



DNA Barcoding for the Assessment of the Taxonomy and Conservation Status of the Fish Bycatch of the Northern Brazilian Shrimp Trawl Fishery

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Trawling is a controversial fishing method due to the perceived lack of selectivity of the net and the resulting capture of a large quantity and diversity of non-target species. Here, we used DNA barcode methods to identify the composition of the bycatch produced by the trawl fishery of the Brazilian North coast. A total of 182 species belonging to 18 orders and 62 families were captured, including 17 species under some degree of threat in the wild according to the IUCN Red List of Threatened Species (IUCN). These results highlight the impact on the marine biodiversity of northern Brazil caused by the bycatch of small-scale industrial and unregulated fishery operations, and support the application of DNA-based methods for the identification of the bycatch species taken by data-poor fisheries, as a powerful tool for the improvement of the quality of fishery catch statistics and more precise bycatch records.

Keywords: conservation, DNA-barcode, genetic identification, bycatch composition, threatened species

INTRODUCTION

Fisheries that trawl for shrimp in tropical regions take an extremely diverse bycatch fauna, but generally provide few historical or biological data for the quantitative assessment of stocks. The constant mortality of bycatch species caused by the fishery industry has a serious impact on the ecosystem and on the ongoing decline of the populations of many marine vertebrate species (Lewison et al., 2004), a pressure that threatens the stability of fish stocks through the overexploitation of many species (Pauly et al., 1998, 2002; Harrington et al., 2006; Worm et al., 2006). Bycatch commonly consists of (a) non-target species that are kept to be eaten or sold, and (b) discards, which are a subset of organisms that represent a wasted fishery resource, and thus attract significant public awareness, especially when including endangered, threatened, or protected species (Gray and Kennelly, 2018). Many groups of species are highly susceptible to bottom trawl fisheries, in particular, commercial shrimping, which unavoidably harvest bottom-dwelling species, such as elasmobranchs and catfishes. Many of these species are endangered or vulnerable, such as

the Largetooth Sawfish *Pristis pristis*, the Long-tail stingray *Hypanus longus*, and the Gillbacker sea catfish *Sciades parkeri* (Chee, 1996; Willems et al., 2016; Schmid and Giarrizzo, 2017).

The poor management of non-target stocks compromises efforts to guarantee sustainability, and may result in a substantial, undocumented removal of biomass. On the northern coast of Brazil, industrial shrimp trawling fleets operate over the continental shelf, leading to the bycatch of a range of fish species from the region's marine fauna, which is still poorly documented (Marceniuk et al., 2019). The recent discovery of a reef system off the mouth of the Amazon River has greatly increased our understanding of the region's marine biodiversity (Moura et al., 2016). The discovery of the occurrence of 73 species of the Elasmobranchii and Actinopterygii in this region reinforces that idea that it does not function simply as a migratory corridor between the Brazilian and Caribbean geographic provinces, but rather that it provides a subsistence habitat for many of these fish, which highlights the functional importance of this Amazonian reef system (Moura et al., 2016). Although a recent study (Marceniuk et al., 2019) provided the first checklist of the bony fish caught by the industrial shrimp trawling operations off the northern coast of Brazil, many of the species (over 15% of the total diversity) were identified through taxonomic keys and photographs taken by onboard observers with no specimens being collected or deposited in museum collections. These authors also overlooked the impact of the trawling operations on the Chondrichthyes, a group of fish that of threatened shark and ray species that are marketed unregulated in Brazil.

Fishery management can be hampered by a lack of reliable species identification (Bornatowski et al., 2014). The traditional morphological approach to species identification has recently been reinforced by the inclusion of DNA-based approaches, such as DNA barcoding. This technique is based on the diversity of the ~650 bp region of the mitochondrial Cytochrome C Oxidase I (COI) gene, which has been used widely to improve the accuracy of fish species identification (Hebert et al., 2003b; Ratnasingham and Hebert, 2007). The DNA barcoding approach has progressed rapidly, and is now widely used, due primarily to its low cost, combined with the need to address critical conservation issues and fishery management questions. In the present study, a DNA barcoding library was compiled in order to investigate the composition of fish species caught as bycatch by the shrimp trawling fleet operating off the North Coast of Brazil, in order to provide a comprehensive update of the region's fish fauna (Chondrichthyes and Teleostei), with comments on the conservation of the species and the unrecognized diversity. The bycatch species were identified at both morphological and molecular levels, generating new data on the fish biodiversity of the study region.

MATERIALS AND METHODS

General Characteristics of the Study Area

The northern coast of Brazil encompasses the region between the Oiapoque River estuary (4°16' N), in Amapá state, and

the Parnaíba River Delta (3°S), in Maranhão state (Figure 1; Isaac and Barthem, 1995; Ekau and Knoppers, 1999). This region has an irregular coastline, being mostly straight in Amapá, but highly indented in Pará and Maranhão, due to the presence of numerous estuaries, interspersed with tidal plains and the largest continuous tract of mangroves on the planet, with a total area of approximately 8,900 km² (Lara and Dittmar, 1999). This region is dominated by the Amazon and Orinoco rivers (Briggs, 1974; Floeter et al., 2008; Briggs and Bowen, 2012), whose total mean annual freshwater discharge into the Atlantic Ocean is 120 × 10³ m³/s to 300 × 10³ m³/s (Ward et al., 2015). This combined discharge results in a plume of low salinity water with a high sediment concentration at the surface, and a muddy-bottomed continental platform stretching approximately 2,500 km of the coast off northwestern South America (Collette and Rützler, 1977; Meade et al., 1985), constituting one of the largest freshwater barrier zones in the Western Atlantic, which is known to form a biogeographic barrier between the coral-dwelling fish faunas of the Caribbean and Brazilian provinces (Briggs, 1995; Floeter and Gasparini, 2000; Rocha, 2003).

Sampling and Morphological Identification

The bycatch specimens were collected during the shrimp trawling operations by onboard technicians of the National Center for the Research and Conservation of the Marine Biodiversity of the North Coast of Brazil (CEPNOR), based in Belém, Pará. This monitoring operation accompanied 229 trawls between July 2015 and May 2017 during bimonthly excursions with a mean duration of 15 days. Were used vessels of the industrial shrimping fleet operating on the North Brazilian Coast, between Marajó Bay (08° S, 47.85° W) and the mouth of the Oiapoque River (4.7° N, 51.17°W), at an approximate mean distance of 80 km from the coast, at depths of 40–80 m. This fleet uses vessel-shaped outboard bottom trawl nets, with a 30 mm × 21 mm mesh size (Figure 1). Specimen collection was authorized by ICMBIO/MMA (permit 69486-1), a branch of the Brazilian Environment Ministry (MMA).

A random subsample of ca. 90 kg of the catch of each monitored trawl was frozen at −18°C and sent to the fish collection of the Aquatic Ecology Group (GEA), at the Federal University of Pará in Belém, Brazil. In the laboratory, the fish species were identified to the lowest possible taxonomic level based on Figueiredo and Menezes (1978, 1980a,b), Carvalho-Filho (1999), and Carpenter et al. (2002a,b), with the scientific names, authority, and year of description following Fricke et al. (2020). The common names used by crew members to refer to the fish were not considered, as they could bias the identification. A small fragment of muscle tissue was taken from between one and eight specimens per species and stored in 96% ethanol for the DNA barcode analysis. The voucher specimens were fixed in a 10% formalin solution in the field, and then stored in a 70% ethanol solution in the laboratory, where they were deposited in the fish collection of the GEA (the GEA catalog is available in Supplementary Table S3). All the material is available to interested researchers on request.

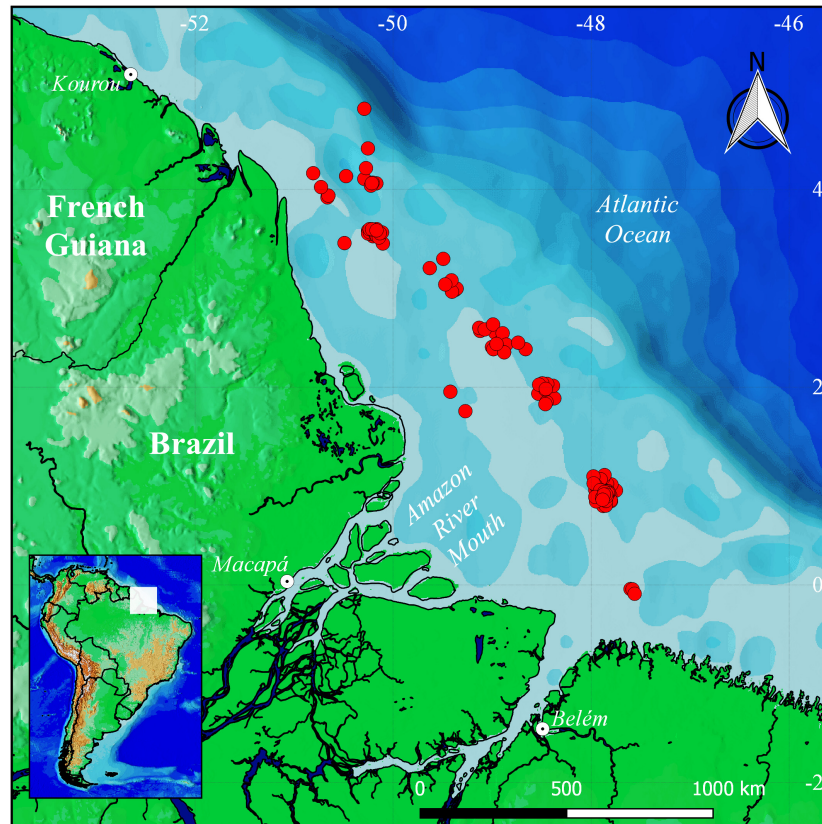


FIGURE 1 | The northern coast of Brazil, showing the location of the sampling sites at which the fish specimens were obtained from the bycatch of shrimp trawling. Map created using QGIS 3.4.0 (Geographic Information System, Open Source Geospatial Foundation) software (<https://qgis.org/en/site/>).

