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MOLECULAR IDENTIFICATION OF SHARK MEAT TRADED AS CAÇÃO IN SOUTHWESTERN STATE OF SÃO PAULO, BRAZIL

Identificação molecular de carne de tubarão vendida como cação no sudoeste do Estado de São Paulo, Brasil

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ABSTRACT

The meat of sharks and rays is commonly traded in Brazil under the generic name of cação. This compromises the monitoring of which species are being traded. In the present study, molecular marker techniques were applied to the species-level identification of elasmobranch meat traded in the southwest of the State of São Paulo. A total of 15 meats purchased in 2019 were partially sequenced (up to 655 base pairs) for the mitochondrial cytochrome c oxidase subunit I (COI) gene. Of these meats, 14 were from Blue Shark, *Prionace glauca*, and one from Shortfin Mako Shark, *Isurus oxyrinchus*. Only two (13,3%) out of the 15 cação meat were species identified on its product label – the other meat was only labeled as cação. Both shark meat that had the scientific name stated on the package label correctly corresponded to the species identification obtained through DNA sequencing. It is suggested that similar studies be conducted in other non-coastal regions of the country to further understanding of the cação trade in locations where elasmobranch consumption is not habitual. The present study was the first to detect the Shortfin Mako Shark sold as cação in Brazil.

Keywords: DNA Barcoding, elasmobranch, molecular marker, conservation.

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RESUMO

A carne de tubarões e raias é comumente comercializada no Brasil sob o nome genérico de cação. Isso compromete um monitoramento das espécies que estão sendo comercializadas. No presente estudo, técnicas de marcadores moleculares foram aplicadas para identificação de espécie de carne de elasmobrânquios comercializada no sudoeste do Estado de São Paulo. Um total de 15 carnes adquiridas em 2019 foi parcialmente sequenciado (até 655 pares de base) para o gene mitocondrial citocromo c oxidase subunidade I (COI). Dessas carnes, 14 eram de tubarão-azul, Prionace glauca, e uma do tubarão-anequim, Isurus oxyrinchus. Das 15 carnes de cação, apenas duas (13,3%) apresentavam a espécie identificada no rótulo do produto – as outras carnes estavam rotuladas apenas como cação. As duas carnes de tubarão que tinham o nome científico informado no rótulo da embalagem correspondiam corretamente à identificação da espécie obtida por meio do sequenciamento de DNA. Sugere-se que estudos semelhantes sejam realizados em outras regiões não litorâneas do país para melhor entender o comércio de cação em locais onde o consumo de tubarões e raias não é habitual. O presente estudo foi o primeiro a detectar o tubarão-anequim vendido como cação no Brasil.

Palavras-chave: *DNA Barcoding, elasmobrânquio, marcador molecular, conservação.*

INTRODUCTION

Currently, the marine environment suffers with several impacts such as climate change, pollution, habitat loss, disorderly occupation of coastal areas, overfishing, and trade exploitation of its resources (Maxwell *et al.*, 2016). This directly affects sharks and rays, a group that helps maintain and regulate marine ecosystems (Myers *et al.*, 2007; Ritchie & Johnson, 2009; Cruz *et al.*, 2021). Sharks and rays usually have biological characteristics, such as slow growth, late sexual maturity, low fertility, and long gestational period, that limit population recovery (Walker, 1998; Dulvy *et al.*, 2014; Feitosa *et al.*, 2018). Unfortunately, sharks and rays have become one of the most endangered groups of marine animals (Stevens *et al.*, 2000; Dulvy *et al.*, 2021).

Sharks and rays, the elasmobranchs, are traded as different products, including meat, fins, gill plates, medicine (cartilage) pills, etc. The meat is appreciated in Brazil due its low cost and for being thornless. Inadvertently though, being sold as meat at the fin market, morphological characteristics necessary for species identification are lost (Wong; Shivji & Hanner, 2009). In Brazil, shark meat is commonly commercialized under the popular name *cação*, used to label many elasmobranchs species (Bornatowski *et al.*, 2015). Under such generic name, consumers can not know if they are consuming an endangered or a species in which fish capture and trade is prohibited (Falcão *et al.*, 2014).

