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A shot in the dark for conservation: Evidence of illegal commerce in endemic and threatened species of elasmobranch at a public fish market in southern Brazil

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Abstract

1. The illegal sale of endangered elasmobranchs has been recorded in a number of different Brazilian states, where sharks and rays are being marketed primarily as 'caçãõ' or 'viola'. Brazil is ranked among the top 10 nations worldwide that harvest most sharks, causing an immeasurable impact on the local elasmobranch populations.
2. The present study applied the DNA barcoding technique, based on the cytochrome C oxidase subunit I gene, for the molecular identification of the elasmobranch species sold as processed products under the generic names of 'caçãõ', 'mangona', 'azul', 'cambeva', 'fiuso' and 'lombo preto', in the fish market of the city of Florianópolis, capital of the state of Santa Catarina, Brazil.
3. Nine elasmobranch species were identified in the 56 samples analysed, including six sharks and three rays, representing six families, the Carcharhinidae, Sphyrnidae, Squatinidae, Arhynchobatidae, Myliobatidae and Gymnuridae. *Prionace glauca*, identified in more than half (56%) of the samples analysed, is listed as Near Threatened by the International Union for the Conservation of Nature.
4. Six species identified in the present study, *Sphyrna zygaena*, *Sphyrna lewini*, *Squatina guggenheim*, *Carcharhinus signatus*, *Gymnura altavela* and *Rioraja agassizii*, are under some level of risk of extinction, while two others (*Rhizoprionodon lalandii* and *Myliobatis goodei*) are listed as Data Deficient.
5. Our results indicate that the commercial exploitation of endemic sharks and rays at risk of extinction is commonplace in southern Brazil. This reinforces the need for more systematic monitoring of the trade in fishery products and more effective application of the environmental legislation and conservation programmes.

KEYWORDS

caçãõ, COI gene, DNA barcode, genetic conservation, mislabelling, overfishing

1 | INTRODUCTION

Sharks and rays occupy a variety of ecological niches (Gardiner et al., 2014; Munroe, Simpfendorfer & Heupel, 2014) and play a fundamentally important role as trophic regulators (as top and meso-predators) of marine ecosystems (Ferretti et al., 2008; Vaudo & Heithaus, 2011; Bornatowski et al., 2014). Although they are well adapted to a range of different environments, many elasmobranchs are among the most endangered vertebrates listed by the International Union for the Conservation of Nature (IUCN), and have been classified as Critically Endangered, Endangered, Vulnerable or Near Threatened in the Red List of Threatened Species (Bräutigam et al., 2015). The unique biological characteristics of elasmobranchs, in particular their low fecundity and late sexual maturation (a characteristic of the life history of most species), exacerbate their vulnerability to fishing pressure, and have led to the widespread depletion of natural stocks and a significant decline in many populations (Ferretti et al., 2010; Dias Neto, 2011; Dulvy et al., 2017).

The overfishing of sharks is problematic, and a number of recent reports have indicated a marked reduction in the stocks of many species, which has often resulted in a demographic collapse at a regional scale (Davidson, Krawchuk & Dulvy, 2016; Taylor et al., 2016; Brown & Roff, 2019; Bargnesi, Lucrezi & Ferretti, 2020). In 1999, the Food and Agriculture Organization (FAO) formally recognized the vulnerability of sharks and rays, and launched an international plan for their conservation and management (Vannuccini, 1999). Despite this initiative, many elasmobranch species continue to be overexploited or threatened by intense fishery activities (Camhi et al., 2009; Cosandey-Godin & Morgan, 2011; Tolotti et al., 2015; Davidson, Krawchuk & Dulvy, 2016; Carvalho et al., 2018; Okes & Sant, 2019).