DNA Extraction, Amplification, and Sequencing of the Genomic Regions

We extracted DNA from the muscle tissue or other parts of the fish using the Wizard Genomic Purification DNA kit (Promega Corporation, Madison, WI, United States), following the instructions provided by the manufacturer. All tissue samples were stored in 95% ethyl alcohol at -20°C and deposited in the fish collection of the GEA at the Federal University of Pará, in Belém (**Supplementary Table S1**). Approximately 650 base pairs (bps) of the COI gene, which was used as the DNA barcode, was amplified via Polymerase Chain Reaction (PCR), using the following primers, described by Ward et al. (2005): FishF1 5'-TCAACCAACCACAAAGACATTGGCAC-3' and FishR1 5'-TAGACTTCTGGGTGGCCAAAGAATCA-3'. The samples were amplified in a final volume of 25 μL , containing 4 μL of dNTP (Deoxyribonucleotide Triphosphate; 1.25 mM), 2.5 μL of 10 \times buffer solution, 1 μL of MgCl_2 (25 mM), 0.25 μL of each primer (200 ng/ μL), 1–1.5 μL of genomic DNA (100 ng/ μL), 1 U of Taq DNA polymerase (5 U/ μL), and purified water to complete the final reaction volume. The amplification conditions were: initial denaturing at 95°C for 5 min; 35 cycles of denaturing at 94°C for 40 s, annealing at 55°C for 35 s, and extension at 72°C for 1 min, with a final extension step at 72°C for 5 min. The PCR products were purified using the ExoSAP-IT enzyme and were

sequenced in an ABI 3500 automatic sequencer using the Big-Dye Terminator Cycle Sequencing Kit (Applied Biosystems).

DNA-Based Species Identification

As described above, morphological identification was conducted based on specific literature. In addition, we used a DNA-based species identification methods were employed for the taxonomic identification of the specimens. The sequences were aligned automatically using the ClustalW software (Thompson et al., 1994), implemented in Bioedit 7.0.9 (Hall, 1999). Following the morphological identification, the sequences of each specimen were compared with those of fish species obtained from the GenBank¹ and BOLD² databases.

The Barcode of Life Data Systems (BOLDSystems) tool (Ratnasingham and Hebert, 2007) was used to assign existing Barcode Index Numbers (BINs) to all the sequences of the specimens analyzed in the present study. The sequence files, institutional data, taxonomic information, images, and collection numbers were all submitted to this platform (see **Supplementary Material**). This permitted the calculation of the number of clusters, to determine the number of BINs, the levels of divergence within and between species, genera, and families, the

¹<http://ncbi.nlm.nih.gov/>

²<http://BOLDsystems.org/>

TABLE 1 | Fish species from the North Coast of Brazil taken as by catch by shrimp trawling vessels.

Order	Family	Genus	Species	Geographic range	Conservation Status		
Carcharhiniformes	Triakidae	<i>Mustelus</i>	<i>Mustelus canis</i>	Southwest and western central Atlantic	NT		
			<i>Mustelus higmani</i>	Western central Atlantic	LC		
			<i>Mustelus</i> sp.	–	–		
	Carcharhinidae	<i>Carcharhinus</i>	<i>Carcharhinus acronotus</i>	Western Central and South America	NT		
			<i>Carcharhinus leucas</i>	Circuntropical	NT		
			<i>Carcharhinus falciformis</i>	Circuntropical	VU		
		<i>Rhizoprionodon</i>	<i>Rhizoprionodon lalandii</i>	Southwest and western central Atlantic	DD		
			<i>Rhizoprionodon porosus</i>	Western Atlantic	LC		
	Sphyrnidae	<i>Sphyrna</i>	<i>Sphyrna lewini</i>	Circuntropical	CR		
			<i>Sphyrna mokarran</i>	Circuntropical	CR		
<i>Sphyrna tiburo</i>			Western Atlantic and Eastern Pacific	LC			
Torpediniformes	Narcinidae	<i>Narcine</i>	<i>Narcine brasiliensis</i>	Southwest Atlantic	DD		
Rhinopristiformes	Rhinobatidae	<i>Pseudobatos</i>	<i>Pseudobatos percellens</i>	Western central Atlantic	NT		
			<i>Pseudobatos horkelii</i>	Southwest Atlantic	CR		
Myliobatiformes	Dasyatidae	<i>Hypanus</i>	<i>Hypanus americanus</i>	Western Atlantic	DD		
			<i>Hypanus guttatus</i>	Western Atlantic	DD		
			<i>Hypanus</i> sp.	–	–		
	Gymnuridae	<i>Gymnura</i>	<i>Gymnura</i> sp.	–	–		
	Myliobatidae	<i>Aetobatus</i>	<i>Aetobatus narinari</i>	Circuntropical	NT		
<i>Rhinoptera</i>		<i>Rhinoptera bonasus</i>	Western Atlantic	NT			
Anguilliformes	Muraenidae	<i>Gymnothorax</i>	<i>Gymnothorax vicinus</i>	Western and Eastern Atlantic	LC		
			<i>Gymnothorax nigromarginatus</i>	Northwest and Central Atlantic	LC		
			<i>Gymnothorax ocellatus</i>	Western Atlantic	LC		
	Chlopsidae	<i>Kaupichthys</i>	<i>Kaupichthys hyoporoides</i>	Western Atlantic and Western Indian Oceans	LC		
	Muraenesocidae	<i>Cynoponticus</i>	<i>Cynoponticus</i> sp.	–	–		
	Ophichthidae	<i>Echiophis</i>	<i>Echiophis punctifer</i>	Western and Eastern Atlantic	LC		
			<i>Ophichthus</i>	<i>Ophichthus cylindroideus</i>	Southwest and western central Atlantic	LC	
		<i>Ophichthus</i>	<i>Ophichthus ophis</i>	Western and Eastern Atlantic	LC		
			<i>Ophichthus</i> sp.	–	–		
	Congridae	<i>Paraconger</i>	<i>Paraconger notialis</i>	Eastern Atlantic	LC		
		<i>Rhynchoconger</i>	<i>Rhynchoconger</i> sp.	–	–		
	Clupeiformes	Pristigasteridae	<i>Odontognathus</i>	<i>Odontognathus mucronatus</i>	Southwest Atlantic	LC	
				<i>Pellona</i>	<i>Pellona harroweri</i>	Southwest and western central Atlantic	LC
<i>Pellona flavipinnis</i>					Coastal Rivers of South America	NE	
Engraulidae		<i>Anchoa</i>	<i>Anchoa spinifer</i>	Western Atlantic and Eastern Pacific	LC		
			<i>Anchoviella</i>	<i>Anchoviella lepidentostole</i>	Southwest Atlantic	LC	
		<i>Cetengraulis</i>	<i>Cetengraulis edentulus</i>	Western Atlantic	LC		
		<i>Lycengraulis</i>	<i>Lycengraulis grossidens</i>	Southwest and western central Atlantic	LC		
Clupeidae		<i>Sardinella</i>	<i>Sardinella brasiliensis</i>	Western Atlantic	NE		
		<i>Rhinosardinia</i>	<i>Rhinosardinia amazonica</i>	Amazon river system	LC		
Siluriformes		Ariidae	<i>Bagre</i>	<i>Bagre marinus</i>	Western Atlantic	LC	
	<i>Bagre bagre</i>			Southwest and western central Atlantic	LC		
	<i>Aspistor</i>			<i>Aspistor quadriscutis</i>	Southwest and western central Atlantic	LC	
	<i>Notarius</i>			<i>Notarius grandicassis</i>	Southwest Atlantic	LC	
	<i>Cathorops</i>			<i>Cathorops agassizii</i>	Coastal Rivers of South America	NE	
	<i>Sciades</i>			<i>Sciades herzbergii</i>	Southwest Atlantic	LC	
	<i>Amphiarus</i>			<i>Amphiarus rugispinis</i>	Western central Atlantic	LC	
	<i>Amphiarus phrygiatus</i>			Western central Atlantic	LC		
	Pimelodidae			<i>Brachyplatystoma</i>	<i>Brachyplatystoma vaillantii</i>	Coastal Rivers of South America	NE
	Aulopiformes			Synodontidae	<i>Saurida</i>	<i>Saurida caribbaea</i>	Western Atlantic
<i>Synodus</i>		<i>Synodus bondi</i>	Western central Atlantic		LC		
		<i>Synodus foetens</i>	Northwest and Central Atlantic		LC		