One way of trying to identify at the species level a shark meat traded as cação would be using molecular markers. These markers are widely used in species genetic identification in food market, including investigations of elasmobranchs products (Nachtigall *et al.*, 2017; Bernardo *et al.*, 2020; Blanco-Fernandez; Garcia-Vazquez & Machado-Schiaffino, 2021; Cruz *et al.*, 2021; Merten-Cruz; Szynwelski & Ochotorena de Freitas, 2021; Fernandes; Amaral & Mafra, 2021). Considering the importance of understanding which elasmobranch species are being trade and the potential of molecular

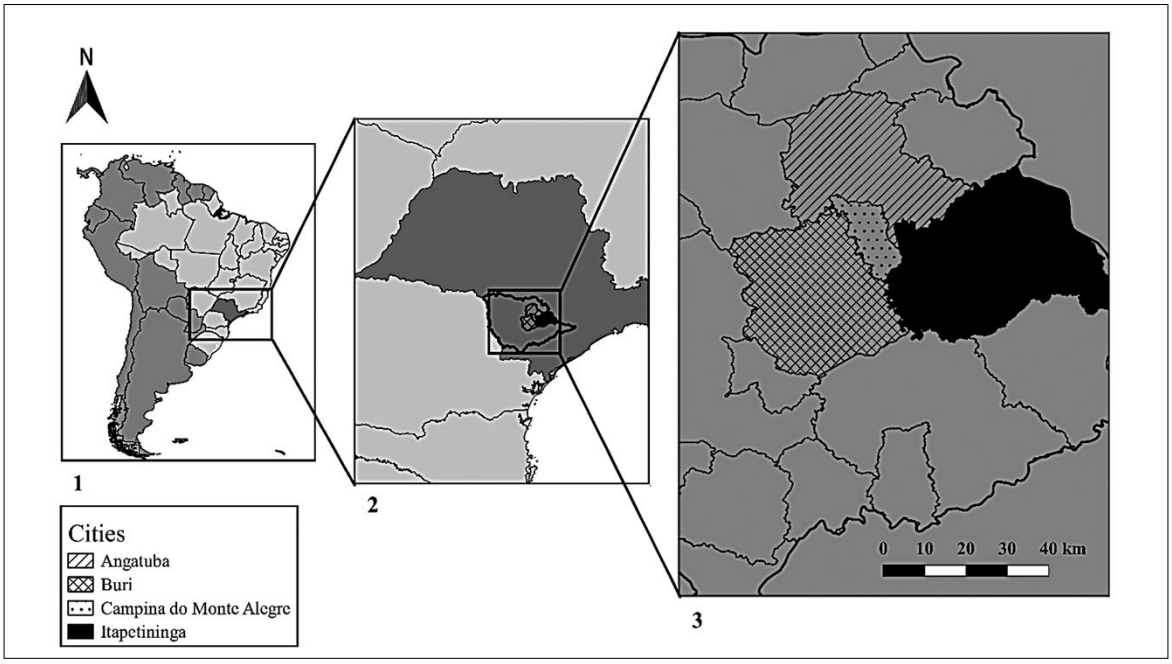
markers for identification of fish products, the aim of this study was to identify species sold as *cação* in southwestern state of São Paulo.

MATERIALS AND METHODS

Tissue Sampling

A total of 21 tissue samples from frozen cação/shark meat was obtained from 13 commercial establishments from Itapetininga (n = 13 samples), Angatuba (n = 4), Buri (n = 2), and Campina do Monte Alegre (n = 2), all of them located in southwestern state of São Paulo, Brazil, in 2019 (Figure 1). Tissue sampling was conducted under SisGen (Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado) permit ADFBA28. Samples were stored in ethanol under freezing conditions (-20 °C) until DNA extraction.

Figure 1 – The four cities located in southwestern State of São Paulo, Brazil, from which *cação*/shark meat were acquired for tissue sampling and species identification through the use of molecular markers



Laboratory procedures

For each sample, 50-100 mg of tissue was used for DNA extraction using the rapid salt-extraction method following Aljanabi and Martinez (1997). DNA was quantified by agarose gel electrophoresis (Low Mass Ladder, Thermo Fisher Scientific). Due to the efficiency of the mitochondrial cytochrome c oxidase subunit I (COI) gene for elasmobranch species identification (Ward *et al.*, 2005; Ward & Holmes, 2007; Ward *et al.*, 2008; Holmes; Steinke & Ward, 2009; Staffen *et al.*, 2017), a 655 bp fragment of this gene was amplified through the primers FISH F1 (5' - TCA ACC AAC CAC AAA GAC ATT GGC AC - 3') and FISH R1 (5' - TAG ACT TCT GGG TGG CCA AAG AAT CA - 3') following Ward *et al.* (2005). For Polymerase Chain Reactions (PCR) were conducted a final volume of 25 µl containing 5 pmol of primer F, 5 pmol of primer R, 12,5 µl of GoTaq Colorless Master Mix

(1x reaction buffer, 3 mM $MgCl_2$, 200 µM of each dNTP 0,5 U Taq DNA polymerase) according to the manufacturer's recommendations (Promega) and 1 µl containing 10-50 ng of DNA. The thermocycler program followed Holmes, Steinke and Ward (2009) protocol, consisting of an initial five-minute cycle at 94 °C for the initial denaturation followed by 40 cycles of 30 s at 94 °C, 45 s at 48 °C, 45 s at 72 °C and, finally, 10 min at 72 °C to final DNA extension. The PCR results were verified through electrophoresis in agarose gel (1%), visualized using a GelRed (Biotium) transilluminator. PCRs were purified using PEG protocol (Lis & Schleif, 1975) and sent to an outsourced company for DNA sequencing using ABI 3730xl DNA Analyzer (Applied Biosystems).

