true level of extinction risk, as well as the failure to understand the real patterns of threats and risks (Butchart and Bird 2010), all of which causes truly threatened data-deficient species to be neglected by conservation programs (Bland et al. 2015). Determining the true conservation status of these species is essential to developing an accurate picture of global biodiversity and enabling the protection of threatened species. In Brazil, according to Ordinance MMA No. 43, January 31, 2014, species assessed as non-threatened should be prioritized for research and conservation measures. The results of this evaluation demonstrate that several shark species in the non-threatened categories face finning pressure, putting at risk of extinction data-deficient species that could already be threatened.

Miscellaneous carcharhinid shark species, accounting for almost 18% of the total seized, were identified as *C. acronotus*, *C. brachyurus*, *C. limbatus*, *C. perezi*, *C. porosus*, *C. signatus*, *C. galapagensis* and/or *C. obscurus*, *G. cuvier*, *R. lalandii*, and *R. porosus*. Many of these species have been previously identified in the international fin trade (Clarke et al. 2006a, b; Steinke et al. 2017; Feitosa et al. 2018; Fields et al. 2018). Among these species, *C. galapagensis* and *C. obscurus* cannot be distinguished by only a single-locus approach since they show a pervasive mitochondrial admixture whereby they could only be distinguished by Single Nucleotide Polymorphisms (SNPs) (Corrigan et al. 2017). Thus, with the COI gene, both species were clustered in the same BIN in BOLD and within the same clade in the phylogenetic reconstruction.

Among the Carcharhinidae family, *C. falciformis* is a common species in purse-seine and pelagic longline fisheries. Currently, their population size has plummeted in the Atlantic Ocean (Barreto et al. 2016; Cortés et al. 2010) by their highly valuable fins. Consequently, in the Atlantic Ocean, the capture and commercialization of this species are prohibited (ICCAT 2011). Globally, this shark species has been recently listed in Appendix II of CITES, and in spite of all these facts, *C. falciformis* is one of the most traded species in Hong Kong (Clarke et al. 2006a, b; Fields et al. 2018). Obviously, we evidenced here that this and other species listed in CITES continue to be illegally captured, landed and traded in Brazil (Almerón-Souza et al. 2018; Barreto et al. 2016).

Other endangered shark species, some also listed in Appendix II of CITES, such as hammerhead sharks (e.g., *S. lewini*, *S. mokarran*, *S. tiburo*, and *S. tudes*) and the bigeye thresher shark, *Alopias superciliosus*, were also identified in lower frequencies. According to the CITES Trade Database (CITES 2018), Hong Kong is the main CITES-listed species importer. Fins reportedly traded in 2015 for the three large hammerhead sharks, *S. lewini* (40.8%) by weight, *S. zygaena*, (35%), and *S. mokarran* (16.8%), account for the majority of fins reportedly traded (Cardeñosa et al. 2018). Regarding *A. superciliosus*, the major threat to thresher sharks is fishery-related mortality, as they are taken mainly for their highly valuable fins. Besides our identification, the species was previously detected in the Hong Kong international fin trade (Clarke et al. 2006a, b; Cardenõsa et al. 2018; Fields et al. 2018), and estimations suggest a huge underreporting of these species (Clarke et al. 2006a).

The most recent and significant genetic assessment of international fin trade of Hong Kong was conducted by Fields et al. (2018) who identified at least 76 different species from 4800 sampled fins. Most traded fins corresponded to the orders Carcharhiniformes and Lamniformes, as also revealed by this study and by other evaluations (Clarke et al. 2006a, b; Steinke et al. 2017). In their results, Fields et al. (2018) reported that almost half of the species were from the family Carcharhinidae, and one-third was categorized as critically endangered. Moreover, in our results, most fins belonged to the Carcharhinidae and Lamnidae families, accounting for 64.8% and 29.8%, respectively. However, almost 40% of the identified species in our evaluation were assessed as EN or VU. The rising consumption of threatened species associated with the high demand for luxury wildlife products, such as the shark fin soup, is putting a heavy pressure on several species increasingly unable to sustain the current levels of exploitation and trade. Lack of proper management and science-based decision making, as well as poor law enforcement, influential lobbying interests from fishing companies, and weak, or no, regulation in the product's source countries and/or demand centers all raise the risk of extinction of exploited species (de Mitcheson et al. 2018).