(Continued)

TABLE 1 | Continued

Order	Family	Genus	Species	Geographic range	Conservation Status	
Holocentriformes	Holocentridae	<i>Holocentrus</i>	<i>Holocentrus adscensionis</i>	Western and Eastern Atlantic	LC	
Batrachoidiformes	Batrachoididae	<i>Amphichthys</i>	<i>Amphichthys cryptocentrus</i>	North coast of South America	LC	
		<i>Batrachoides</i>	<i>Batrachoides surinamensis</i>	Southwest and western central Atlantic	LC	
		<i>Porichthys</i>	<i>Porichthys oculo-frenum</i>	Coast of Venezuela and Amapá	DD	
			<i>Porichthys pauciradiatus</i>	Western central Atlantic	LC	
		<i>Thalassophryne</i>	<i>Thalassophryne maculosa</i>	Western central Atlantic	LC	
Mugiliformes	Mugilidae	<i>Mugil</i>	<i>Mugil curema</i>	Western Atlantic, Eastern Atlantic and Eastern Pacific	LC	
			<i>Mugil hospes</i>	Western Atlantic and Eastern Pacific	LC	
Beloniformes	Exocoetidae	<i>Cheilopogon</i>	<i>Cheilopogon cyanopterus</i>	Circuntropical	LC	
Carangiformes	Rachycentridae	<i>Rachycentron</i>	<i>Rachycentron canadum</i>	Circuntropical	LC	
	Echeneidae	<i>Echeneis</i>	<i>Echeneis naucrates</i>	Circuntropical	LC	
		<i>Remora</i>	<i>Remora australis</i>	Circuntropical	LC	
	Carangidae	<i>Caranx</i>	<i>Caranx crysos</i>	Western and Eastern Atlantic and Mediterranean	LC	
			<i>Caranx hippos</i>	Western and Eastern Atlantic	LC	
			<i>Caranx latus</i>	Western and Eastern Atlantic	LC	
			<i>Chloroscombrus</i>	<i>Chloroscombrus chrysurus</i>	Western and Eastern Atlantic	LC
			<i>Decapterus</i>	<i>Decapterus tabl</i>	Circuntropical	LC
			<i>Hemicaranx</i>	<i>Hemicaranx amblyrhynchus</i>	Western Atlantic	LC
			<i>Oligoplites</i>	<i>Oligoplites palometa</i>	Southwest and western central Atlantic	LC
				<i>Oligoplites saliens</i>	Western Atlantic	LC
			<i>Selar</i>	<i>Selar crumenophthalmus</i>	Circuntropical	LC
			<i>Selene</i>	<i>Selene brownii</i>	Western Atlantic	LC
				<i>Selene setapinnis</i>	Western Atlantic	LC
				<i>Selene vomer</i>	Western Atlantic	LC
				<i>Seriola</i>	<i>Seriola rivoliana</i>	Circuntropical
		<i>Trachurus</i>	<i>Trachurus lathami</i>	Western Atlantic	LC	
Istiophoriformes	Sphyracidae	<i>Sphyracna</i>	<i>Sphyracna barracuda</i>	Circuntropical	LC	
			<i>Sphyracna guachancho</i>	Western and Eastern Atlantic	LC	
Pleuronectiformes	Paralichthyidae	<i>Cyclosetta</i>	<i>Cyclosetta chittendeni</i>	Western Atlantic	LC	
		<i>Citharichthys</i>	<i>Citharichthys spilopterus</i>	Western Atlantic	LC	
		<i>Syacium</i>	<i>Syacium gunteri</i>	Northwest and central Atlantic	LC	
	Bothidae	<i>Bothus</i>	<i>Bothus ocellatus</i>	Western Atlantic	LC	
		<i>Trichopsetta</i>	<i>Trichopsetta ventralis</i>	Western Atlantic North	LC	
	Achiridae	<i>Achirus</i>	<i>Achirus achirus</i>	Southwest Atlantic	LC	
		<i>Apionichthys</i>	<i>Apionichthys dumerili</i>	Southwest Atlantic	LC	
		<i>Gymnachirus</i>	<i>Gymnachirus nudus</i>	Western Atlantic	LC	
		<i>Trinectes</i>	<i>Trinectes paulistanus</i>	Southwest and western central Atlantic	LC	
			<i>Trinectes micropthalmus</i>	Southwest and western central Atlantic	LC	
	Cynoglossidae	<i>Symphurus</i>	<i>Symphurus plagiusa</i>	Western Atlantic North	LC	
			<i>Symphurus rhytisma</i>	Bahamas, Belize, Curaçao, and Espírito Santo	LC	
			<i>Symphurus tessellatus</i>	Western Atlantic	LC	
Scorpaeniformes	Dactylopteridae	<i>Dactylopterus</i>	<i>Dactylopterus volitans</i>	Western and Eastern Atlantic	LC	
Gasterosteiformes	Fistulariidae	<i>Fistularia</i>	<i>Fistularia petimba</i>	Circuntropical	LC	
Scombriformes	Trichiuridae	<i>Trichiurus</i>	<i>Trichiurus lepturus</i>	Circuntropical	LC	
	Scombridae	<i>Auxis</i>	<i>Auxis rochei</i>	Circuntropical	LC	
		<i>Scomberomorus</i>	<i>Scomberomorus brasiliensis</i>	Southwest and western central Atlantic	LC	
			<i>Scomberomorus cavalla</i>	Western Atlantic	LC	
	<i>Stromateidae</i>	<i>Peprius</i>	<i>Peprius paru</i>	Western Atlantic	LC	
Labriformes	Labridae	<i>Halichoeres</i>	<i>Halichoeres</i> sp.	–	–	
Perciformes	Centropomidae	<i>Centropomus</i>	<i>Centropomus undecimalis</i>	Western Atlantic	LC	
	Gerreidae	<i>Diapterus</i>	<i>Diapterus auratus</i>	Western Atlantic	LC	
			<i>Diapterus rhombeus</i>	Western Atlantic	LC	
		<i>Eucinostomus</i>	<i>Eucinostomus argenteus</i>	Western Atlantic	LC	

(Continued)