Data analysis

In total, 15 of the 21 tissue samples included in this study were successfully DNA sequenced. The obtained DNA sequences were assessed and trimmed using Chromas 2.6.5. The DNA sequences (up to 655 bp; available at https://drive.google.com/drive/folders/1_mRSOIi97QDU1ocRxA9S0jWGo5vpFVdY?usp=sharing) were then submitted to Basic Local Alignment Search Tool (BLAST) analysis (Altschul *et al*., 1990), which is available on the website of the National Center for Biotechnology Information - NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi). To minimize the chance of incorrect species attribution, and identity percentage threshold of ≥98% was assumed (Sanches *et al*., 2012; Coughlan *et al*., 2011).

RESULTS

Two shark species were identified: the Blue Shark, *Prionace glauca* (Linnaeus, 1758) (n = 14) (Carcharhinidae) and the Shortfin Mako Shark, *Isurus oxyrinchus* (Rafinesque, 1810) (n = 1) (Lamnidae) (Table I). Among the 15 *cação* meat samples successfully sequenced, only two had its package labeled with species identity and source country. Both were labeled as *Prionace glauca*, one of them being imported from Portugal (BQ03) and the other from Spain (BQ12). In both cases the species identification was correct (Table I). Information about origin and species were not attributed to the other samples.

Table I – Percent identity values between sequences of the mitochondrial DNA cytochrome oxidase c subunit I (COI) gene obtained in the present study for tissue samples obtained from *cação*/shark meat traded in southwestern state of São Paulo, Brazil and DNA sequences available in GenBank. Percent identity values were obtained after Basic Local Alignment Search Tool (BLAST)

Sample id.	Species identification information on meat label	Species molecular identification	[1] BLAST Percent identity	[2] GenBank Accession number/ locality of the sample
BQ01	none	*Prionace glauca*	100.00%	MH194484.1/ Peru
BQ02	none	*Prionace glauca*	100.00%	MH719984.1/ Brazil
BQ03	none	*Prionace glauca*	100.00%	FJ518955.1/ USA
BQ05	none	*Prionace glauca*	100.00%	MH719984.1/ Brazil
BQ06	none	*Prionace glauca*	100.00%	MH719984.1/ Brazil
BQ07	none	*Prionace glauca*	100.00%	MH719984.1/ Brazil

(continuation Table I)

Sample id.	Species identification information on meat label	Species molecular identification	[1] BLAST Percent identity	[2] GenBank Accession number/ locality of the sample
BQ08	none	*Prionace glauca*	100.00%	MH719984.1/ Brazil
BQ09	none	*Prionace glauca*	100.00%	MH719984.1/ Brazil
BQ10	none	*Prionace glauca*	100.00%	MH719984.1/ Brazil
BQ11	none	*Prionace glauca*	98.22%	MH243144.1/ Singapore
BQ12	*Prionace glauca*	*Prionace glauca*	99.82%	MH194481.1/ Peru
BQ13	none	*Prionace glauca*	99.78%	MH194481.1/ Peru
BQ14	*Prionace glauca*	*Prionace glauca*	99.46%	MH194480.1/ Peru
BQ16	none	*Prionace glauca*	99.52%	MH243166.1/ Singapore
BQ17	none	*Isurus oxyrinchus*	100.00%	KF793722.1/ Indonesia

[1] "Percent identity" is the highest identity obtained by a *High Scoring Segment Pairs* (HSP).
[2] "Locality of the sample" refers to the country considered to be the source or capture locality of the specimen from which a DNA sequence deposited at GenBank had the highest similarity with a correspondent DNA sequence obtained from any given sample.

DISCUSSION

The genetic identification shows that at least two shark species are traded as cação in southwestern state of São Paulo. One of them is the Shortfin Mako Shark, Isurus oxyrinchus. This species has already been molecularly identified in shark products traded in Italy (Filonzi *et al.*, 2010) and USA (Hellberg; Isaacs & Hernandez, 2019), but the present study reports a shortfin mako shark molecularly identified from a cação meat traded in Brazil for the first time. Under the IUCN's Red List criteria, this species is considered Near Threatened (NT) at a national (regional) level in Brazil (ICMBio, 2018) and considered Endangered (EN) at a global (Rigby *et al.*, 2019a). This shark has been captured by pelagic longline in the Atlantic, Indian, and Pacific Oceans and has its meat and fins traded (Dulvy *et al.*, 2008). The species has been through a 70% population decline in the North Atlantic Ocean (Dulvy *et al.*, 2008). In Brazil, it is estimated that its population declines may reach up to 30% due overfishing, leading the species to vulnerability category (VU) in a near future according to ICMBio (2016). As an answer to the declines, the species was added to CITES Appendix II in August 2019, a huge step towards its conservation.