Hong Kong has been traditionally a major consumer of shark fins, as well as the principal international hub for about 50% of globally traded dried shark fins, much of which is re-exported to mainland China (Clarke et al. 2006a, b; de Mitcheson et al. 2018; Shea and To 2017). Imports of dried shark fins into Hong Kong grew six-fold between 1960 and 2002, declining to one-third of 1960 levels by 2016 to almost 6000 tons (de Mitcheson et al. 2018). Hong Kong government data show a drop in shark fin re-exports to China of nearly 80% in the last decade. The overall consumption outside mainland China has not declined significantly, nor has commercial fishing pressure on sharks (Clarke 2004; Clarke et al. 2007; Steinke et al. 2017; Fields et al. 2018; Cardeñosa et al. 2018). Furthermore, trade in Hong Kong appears to be declining, possibly from consumer and retailer educational campaigns around shark fin consumption, and evidence of this also comes from other trade hubs, such as Macau and Vietnam (Fabinyi et al. 2012).

On the other hand, Brazil is one of the many worldwide fin exporters to Hong Kong, and since 2004, more than 83 tons of fins were seized in Brazil and companies summarily charged with illegal finning (Barreto et al. 2017). Of the 20 identified shark species studied, the outcome of our genetic evaluation shows that most are threatened with extinction. Some are even CITES-listed as internationally trade-regulated, and a fifth is protected. These results represent the first DNA-based assessment of finning seizures from Brazil and demonstrate that IUU fisheries have an impact on endangered shark species when coupled with illegal practices like finning, raising, in turn, concerns about the lack of fishery surveillance in developing countries which continue to supply the international shark fin trade. In 2016, Brazil was ranked as the 10th country for reported shark landings, accounting for almost 18,700 tons, while it is the 11th shark fin exporter (FAO 2018).

Brazil has its own set of regulations for shark landings and transportation on board, namely requiring that all fins be attached to the shark during landings (MPA/MMA No. 14, November 26, 2012). Despite this, no nationally standardized fisheries data collection system has been available since 2007 in Brazil, and total catches may be almost two-fold those reported to FAO (Freire et al. 2015). The absence of fisheries species-specific catch data hinders efforts to monitor the capture of threatened and protected species, to implement fisheries good practices, and to maintain sustainable stocks (Di Dario et al. 2015). According to Barreto et al. (2017), only a few companies are authorized to export shark fins. However, no reliable information specifies which species or quantities are being traded. In addition, at least fifteen raids to disrupt illegal fin trading in Brazil were carried out between 2004 and 2013. Therefore, owing to the absence of a reliable standardized fisheries species-specific collection data system and the widespread presence of IUU fisheries, we can conclude that Brazil is currently one of the major hotspots for the international shark fin black market.

The Brazilian fisheries management system is obsolete and cannot promote sustainable fisheries or prevent overexploitation in the absence of shared strategic management in the southwestern Atlantic (Griffith 2008; Symes 2006). Furthermore, fisheries legislation is complex, conservation efforts are weak, as are surveillance and law enforcement (Cochrane and Doulman 2005; Pinheiro et al. 2015; Fiedler et al. 2017). Therefore, an efficient fisheries management policy should not be based on models that do not account for the complex effects of the interaction among the marine ecosystem, capture quotas, and the exploited species. Instead, they should be based on scientific data for mid- and long-term sustainable management. This will require an investment in scientific research, new technologies and the application of viable public policies, conservation plans and fisheries management (Walters 2007; Fromentin et al. 2014; Fiedler et al. 2017) to the exclusion of fisheries lobbying interests.

To conclude, the results demonstrate the efficiency of DNA-based identification methodologies and evidence the disastrous impact of finning on marine ecosystems and developing countries. Endangered and protected species are illegally traded and demand new tools for proper surveillance, highlighting the importance of public–private partnerships to encourage the implementation of DNA-based technology for effective law enforcement and monitoring of wildlife trade, as well as the conservation of protected and endangered species and the detection of commercial fraud.

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