TABLE 1 | Continued

Order	Family	Genus	Species	Geographic range	Conservation Status
			<i>Eucinostomus melanopterus</i>	Western and Eastern Atlantic	LC
	Mullidae	<i>Pseudupeneus</i>	<i>Pseudupeneus maculatus</i>	Western Atlantic	LC
		<i>Upeneus</i>	<i>Upeneus parvus</i>	Western Atlantic	LC
	Epinephelidae	<i>Alphestes</i>	<i>Alphestes afer</i>	Southwest and western central, Eastern Atlantic	LC
		<i>Cephalopholis</i>	<i>Cephalopholis fulva</i>	Western Atlantic	LC
		<i>Dermatolepis</i>	<i>Dermatolepis inermis</i>	Western Atlantic	NT
		<i>Epinephelus</i>	<i>Epinephelus itajara</i>	Western and Eastern Atlantic	VU
	Serranidae	<i>Paralabrax</i>	<i>Paralabrax dewegeri</i>	Western central Atlantic	LC
		<i>Rypticus</i>	<i>Rypticus randalli</i>	Western Atlantic	LC
			<i>Rypticus saponaceus</i>	Western and Eastern Atlantic	LC
	Priacanthidae	<i>Priacanthus</i>	<i>Priacanthus arenatus</i>	Western and Eastern Atlantic, Mediterranean and Black sea	LC
	Chaetodontidae	<i>Chaetodon</i>	<i>Chaetodon ocellatus</i>	Western Atlantic	LC
	Pomacanthidae	<i>Holacanthus</i>	<i>Holacanthus ciliaris</i>	Western and Eastern Atlantic	LC
			<i>Holacanthus tricolor</i>	Western Atlantic	LC
		<i>Pomacanthus</i>	<i>Pomacanthus paru</i>	Western and Eastern Atlantic	LC
	Malacanthidae	<i>Malacanthus</i>	<i>Malacanthus plumieri</i>	Western and Eastern Atlantic	LC
	Haemulidae	<i>Anisotremus</i>	<i>Anisotremus surinamensis</i>	Western Atlantic	DD
			<i>Anisotremus virginicus</i>	Western Atlantic	LC
		<i>Conodon</i>	<i>Conodon nobilis</i>	Western Atlantic	LC
		<i>Genyatremus</i>	<i>Genyatremus luteus</i>	Southwest and western central Atlantic	NE
		<i>Haemulon</i>	<i>Haemulon aurolineatum</i>	Western Atlantic	LC
			<i>Haemulon parra</i>	Western Atlantic	LC
			<i>Haemulon plumieri</i>	Western Atlantic	LC
			<i>Haemulon steindachneri</i>	Western Atlantic and Eastern Pacific	LC
		<i>Haemulopsis</i>	<i>Haemulopsis corvinaeformis</i>	Southwest and western central Atlantic	LC
		<i>Orthopristis</i>	<i>Orthopristis rubra</i>	Southwest Atlantic	LC
	Lutjanidae	<i>Lutjanus</i>	<i>Lutjanus analis</i>	Western Atlantic	NT
			<i>Lutjanus purpureus</i>	Western Atlantic	VU
			<i>Lutjanus jocu</i>	Western and Eastern Atlantic	DD
			<i>Lutjanus synagris</i>	Western Atlantic	NT
	Polynemidae	<i>Polydactylus</i>	<i>Polydactylus oligodon</i>	Western Atlantic	LC
			<i>Polydactylus virginicus</i>	Western Atlantic	LC
	Sciaenidae	<i>Micropogonias</i>	<i>Micropogonias furnieri</i>	Southwest and western central Atlantic	LC
		<i>Bairdiella</i>	<i>Bairdiella ronchus</i>	Southwest and western central Atlantic	LC
		<i>Ctenosciaena</i>	<i>Ctenosciaena gracilicirrhus</i>	Southwest and western central Atlantic	LC
		<i>Cynoscion</i>	<i>Cynoscion acoupa</i>	Southwest and western central Atlantic	LC
			<i>Cynoscion jamaicensis</i>	Southwest and western central Atlantic	LC
			<i>Cynoscion leiarchus</i>	Southwest Atlantic	LC
			<i>Cynoscion microlepidotus</i>	Southwest Atlantic	LC
			<i>Cynoscion virescens</i>	Southwest and western central Atlantic	LC
		<i>Equetus</i>	<i>Equetus lanceolatus</i>	Western Atlantic	LC
		<i>Isopisthus</i>	<i>Isopisthus parvipinnis</i>	Southwest and western central Atlantic	LC
			<i>Isopisthus</i> sp.	–	–
		<i>Macrodon</i>	<i>Macrodon ancylodon</i>	Southwest Atlantic	LC
		<i>Menticirrhus</i>	<i>Menticirrhus americanus</i>	Western Atlantic	LC
		<i>Nebris</i>	<i>Nebris microps</i>	Southwest and western central Atlantic	LC
		<i>Paralonchurus</i>	<i>Paralonchurus brasiliensis</i>	Southwest and western central Atlantic	LC
		<i>Stellifer</i>	<i>Stellifer microps</i>	Southwest and western central Atlantic	LC
			<i>Stellifer stellifer</i>	Southwest Atlantic	DD
			<i>Stellifer naso</i>	Western central Atlantic	LC
			<i>Stellifer brasiliensis</i>	Southwest Atlantic	NE
		<i>Umbrina</i>	<i>Umbrina canosai</i>	Southwest Atlantic	NE
			<i>Umbrina coroides</i>	Western Atlantic	LC

(Continued)

TABLE 1 | Continued

Order	Family	Genus	Species	Geographic range	Conservation Status
Acanthuriformes	Acanthuridae	<i>Acanthurus</i>	<i>Acanthurus chirurgus</i>	Western Atlantic	LC
Scorpaeniformes	Scorpaenidae	<i>Scorpaena</i>	<i>Scorpaena bergii</i>	Northwest and Central Atlantic	LC
			<i>Scorpaena dispar</i>	Western Atlantic	LC
			<i>Prionotus punctatus</i>	Western Atlantic	LC
Ephippiformes	Ephippidae	<i>Chaetodipterus</i>	<i>Chaetodipterus faber</i>	Western Atlantic	LC
Spariformes	Sparidae	<i>Calamus</i>	<i>Calamus penna</i>	Western Atlantic	LC
Lophiiformes	Atennariidae	<i>Antennarius</i>	<i>Antennarius striatus</i>	Circuntropical	LC
	Ogcocephalidae	<i>Ogcocephalus</i>	<i>Ogcocephalus pumilus</i>	Western central Atlantic	LC
			<i>Ogcocephalus vespertilio</i>	Western central Atlantic	NE
Tetraodontiformes	Ostraciidae	<i>Acanthostracion</i>	<i>Acanthostracion polygonius</i>	Western Atlantic	LC
	Balistidae	<i>Balistes</i>	<i>Balistes capriscus</i>	Western and Eastern Atlantic	VU
			<i>Balistes vetula</i>	Western and Eastern Atlantic	NT
			<i>Aluterus monoceros</i>	Circuntropical	LC
	Tetraodontidae	<i>Canthigaster</i>	<i>Canthigaster figueiredoi</i>	Southwest and western central Atlantic	LC
			<i>Lagocephalus laevigatus</i>	Western and Eastern Atlantic	LC
	<i>Sphoeroides</i>	<i>Sphoeroides greeleyi</i>	Western Atlantic	LC	
		<i>Sphoeroides spengleri</i>	Western Atlantic	LC	
		<i>Sphoeroides testudineus</i>	Western Atlantic	LC	
		<i>Xanthichthys ringens</i>	Western and Eastern Atlantic	LC	
		<i>Colomesus psittacus</i>	Cuba and Southwest Atlantic	LC	
		<i>Chilomycterus antillarum</i>	Southwest and western central Atlantic	LC	
	Diodontidae	<i>Chilomycterus</i>	<i>Chilomycterus reticulatus</i>	Circuntropical	LC
			<i>Chilomycterus spinosus</i>	Western Atlantic	LC

barcode gap, and to construct a Neighbor-Joining (NJ) tree based on the Kimura 2-parameter (K2P) approach (Kimura, 1980), using 1,000 bootstrap pseudoreplicates. This analysis was run in the BOLD Workbench application (version 3.6).

In addition, phylogenetic relationships were analyzed using Bayesian Inference and the Maximum Likelihood approach. The BI approach was run in MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001), while the ML analyses were performed in RAxML 7.2.7 (Stamatakis, 2006). *A priori*, the best nucleotide substitution model was selected by jModeltest (Darrriba et al., 2012). The BI approach had two independent runs, four chains, and 10 million generations, for which one tree was archived every 100 generations, with the first 25% of the trees being discarded as burn-in. The performance of the runs was evaluated using Tracer 1.5 (Rambaut and Drummond, 2009), and the *a posteriori* probability was calculated as the percentage of samples recovered in a clade ($\geq 95\%$ significant support; Huelsenbeck and Ronquist, 2001). The ML analysis was run using the GTRGAMMA model, and the confidence of the branches of the best tree was analyzed in greater depth, based on 1,000 bootstrap replicates.

Species Delimitation Analysis

The DNA barcoding analysis was complemented by performing species-delimitation procedures based on a single locus. These methods have been useful in resolving relationships among groups of species that have cryptic taxa and hence, taxonomic uncertainty (Conte-Grand et al., 2017; Araújo et al., 2019). We

selected three widely used methods to delimit species based on a single locus: the Generalized Mixed Yule Coalescent (GMYC) (Pons et al., 2006), AGBD (Automatic Barcode Gap Discovery) (Puillandre et al., 2012), and BPTP (Bayesian Poisson Tree Processes) methods (Zhang et al., 2013).

For the GMYC approach, we used the topology inferred by MrBayes for single-locus species delimitation. The analyses were performed using the *Splits* package (Ezard et al., 2009) in R 3.5.2 (R Core Team, 2018), and by using a single *temporal* threshold. The bPTP was run on the webserver³ and was based on the best unrooted ML tree identified by RaxML as the input, over 500,000 generations (thinning = 500) and with other default parameters. The AGBD analysis was run online at the <http://www.wabi.snv.jussieu.fr/public/abgd/abgdweb.html> interface, using the *.mega* format matrix of the pairwise K2P distances between the *Sparisoma* specimens. This analysis had a relative gap width of 1.5 and interspecific divergence ranging from 0.001 to 0.1.