The Convention on International Trade of Endangered Species (CITES) of Wild Flora and Fauna is a major international agreement among governments that bans the international trade in threatened species, even so, there is evidence of high levels of trade in threatened species of both sharks and rays (Palmeira et al., 2013; Sarmiento-Camacho, Valdez-Moreno & Adamowicz, 2018; Hobbs et al., 2019; Rodrigues Filho et al., 2020), as well as systematic fraud, with higher-priced species being substituted by cheaper ones (Carvalho et al., 2015; Harris, Rosado & Xavier, 2016; Staffen et al., 2017; Horreo et al., 2019; Mizrahi et al., 2019).

Between 2007 and 2017, Brazil was ranked ninth among the top 10 nations worldwide that harvest most sharks, worldwide (Okes & Sant, 2019). The illegal trade in sharks and rays is known to be widespread in the different Brazilian coastal states, with the fish being sold as 'cação' or 'viola', generic terms applied to elasmobranchs (Almerón-Souza et al., 2018). This illegal trade has been recorded in the Brazilian states of Pará, Rio Grande do Norte, Bahia, Rio de Janeiro, São Paulo, Paraná, Santa Catarina and Rio Grande do Sul (Rodrigues-Filho et al., 2009; De-Franco et al., 2012; Almerón-Souza et al., 2018; Ferrette et al., 2019; Alvarenga, 2020, unpublished data; Bernardo et al., 2020).

The morphological identification of species is virtually impossible when a fish is marketed in the form of processed products, such as

fillets or steaks, given that features such as the head and swim bladder, which are important for the identification of the species, are missing (Bornatowski et al., 2015; Staffen et al., 2017). In many cases, these products are labelled inadequately (Barreto et al., 2017; Staffen et al., 2017; Camacho-Oliveira et al., 2020), and even when the species is identified on the label, errors are common, and are often intentional. The incorrect labelling of fishery products hampers conservation programmes by altering fishery statistics, but also has a potential impact on public health owing to the marketing of allergenic or contaminated products (Sheth et al., 2010; Di Pinto et al., 2015; Harris, Rosado & Xavier, 2016; Staffen et al., 2017; Mizrahi et al., 2019; Camacho-Oliveira et al., 2020).

The DNA barcode is a molecular tool which is widely used to identify biological species (Hebert et al., 2003; Ward et al., 2005; Trivedi et al., 2016; Rosas et al., 2018; Guimarães-Costa et al., 2019). The DNA barcode can provide an accurate determination of the species from live organisms, dead specimens or even processed food (Chuang et al., 2016; Steinke et al., 2017; Wainwright et al., 2018; Hellberg, Isaacs & Hernandez, 2019; Camacho-Oliveira et al., 2020; Cardeñosa et al., 2020). In the present study, the DNA barcode technique was used to identify the elasmobranch species on sale in a fish market in southern Brazil.

2 | METHODS

Samples of processed elasmobranch meat were obtained from the main public market in Florianópolis, capital of Santa Catarina state, Brazil (<http://www.mercadopublicofloripa.com.br/>). The samples were collected monthly from January to December 2016, and the meat was labelled with a number of generic names, e.g. 'cação', 'roliço', 'mangona', 'azul', 'cambeva', 'fuso', and 'lombo preto' all of which refer to elasmobranchs. Sixty-four samples of muscle tissue (~1.0 cm³) were extracted from the fillets or steaks and preserved in 96% ethanol at -18°C for subsequent molecular analyses at the UNESP Laboratory of Fish Biology and Genetics in Botucatu, São Paulo, Brazil.

The total DNA was extracted from the tissue samples following the protocol established by Ivanova, Dewaard & Hebert (2006). Partial sequences of approximately 650 base pairs (bp) were obtained by PCR amplification using the fish-specific primers (F1/R1; Ward et al., 2005) of the cytochrome C oxidase subunit I (COI) gene. Aliquots of 10 µl of reaction solution were composed of 1 unit of the DNA and 0.25 µM of each primer, 5× Reaction Buffer B (Solis Biodyne), with 1 unit of FIREPol® DNA polymerase, 12.5 mM of MgCl₂ and 1 mM of the dNTPs (200 µM of each). The PCR products were amplified in the Veriti Thermocycler (Applied Biosystems) through the following conditions: initial denaturation at 95°C for 2 min, followed by 30 cycles of denaturation at 95°C for 20 s, annealing at 52°C for 30 s, and extension at 72°C for 1 min, with final extension at 72°C for 5 min.