RESULTS

Composition and Geographical Ranges

A total of 182 fish species were recognized, representing two subclasses, 26 taxonomic orders, 62 families, and 123 genera (Table 1). These species included 20 elasmobranchs and 162

³<https://species.h-its.org/ptp/>

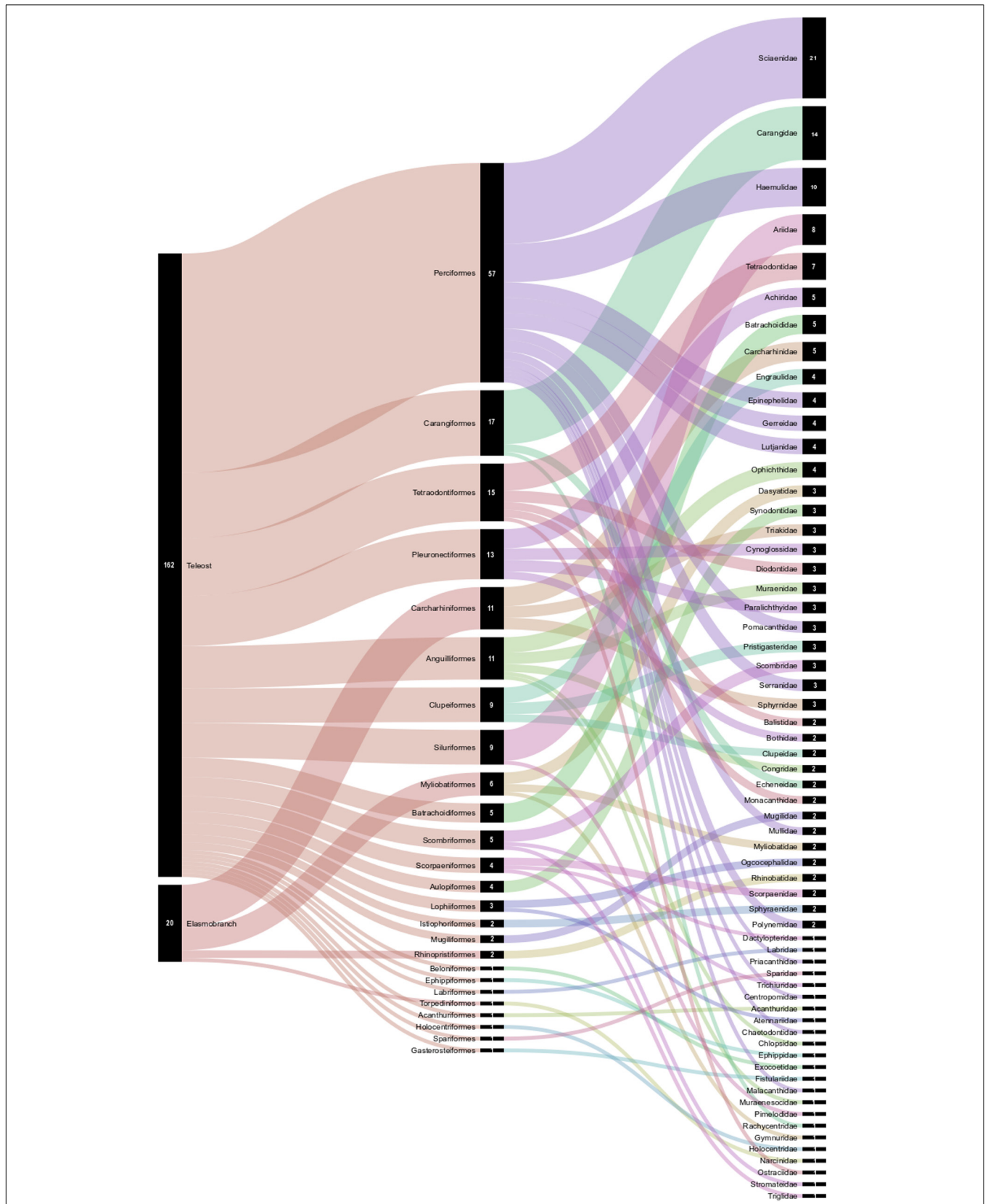


FIGURE 2 | Orders and families of teleosts and elasmobranchs taken as bycatch by the northern Brazilian shrimp trawl fisheries. The numbers in the black columns represents the richness of the taxa in each order and family. The chart was created using the free online software RAWGraphs (<https://rawgraphs.io/>).

teleosts. The most diverse fish families represented in the bycatch were Sciaenidae (21 species), Carangidae (14 species), Haemulidae (10 species), Ariidae (8 species), and Tetradontidae (7 species), while none of the other families were represented by more than six species (Table 1 and Figure 2).

According to Fricke et al. (2020), the composition of the bycatch was dominated by species with a wide geographical distribution: 20 species, including sharks (*Carcharhinus falciformis*, *Carcharhinus leucas*, *Sphyrna mokarran*, and *Sphyrna lewini*), rays (*Aetobatus narinari*), and members of the families Carangidae (*Selar crumenophthalmus*, *Seriola rivoliana*, and *Decapterus tabl*), Scombridae (*Auxis rochei*), Sphyrnaeidae (*Sphyrna barracuda*), Exocoetidae (*Cheilopogon cyanopterus*), Echeneidae (*Remora australis* and *Echeneis naucrates*), and Monacanthidae (*Aluterus monoceros* and *Aluterus scriptus*) have a circumtropical distribution (see Table 1). In addition, 57 species identified are distributed throughout the Western Atlantic Ocean, while another 23 species are amphi-Atlantic, including species also distributed in the Mediterranean, the Black Sea (*Priacanthus arenatus* and *Caranx crysos*), and in the Western Indian Ocean (*Kaupichthys hyoprroides*). We identified some species that also occur in both the Atlantic and the eastern Pacific, including the shark *Sphyrna tiburo* (Sphyrnidae), the hamulid *Haemulon steindachneri*, the mugilids *Mugil hospes* and *Mugil curema*, and the engraulid, *Anchoa spinifer*. Finally, the remainder species have a more restricted distribution, with 27 occurring in the southwestern and western central Atlantic, four in the northwestern and central Atlantic, 11 only in the western central Atlantic, and 15 found only in the southwestern Atlantic, including 13 new occurrences for the North coast of Brazil (Table 1). Species that have an affinity with the freshwater system were also recorded, including *Brachyplatystoma vaillantii*,

Cathorops agassizii, *Rhinosardinia amazonica*, and *Pellona flavipinnis*, which are found in the Amazon basin and coastal rivers of South America.

DNA Barcoding

We were able to obtain usable DNA sequences from 112 of the 182 morphospecies identified during the present study. We obtained 624 bp of the COI gene from 557 fish specimens, and no deletions, insertions, and stop codons were detected in the final alignment. All the sequences were submitted to BOLD Systems, and details of the specimen list and BINs are provided in **Supplementary Table S2**. The Kimura 2-Parameter (K2P) model revealed a hierarchical increase in nucleotide divergence, with a mean divergence of 0.10% between members of the same species, 12.84% between species of the same genus, and 19.10% between species of the same family (Table 2).

The lowest mean interspecific divergence was observed between *Carcharhinus acronotus* and *Carcharhinus falciformis* (4.47%), while the highest value was recovered between *Prionotus punctatus* and *Aluterus monoceros* (23.41%). In general, the nucleotide divergence between congeners was 1.3 times greater than that between specimens of the same species, which confirms the existence and magnitude of the Barcode Gap (Table 2 and Figure 3).

The Bayesian phylogenetic tree (Figure 4) was effective for the separation of all the identified sequences of the different species into 112 clusters, or OTUs (Supplementary Tables S1, S2) supported by high bootstrap values (>90), although the three methods of species delimitation (ABGD, GMYC, and bPTP) indicated the formation of 113 clusters. These methods indicated that the nine specimens identified as *Notarius grandicassis*, based on their external morphology, may

TABLE 2 | Distribution of the K2P nucleotide divergence at each taxonomic level.

	n	Taxa	Comparison	Minimum distance (%)	Mean distance (%)	Maximum distance (%)	Standard error (%)
Within species	518	105	1036	0.00	0.10	3.40	0.00
Within genus	186	16	763	4.47	12.84	19.63	0.00
Within family	294	13	3664	6.29	19.10	26.95	0.00