The results of the amplification were verified in 1% agarose gel, and the PCR products were purified and labelled for sequencing

using the Big Dye® Terminator v. 3.1 kit. The labelling reaction was run in a Veriti thermocycler (Applied Biosystems) with the following conditions: an initial denaturation of 1 min at 96°C, followed by 30 cycles of denaturation for 30 s at 96°C, annealing for 15 s at 54°C and extension for 4 min at 60°C, and then sequencing in the ABI 3130X1 Genetic Analyzer system (Applied Biosystems). These sequences were curated and edited in Geneious Pro 4.8.5 (Kearse et al., 2012).

The consensus sequence was then compared with those available in the NCBI database (<http://www.ncbi.nlm.nih.gov/>) using the Basic Local Alignment Search Tool – Nucleotide (BLASTn). Following species confirmations, the genetic distances between the species identified were calculated using the Kimura-2-parameter (K2P) model (Kimura, 1980) in MEGA v 7.0 (Kumar, Stecher & Tamura, 2016).

The multiple species alignments consisted of pseudoreplicates of the COI gene in 81 samples, of which 56 sequences were obtained in the present study, and 25 from the NCBI database (Table S1). At least two sequences of each species were obtained from the NCBI database. All the sequences were aligned in the Geneious program using MUSCLE (Edgar, 2004), and the tree was reconstructed using the maximum likelihood (ML) approach in MEGA (Kumar, Stecher & Tamura, 2016) and was tested using the bootstrap procedure with 1,000 pseudoreplicates (Felsenstein, 1985), based on the GTR + G + I nucleotide substitution model selected by MEGA model selection. The sequences obtained in the present study have been deposited in GenBank (accession numbers MT757692–MT757747).

3 | RESULTS

Fifty-six of the 64 samples were sequenced successfully, with high-quality sequences of 650 bp, which were compared with the NCBI sequences (Table 1). DNA barcode analysis identified nine species of elasmobranch (Figure 1) from the 56 samples, with 87.5% ($n = 49$) being identified as sharks and 12.5% ($n = 7$) as rays, representing six families, Carcharhinidae, Sphyrnidae, Squatinidae, Arhynchobatidae, Myliobatidae and Gymnuridae. All of the sequences obtained in the present study matched the NCBI references, with a high degree of similarity (>99%). Most of the species identified were clustered in well-supported clades in the ML tree, reinforcing the success of the genetic identification based on the DNA barcode (Figure 2).

The most abundant of the six shark species identified was *Prionace glauca* (51.78%), followed by *Sphyrna zygaena* (19.64%). The other sharks were less frequent, with *Sphyrna lewini* being recorded in 8.9% of the samples and *Squatina guggenheim*, *Rhizoprionodon lalandii* and *Carcharhinus signatus* each being recorded in a single sample. The most abundant ray was *Myliobatis goodei*, identified in 7.1% of the samples, followed by *Gymnura altavela*, found in 3.7% of the samples, and *Rioraja agassizii* represented in a single sample (Table 1). The mean K2P value for the genetic distance among the nine species was 13.5% (see Table 2), with between-species distances ranging from 5.8%, between *P. glauca* and *C. signatus* to 28% between pairs of species such as *M. goodei* and *S. lewini*.

4 | DISCUSSION

The illegal commercial trade in sharks and rays is known to occur in many different parts of the world, reinforced by the difficulty of identifying the species of processed fish products which, in Brazil, are marketed under generic names, such as 'cação' and 'viola' (Bornatowski, Braga & Barreto, 2018). The results of the present study confirm the sale of nine elasmobranch species, including six sharks (*R. lalandii*, *S. lewini*, *S. zygaena*, *P. glauca*, *S. guggenheim* and *C. signatus*) and three rays (*G. altavela*, *R. agassizii* and *M. goodei*), in a fish market in southern Brazil, based on a DNA barcoding analysis. According to Barreto et al. (2016), most shark populations in Brazil are currently depleted, mainly ones that have been caught by local and international longline fishing fleets since the 1950s, among them are listed *P. glauca*, *C. signatus*, *S. lewini* and *S. zygaena*.

With the exception of *R. lalandii* (represented by a single sample), which is classified as Data Deficient (DD) by the IUCN, all of the shark species identified in the present study are considered to be under some threat of extinction, owing primarily to population decline. The most threatened is *S. lewini* ($n = 5$ specimens/8.9% of the total), which is classified as Critically Endangered (CR), followed by *S. guggenheim* (represented by a single sample), a species classified as Endangered (EN). Two species, *S. zygaena* (11/19.6%) and *C. signatus* (one sample), are classified as Vulnerable (VU), while the sixth, *P. glauca* ($n = 22/39.2%$), is classified as Near Threatened.

Four of these species, *P. glauca*, *C. signatus*, *S. lewini* and *S. zygaena*, are also known to be sold in other Brazilian states, including Pará, Rio Grande do Norte, Rio de Janeiro, São Paulo, Paraná, Santa Catarina and Rio Grande do Sul (Rodrigues-Filho et al., 2009; Almerón-Souza et al., 2018; Ferrette et al., 2019; Alvarenga, 2020, unpublished data; Bernardo et al., 2020). More than half (52%; 29/56; Table 1) of the samples were identified as *P. glauca*, a percentage consistent with the predominance of this species (56%) in the worldwide catch of pelagic sharks, as recorded by Camhi et al. (2009) and Dulvy et al. (2017), with an estimated 103,528 metric tons being harvested globally in 2017 (Okes & Sant, 2019). Despite this pressure, Bornatowski, Braga & Barreto (2018) believe that the stocks of this species have yet to enter into a state of collapse.

One possible reason for the predominance of the blue shark (*P. glauca*) in elasmobranch catches worldwide is the fecundity of this species, with 4–135 pups being produced per litter (Compagno, 1984) and an average of 38 pups per litter in the Atlantic Ocean (Castro & Mejuto, 1995), in addition to its relatively early maturation (5–7 years) in comparison with other pelagic sharks (Pratt, 1979). Almerón-Souza et al. (2018) observed the sale of this species in different regions of Santa Catarina, and its predominance was confirmed by Ferrette et al. (2019), who found that it was the shark species most often sold in many different Brazilian states. However, Feitosa et al. (2018) did not identify *P. glauca* in any of the 427 samples obtained from fish markets in the states of Pará and Maranhão, which may reflect possible differences in the distribution and composition of the stocks of shark species in different regions.

TABLE 1 Species identification after DNA barcoding of samples obtained in the commercial market