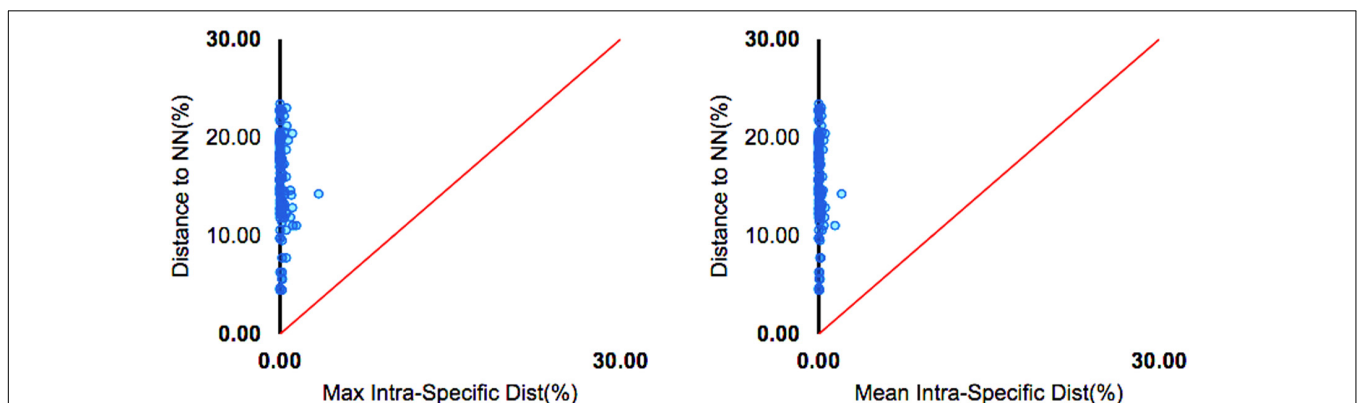
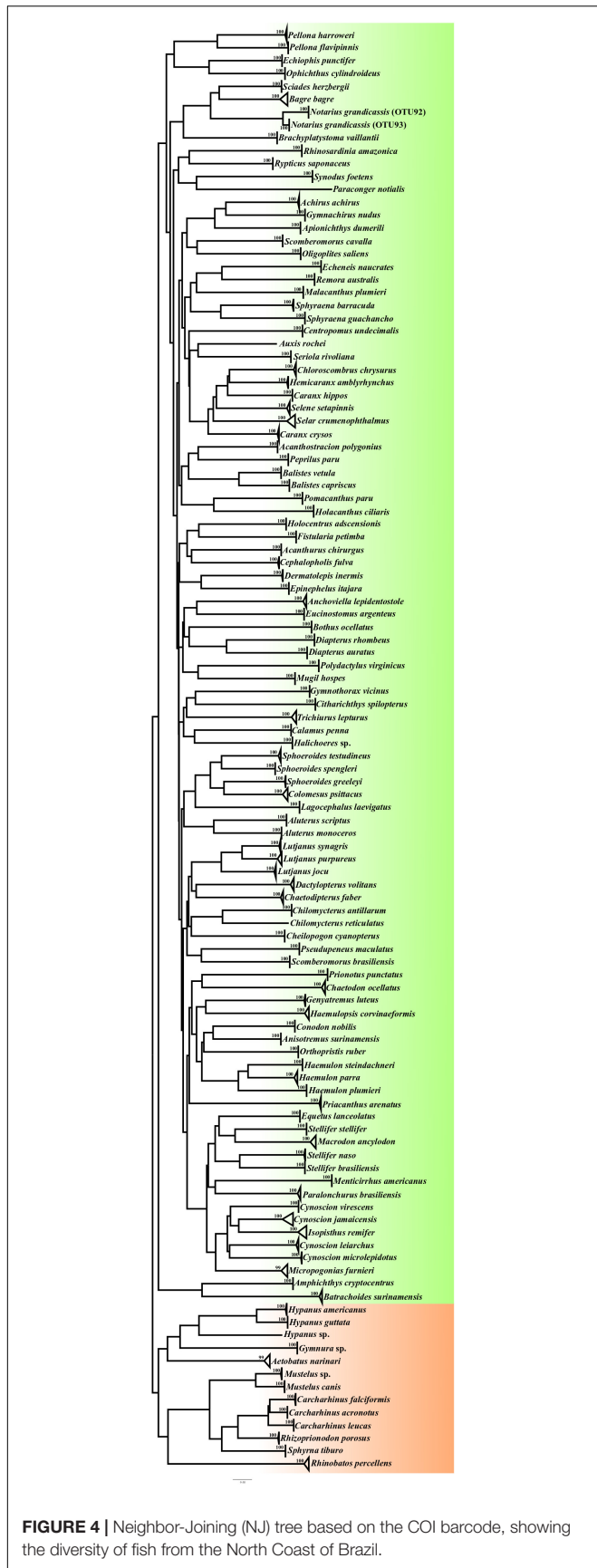


FIGURE 3 | Barcode gap analysis of the COI sequences of the fish taken as bycatch by shrimp trawling vessels off the North Coast of Brazil. The dot plot shows the maximum intra-specific distances vs. the inter-specific (nearest neighbor) distances.



actually belong to two different groups, that is, OTUs 92 and 93 (Supplementary Table S1).

New Occurrences on the Northern Coast of Brazil and in the Western Atlantic

We also recorded species not previously known to occur on the northern coast of Brazil or in the western Atlantic. In particular, we identified *Paraconger notialis* (Congridae), a demersal conger eel, originally assumed to be endemic to the coastal regions of the eastern Atlantic Ocean, between Senegal and Angola (Kanazawa, 1961; Sylla et al., 2016). Our molecular diagnosis was confirmed using the ultrametric topology (Figure 5) generated in the BEAST program, in which our specimens grouped in the *P. notialis* cluster of the sequences from the complete mitochondrial genome of this species downloaded from the BOLD Systems and GenBank databases. All three species delimitation methods (GMYC, ABGD, and bPTP) also confirmed the formation of the *P. notialis* cluster.

Other species known to occur in the Atlantic Ocean were also recorded for the first time on the northern coast of Brazil (see previous records in Table 1). The teleosts *Umbrina canosai* (Sciaenidae), *Dermatolepis inermis* (Serranidae), *Syacium gunteri* (Paralichthyidae), *Synodus foetens* (Synodontidae), *Scorpaena bergii* (Scorpaenidae), *Gymnothorax nigromarginatus* (Muraenidae), *Trichopsetta ventralis* (Bothidae), and *Symphurus rhytisma* (Cynoglossidae). Three elasmobranchs, *Mustelus canis* (Triakidae), *Narcine brasiliensis* (Narcinidae), and *Rhinobatos horkelii* (Rhinobatidae), were also recorded on the northern Brazilian coast for the first time. These species have been recorded previously mainly in the southwestern Atlantic, south of the Brazilian state of Rio de Janeiro, southwards to Argentina (Fricke et al., 2020).

Species Delimitation of the Genus Isopisthus in the Bycatch

The different species delimitation approach produced unexpected results for the COI sequences of the *Isopisthus* (Sciaenidae) bycatch specimens, when compared with those downloaded from BOLDSystems. The GMYC (single threshold), bPTP, and ABGD analyses ($P = 0.035938$) recovered three species-level *Isopisthus* clusters. Cluster 1 was composed of *Isopisthus parvipinnis*, a species found in the Atlantic Ocean (Figure 6). However, cluster 2 was formed by *Isopisthus remifer*, a species known previously to occur in the eastern Pacific Ocean grouped specimens identified as *I. remifer*, which were collected in the Atlantic (collected by C. O. Data available at Boldsystems)⁴.

Conservation Status

The IUCN Red List classifies the 20 elasmobranchs identified in the present study in different categories, including five species as Data Deficient (DD) and three as Least Concern (LC). However, six other species—*Aetobatus narinari* (Aetobatidae), *Carcharhinus acronotus*, *C. leucas* (Carcharhinidae), *Mustelus canis* (Triakidae), *Rhinoptera bonasus* (Rhinopteridae), and

⁴<http://boldsystems.org/>

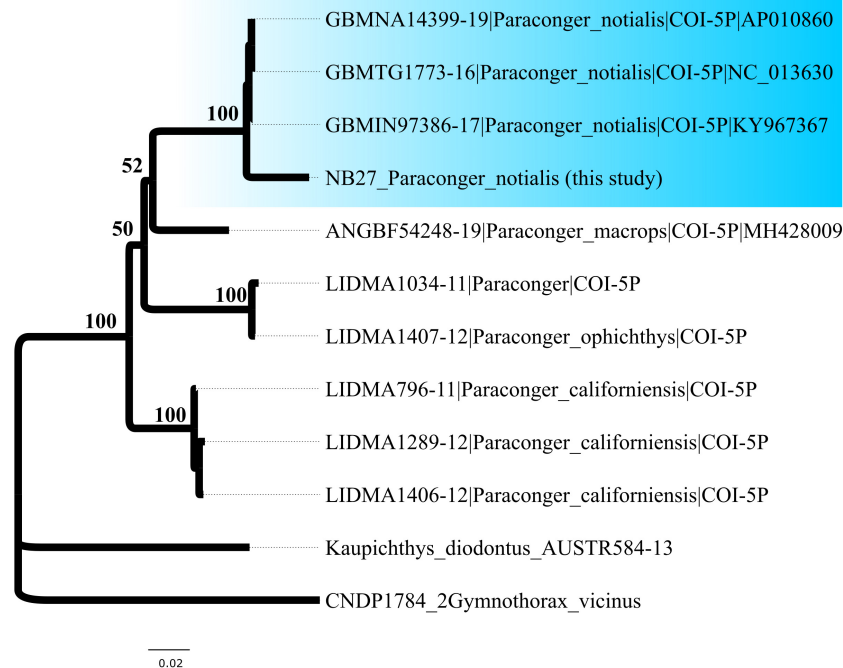


FIGURE 5 | New occurrence of *Paraconger notialis* in the western Atlantic. The topology of the genus *Paraconger* generated in RAXML indicates the position (blue clade) of the specimen NB27 captured at the mouth of the Amazon in the *P. notialis* cluster. The delimitations based on the GMYC, ABGD, and bPTP approaches all confirmed the validity of this cluster.