| Superorder | Family | Species | Common names | IUCN status | Names of sharks and rays labelled | | | | | | Total |
|------------------------|---------------------------------------|---|---|-------------|-----------------------------------|---------|------|---------|-------|-------------|-------|
| | | | | | Cação | Mangona | Azul | Cambeva | Fiusa | Lombo preto | |
| Batoidea (rays) | Gymnuridae | <i>Gymnura altavela</i> (Linnaeus, 1758) | Spiny butterfly ray, raia-borboleta | VU | 1 | | | | | 1 | 2 |
| | Myliobatidae | <i>Myliobatis goodii</i> (Garman, 1885) | Southern eagle ray, raia-sapo | DD | 1 | 2 | 1 | | | | 4 |
| | Arhynchobatidae | <i>Rioraja agassizii</i> Müller & Henle, 1841 | Rio skate, raia-santa | VU | 1 | | | | | | 1 |
| Selachimorpha (sharks) | Squatinae | <i>Squatina guggenheim</i> Marini, 1936 | Angular angel shark, cação-anjo | EN | 2 | | | | | | 2 |
| | Sphyrinae | <i>Sphyrna lewini</i> Griffith & Smith, 1834 | Scalloped hammerhead, tubarão-martelo | CR | 4 | 1 | | | | | 5 |
| | Carcharhinidae | <i>Sphyrna zygaena</i> Linnaeus, 1758 | Smooth hammerhead, cambeva | VU | 8 | 2 | 1 | | | | 11 |
| | | <i>Rhizoprionodon lalandii</i> Müller & Henle, 1839 | Brazilian sharpnose shark, cação-frango | DD | 1 | | | | | | 1 |
| | | <i>Carcharhinus signatus</i> Poey, 1868 | Night shark, cação-noturno | VU | | | | 1 | | | 1 |
| | <i>Prionace glauca</i> Linnaeus, 1758 | Blue shark, tubarão-azul | NT | 13 | 12 | 2 | 1 | 1 | | | 29 |
| | Total | | | | 32 | 15 | 6 | 1 | 1 | 1 | 56 |

IUCN, International Union for the Conservation of Nature; CR, Critically Endangered; EN, Endangered; VU, Vulnerable; NT, Near Threatened; DD, Data Deficient.

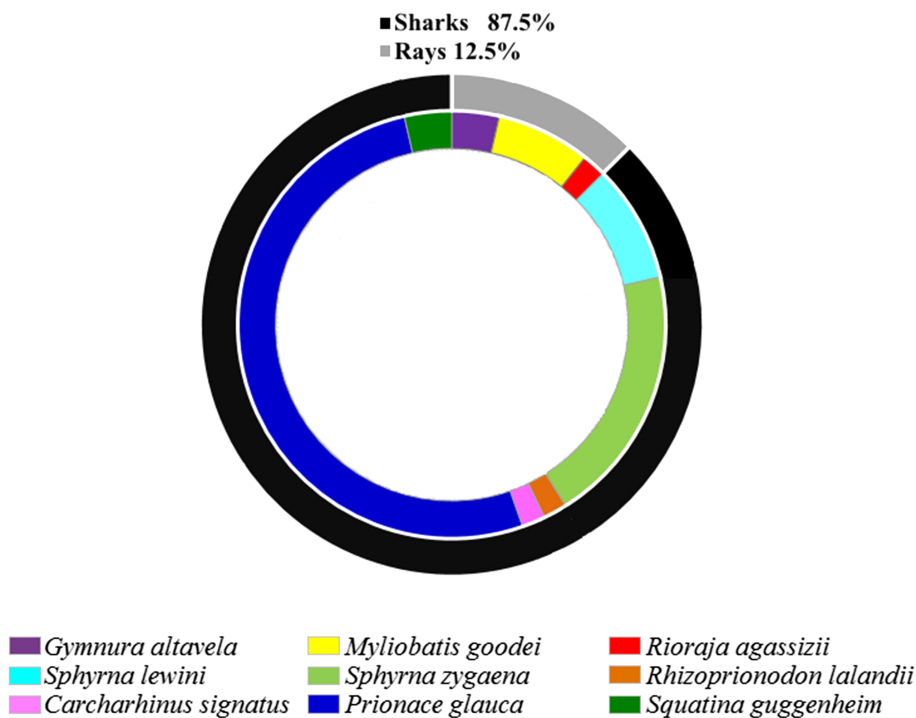


FIGURE 1 Graphic showing the identified composition of the marketed elasmobranch samples analysed in the present study

Clarke et al. (2006) estimated that 1–3 million hammerhead sharks (*S. lewini*, CR, and *S. zygaena*, VU) are harvested annually, worldwide, but the actual contribution of each species to this total catch remains unknown. In southern Brazil, hammerhead shark populations decreased by more than 90% between 2000 and 2012, probably owing to overfishing and the illegal harvesting of these species, primarily for the fin trade (Chapman, Pinhal & Shivji, 2009; Barreto et al., 2017). Here, 28.6% (16/56) of the samples analysed were of hammerhead shark meat, exactly the same percentage (66/231 samples) recorded by Bernardo et al. (2020) in samples collected in the Brazilian state of Paraná. It is important to note that, as these species are endangered, their harvesting is prohibited in Brazil under Federal Ordinance 445 (Brasil, 2014), although, in practice, they are still widely sold.

One other species recorded in the present study, the angel shark, *S. guggenheim* (EN), is endemic to the Southern Atlantic Ocean (Cousseau & Figueroa, 2001; Vooren & Sandro, 2005). This species has become a target of commercial fisheries throughout its area of occurrence since the 1990s (Villwock & Vooren, 2003; Chiaramonte, Vooren & IUCN, 2007), and it is still widely harvested in this region. As an endangered species, *S. guggenheim* is also covered by Federal Ordinance 445, although illegal trade is widely reported in the Brazilian states of Rio de Janeiro, São Paulo, Paraná, Santa Catarina and Rio Grande do Sul (Almerón-Souza et al., 2018; Bunholi et al., 2018; Alvarenga, 2020, unpublished data; Bernardo et al., 2020). It is important to note that the endemism of this species is a factor that increases its potential extinction risk.

A number of studies have detected errors in the identification of shark meat sold in commercial establishments, including public markets, identification errors may arise as the result of a number of

different factors, such as the incorrect identification of species with similar phenotypes (Bunholi et al., 2018), the use of different common names for a single species (Cawthorn, Steinman & Witthuhn, 2012; Staffen et al., 2017) and the use of a single generic name for multiple species, that is, an ‘umbrella’ label that may include species with widely varying conservation statuses (Bornatowski, Braga & Vitule, 2013; Bornatowski et al., 2015; Staffen et al., 2017). In the present study, approximately 57% of the samples were marketed as ‘cação’ and 26.7% as ‘mangona’ (the latter the common name for *Carcharias taurus*) (Mayer, 2017, 2019).

Three species of ray (superorder Batoidea), *Gymnura altavela* (VU), *Rioraja agassizii* (EN) and *Myliobatis goodei* (DD), were identified in the present study. The small number of ray samples collected in the present study reflects the reduced demand for this meat, which is undervalued locally and in many other parts of the world (Dent & Clarke, 2015). In Brazil, ray meat is considered to be a cheap product, which is rarely in demand, and when it is sold, it is often given the generic name ‘cação’ or ‘viola’. It is a very common product in the fish markets of Rio de Janeiro (De-Franco et al., 2012; Alvarenga, 2020, unpublished data; Rodrigues Filho et al., 2020).

Both *G. altavela* and *M. goodei*, which have a wide geographic distribution, have already been reported as being sold in markets in Santa Catarina state by Almeirón-Souza et al. (2018), although in smaller quantities when compared with the present study. *Rioraja agassizii* was detected in only one of the samples analysed in the present study, however, and no data are available on its fishery exploitation in other states of Brazil. As this species is endemic to the area from southern Brazil to northern Argentina, there is a clear need for the more systematic monitoring of the trade in this species in order to offset eventual population decline.