Pseudobatos percellens (Rhinobatidae)—are Near Threatened (NT). The Silky Shark *Carcharhinus falciformis* is classified as Vulnerable (VU), while *Sphyrna lewini* (Sphyrnidae) and *Pseudobatos horkelii* are Critically Endangered (CR) in the wild (Figure 7).

The vast majority of the teleost species identified in the bycatch in the present study are classified as Least Concern (LC) by the IUCN (Figure 7). However, some epinephelids, lutjanids, and balistids have been assigned to the Near Threatened (*Dermatolepis inermis*, *Lutjanus synagris*, *L. analis*, and *Balistes vetula*) or Vulnerable (*Balistes capriscus*, *Epinephelus itajara*, and *Lutjanus purpureus*) categories.

The Brazilian List of Endangered Fauna (MMA ordinance numbers 444/2014 and 445/2014) classifies the elasmobranchs *M. canis* and *S. mokarran* as Endangered (EN), and *S. tiburo*, *S. lewini*, *P. horkelii* as Critically Endangered (CR). This list also includes the teleost *L. purpureus* as Vulnerable (VU) and *E. itajara* as Critically Endangered (CR).

DISCUSSION

DNA Barcoding

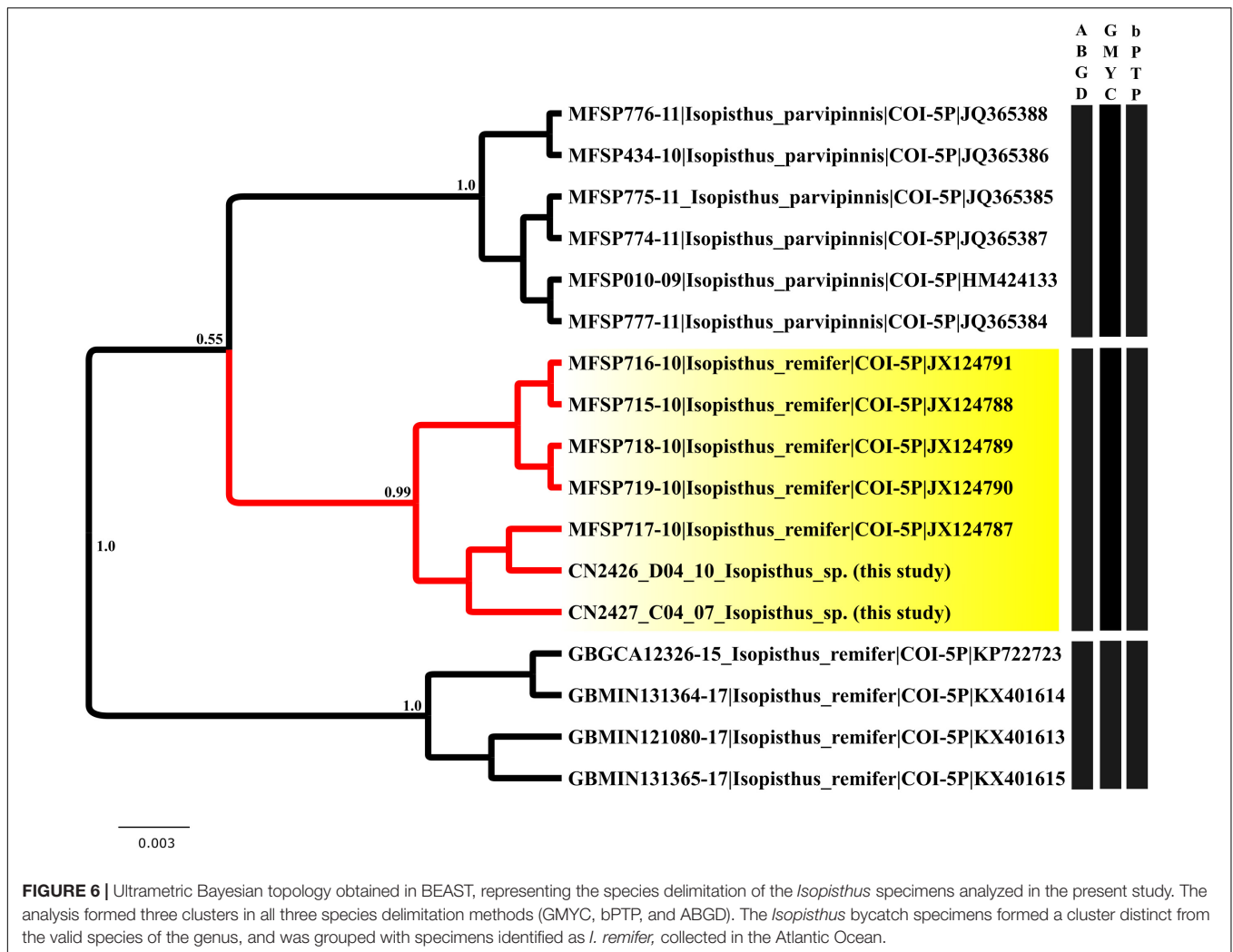
The COI gene (Barcoding region) was effective for the taxonomic delimitation of the 557 fish specimens analyzed in the present study using the most popular species delimitation methods. Our results revealed details of the little-known diversity of the biogeographic region located between the Caribbean and Brazilian provinces (Briggs and Bowen, 2012). The combined

analysis of morphological characteristics and COI sequences nevertheless resulted in a slight divergence, given that, whereas 112 morphospecies were identified in the BOLD analysis, 113 potential species were recovered by the three species delimitation methods. This divergence was due to the separation of the *Notarius grandicassis* (Ariidae) specimens into two OTUs, 92 and 93 (Supplementary Table S1) with a 3.2% genetic divergence, which supports the existence of cryptic species in this region, which require further assessment.

The effectiveness of the COI gene for the delimitation of species is related directly to the Barcode Gap, that is, the difference between the maximum and minimum intraspecific distances in the COI sequences (Meyer and Paulay, 2005). In the present study, we did not detect any overlap between the maximum intraspecific distance (3.40%) and the minimum interspecific distance (4.47%), which is consistent with the existence of the Barcode Gap. The clusters of the NJ tree were supported by high bootstrap values (>90). With the gene used in the present study for cluster analyses, we were able to observe a phylogenetic signal for the delimitation of some monophyletic groups, such as the Siluriformes, Tetraodontidae, and Sciaenidae, an approach that appears to be effective, as discussed by Hebert et al. (2003a).

New Species Occurrences From the Northern Coast of Brazil

The findings of the present study contribute to a better understanding of the diversity and distribution of fish species