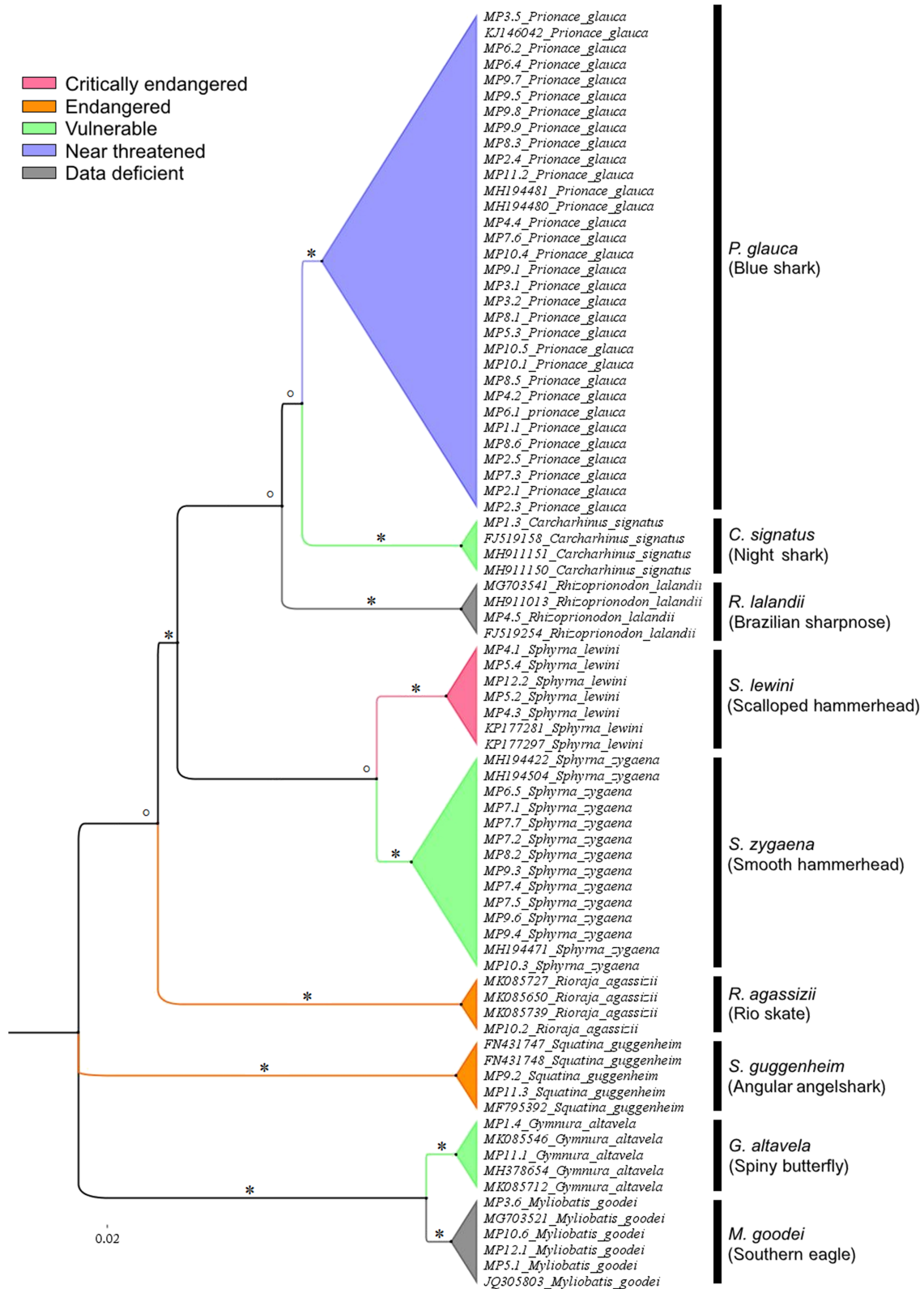


FIGURE 2 Maximum likelihood tree with 1,000 bootstrap pseudoreplicates of the cytochrome C oxidase subunit I (COI) gene – 81 samples: 56 from this study and 25 from the NCBI database. The species are categorized as Critically Endangered, Endangered, Vulnerable, Near Threatened and Data Deficient according to the Red List of Threatened Species of the International Union for the Conservation of Nature. * 100% support; ° values between 56 and 81%