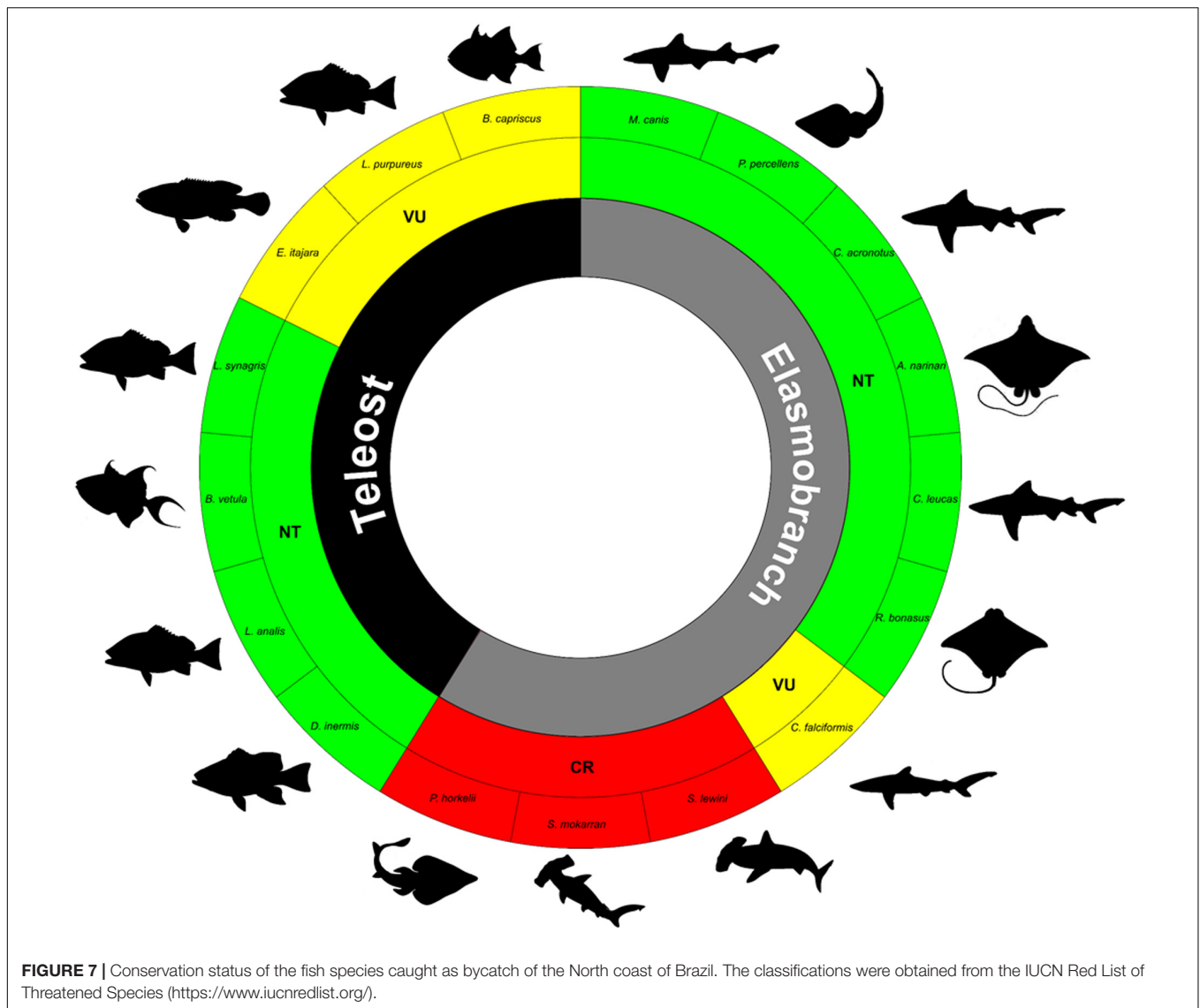


in this poorly studied region of the Brazilian coast. In particular, the data resolve a distribution gap in the known occurrence of *Mustelus canis* (Triakidae) and *Dermatolepis inermis* (Serranidae), previously restricted to the northwestern Atlantic, between Massachusetts, the Gulf of Mexico, and the Caribbean, and southeastern Brazil, with a gap in the central Atlantic, which coincides with the Amazon coast (Conrath, 2009; Claro et al., 2015). We also confirmed (i) the expansion of the known geographic distribution of three species (*Narcine brasiliensis*, *Rhinobatos horkelii*, and *Umbrina canosai*) previously known only from southeastern Brazil, (ii) the occurrence of three species (*Syacium gunteri*, *Synodus foetens*, *Scorpaena bergii*, and *Gymnothorax nigromarginatus*) recorded previously only in the northern and central regions of the western Atlantic, but not on the northern coast of Brazil, and (iii) the occurrence in the region of a species (*Trichopsetta ventralis*) recorded previously only in the western North Atlantic (Table 1).

The bycatch analyzed in the present study also provided the first record of the Guinea conger *Paraconger notialis* (Congridae) in the western Atlantic. The genus *Paraconger* Kanazawa, 1961, currently includes seven recognized species of which only two—*Paraconger caudilimbatus* Poey, 1867 and *Paraconger guianensis*

Kanazawa, 1961—had been recorded previously in the western Atlantic Ocean (Nolf and Aguilera, 1998; Aguilera and Lundberg, 2010). As only a single specimen of *P. notialis* was collected in the present study, however, it remains unclear whether the species is established in the western Atlantic Ocean.

One other important finding of the present study was the collection of an unknown species of *Isopisthus* (Sciaenidae). Two valid *Isopisthus* species are known to inhabit the coastal and estuarine waters of South America, *I. parvipinnis*, which is found in the western Atlantic, in Central and South America, and *I. remifer*, previously assumed to be endemic to the eastern Pacific. The molecular evidence from the present study indicates that two individuals of the genus *Isopisthus* formed a third, divergent cluster. All three species delimitation methods (GMYC, bPTP, and ABGD) applied in the present study confirmed the existence of this cluster (Figure 6). Although the geographic distribution of this new lineage is unclear, these findings indicate the existence of an as yet unknown *Isopisthus* species in the western Atlantic, which may further increase the diversity of one of the most diverse families of Perciformes. There is thus a clear need for the reassessment of the taxonomy and zoogeography of this group.



Few studies have evaluated the diversity of the fish bycatch taken by the shrimp trawling fleets of the Brazilian coast. While these operations appear to be decimating the fish diversity of Brazilian waters, there is little official monitoring or control of these activities. A larger number of taxa (182 species, 123 genera, and 62 families) were identified in the bycatch analyzed in the present study, which is consistent with the findings on other industrial fisheries, in northern (Marceniuk et al., 2019, $N = 201$ species), northeastern (Silva-Júnior et al., 2018, $N = 51$ species), and southern Brazil (Vianna and Almeida, 2005, $N = 91$ species; Branco et al., 2015, $N = 124$ species). This further highlights the impact of trawling fisheries on the biodiversity of the Brazilian coast. Trawling is the most common shrimping method used in the region, which impacts most the fauna of sciaenids and haemulids, the families that make up a large part of the fish bycatch taken off the coast of Brazil (see Vianna and Almeida, 2005; Branco et al., 2015; Silva-Júnior et al., 2013, 2015; Marceniuk et al., 2019). This pressure from shrimp trawling operations, combined with the overfishing of some of

the commercially important species may lead to widespread ecological impacts and the decline of stocks.

In the present study, the bycatch fauna included 20 elasmobranch species, of which nine are subject to some level of global threat, as indicated by the IUCN Red List, while five species are also included in the Brazilian list of endangered fauna. Most of the bycatch harvested in this tropical region is made up of fish, which increases the fishing pressure on many of these species (Wassenberg and Hill, 1989). In addition to bycatch, the demand for shark fins has driven an increase in the commercial harvesting of these species in the world (Musick et al., 2000; Stobutzki et al., 2001; Feitosa et al., 2018).

The predominance of teleosts, in particular lutjanids, serranids, and balistids, should be a priority for research and management initiatives, given that these families tend to already suffer pressure from overfishing. A prime example is the Atlantic goliath grouper, *E. itajara*, which is classified globally as Vulnerable (VU), but in Brazil, this species has suffered a significant population decline due to fishing

pressure, primarily on juvenile individuals, which has led to its classification as Critically Endangered (CR) in the Brazilian list of endangered fauna.

In addition to these considerations, the analysis of the composition of the shrimping bycatch provided important insights into the marine biodiversity of the northern coast of Brazil. This region encompasses the Amazon Plume, formed by sediment a persistent and massive discharge (Moura et al., 2016). The complexity of the local aquatic habitats accounts for the diversity of local fish assemblages, which include freshwater, estuarine, and marine species, some of which have extremely limited geographic distributions.

CONCLUSION

The DNA barcoding technique was highly effective for the delimitation of the fish species taken as bycatch by shrimp trawling operations off the northern coast of Brazil. This region not only has a rich fauna of widely distributed species, but the study also revealed the occurrence of a number of species in the western Atlantic for the first time. The analyses also revealed the presence in the bycatch of a number of elasmobranchs and teleosts that have an at risk conservation status.

On the Brazilian coast, there is no systematic monitoring of fisheries in terms of catches or biomass that would permit a more reliable evaluation of the potential threat of bycatch (see debate in Reis-Filho and Leduc, 2017; Reis-Filho, 2019). We thus conclude that local and regional measures of management and control will be more effective when supported by: (i) systematic surveillance to assess the intensity of the impact of bottom trawling on non-target species, and provide basic data on the impact of these fisheries; (ii) the implementation of continuous monitoring to determine the spatio-temporal patterns in catches and fishing effort, and (iii) the evaluation and implementation of Bycatch Reduction Devices (BRDs) to minimize the accidental catches in shrimp trawls. The need for collaboration and feedback between the trawling fleet and resource managers cannot be overstated, nor can the importance of more reliable data, such as those presented in this study. Clearly, the success of any initiative of this type will depend on the constant evaluation of management strategies and the trustworthy involvement of the stakeholders.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in the online repositories. The names of the repository/repositories

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and accession number(s) can be found in the article/**Supplementary Material**.

ETHICS STATEMENT

The animal study was reviewed and approved by the Instituto Chico Mendes de Conservação da Biodiversidade.

AUTHOR CONTRIBUTIONS

TG: design and conception of the experiments. FM, MA, EC, and TG: data collection and specimen processing. AG-C, RA, and IS: molecular analyses. AG-C, FM, RA, JR-F, MA, EC, IS, and TG: production and review of the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2020.566021/full#supplementary-material>

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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