TABLE 2 Genetic distances (Kimura-2-parameter, K2P) among the elasmobranch species identified in the processed fish products sold in southern Brazil, based on the analysis of cytochrome C oxidase subunit I sequences

| Species | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|-----------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|---|
| 1. <i>Gymnura altavela</i> | | | | | | | | | |
| 2. <i>Myliobatis goodei</i> | 0.170 | | | | | | | | |
| 3. <i>Squatina guggenheim</i> | 0.248 | 0.273 | | | | | | | |
| 4. <i>Rioraja agassizii</i> | 0.270 | 0.236 | 0.259 | | | | | | |
| 5. <i>Sphyrna lewini</i> | 0.249 | 0.281 | 0.223 | 0.230 | | | | | |
| 6. <i>Rhizoprionodon lalandii</i> | 0.245 | 0.272 | 0.205 | 0.235 | 0.116 | | | | |
| 7. <i>Sphyrna zygaena</i> | 0.260 | 0.255 | 0.212 | 0.195 | 0.090 | 0.119 | | | |
| 8. <i>Carcharhinus signatus</i> | 0.229 | 0.253 | 0.212 | 0.211 | 0.109 | 0.085 | 0.084 | | |
| 9. <i>Prionace glauca</i> | 0.245 | 0.245 | 0.215 | 0.197 | 0.113 | 0.095 | 0.089 | 0.058 | |

Despite the fact that there is reduced demand for ray meat, different species of ray are known to be sold in varying proportions in different regions of Brazil. This probably reflects the relatively ample distribution of most species, as well as the occurrence of endemism in some regions. In a DNA barcode study of 118 samples obtained from fish markets in Pará and Maranhão (Rodrigues Filho et al., 2020), 41.5% were identified as the genus *Hypanus*. De-Franco et al. (2012) identified species of the genus *Pseudobatos* as the rays sold most often at numerous locations around the Brazilian coast, including Bahia, Rio de Janeiro, São Paulo, Paraná, Santa Catarina and Rio Grande do Sul, while in the neighbouring state of Paraná, Bernardo et al. (2020) analysed 231 samples labelled as 'caçõ', and identified *Pseudobatos percellens* in the largest number of samples ($n = 33$, 14.3% of the total).

The results of the present study reinforce the value of the DNA barcode as a tool for the identification of elasmobranch species, and given the marked efficiency and reliability of this molecular tool, it has been employed in a growing number of studies in a range of countries in recent years. In particular, many studies have revealed the sale of fish species not previously known to have been targeted by commercial fisheries (Johri et al., 2019; Muttaqin et al., 2019; Pazartzi et al., 2019; Abdullah et al., 2020; Cardeñosa et al., 2020). These findings reinforce the need for the molecular identification of fishery products, not only in Brazil, but also in other countries in which elasmobranch meat is consumed, in order to provide reliable data to support the implementation of effective conservation measures. In particular, it is important to minimize the exploitation of endangered species or redirect consumption towards more sustainable species, as well as establishing species-specific commercial names that facilitate the monitoring and tracking of protected species (Jacquet & Pauly, 2008; Barbuto et al., 2010; Bornatowski, Braga & Vitule, 2013).

The present study demonstrates clearly that, despite the illegal nature of the trade in endangered elasmobranch species, many of these species are still harvested in many parts of Brazil, at rates that may threaten the long-term survival of stocks. In this context, the incorrect identification or mislabelling of fishery products may preclude or hinder informed decision-making for the effective conservation of elasmobranchs (Barbuto et al., 2010; Bornatowski,

Braga & Vitule, 2013; Bornatowski, Braga & Barreto, 2018). In the present day, molecular biology is a valuable and feasible diagnostic tool, in both logistical and financial terms, for the monitoring of the commerce in endangered species, and it should thus play a fundamental role in programmes of conservation and management for a wide range of organisms.

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CONFLICT OF INTEREST


None.

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