The other species traded in southwestern state of São Paulo, the Blue Shark, *Prionace glauca*, was the species most detected in this trade. The Blue Shark has been already molecularly identified from *cação* meat traded in Brazil (Staffen *et al.*, 2017; Almerón-Souza *et al.*, 2018; Cruz *et al.*, 2021; Merten-Cruz; Szynwelski & Ochotorena de Freitas, 2021) as well as also abroad for shark products (Filonzi *et al.*, 2010; Hellberg *et al.*, 2019). Under the IUCN's Red List criteria, this species is considered Near Threatened (NT) at a national

(regional) level in Brazil (ICMBio, 2018) and at a global level (Rigby *et al.*, 2019b). Blue shark is the most caught and commercialized shark species in Brazil, where the imports of its meat was close to the national production of all shark and ray species (ca. 21 thousand tones; Barreto *et al.*, 2017). In addition, the Blue Shark represents 56% of all shark catches made especially by industrial fisheries vessels in pelagic habits (Almerón-Souza *et al.*, 2018). Brazil imported almost all of the Blue Shark production of Uruguay between 2002 and 2012 (Barreto *et al.*, 2017). Besides that, Brazil imports shark meat from other nations, such as Spain, Taiwan, China, and Portugal; countries that fish in Brazilian waters (Barreto *et al.*, 2017); in fact, three shark meat acquired in the present study – and molecularly identified as Blue Shark – were imported from Spain (BQ12 and BQ14) and Portugal (BQ03). About 49-86% of all longline catches are composed of blue sharks in Brazil, depending on the local and period of the year (Barreto *et al.*, 2017). The blue shark has one of the largest annual population growing when compared to other species (Dulvy *et al.*, 2008). Its catch frequency reflects its wide distribution and abundance, which provides it the title of the most captured shark species also at a global level (Dulvy *et al*., 2008; Almerón-Souza *et al.*, 2018). The Blue Shark is the most commonly traded species in the largest shark fin market in the world, in Hong Kong (Clarke *et al.*, 2006). These authors estimate about 10.7 million individuals (0.36 mt) caught per year for this purpose; inadvertently, the meat associated to these sharks end up being consumed in developing countries, such as Brazil.

The use of generic name like *cação* facilitates trade and consumption of elasmobranchs. As previously mentioned, sharks become more commercially attractive when sold in pieces as flitch or fillet, with whitish and thornless meat, without their morphological attributes (Barreto *et al.*, 2017). Often commercialized frozen when far from coastal areas, shark meat may also be imported from other countries (Bornatowski *et al.*, 2015; Barreto *et al.*, 2017), such as the cação meat acquired for the present study (BQ03, BQ12, and BQ14 samples, imported from Spain). As consequence, the widespread use of the term *cação* hinders conservation efforts for the group, since it enables the consumption of threatened species by unaware consumers (Almerón-Souza *et al.*, 2018). In the present study, only two (13,3%) out of 15 cação meat were species identified on its product label, which is suggestive of how widespread this problem in the region is.

In the present study, both shark meat (BQ03 and BQ12) that had the scientific name stated on the package label correctly corresponded to the species identification obtained through DNA sequencing. Therefore, no mislabeling were detected, although sample size was minimum. Mislabeling of shark meat can occur due to several reasons. Intentional species mislabeling may occur due to commercial interests. The most common situation is the trade of sharks and rays named as bony fishes of higher commercial value, such as grouper, swordfish, salmon, or croaker (Filonzi *et al.*, 2010; Barreto *et al.*, 2017; Staffen *et al.*, 2017). But, mislabeling may also occur simply due to non-intentional species misidentification; for example, shark meat can be sold mislabeled as weakfish, a teleost species of similar commercial value (Staffen *et al.*, 2017). Substitution may also occur among shark species (Filonzi *et al.*, 2010; Staffen *et al.*, 2017); for example, in Brazil, meat from a sand tiger shark, *Carcharias taurus*, was once detected being sold as blue shark (Staffen *et al.*, 2017).

It is suggested that the scientific and common names of sharks being traded be stated on the package label. This would permit consumers to be aware and make informed choices in face of shark conservation issues. For example, the critically endangered hammerhead

shark, *Sphyrna lewini*, has been molecularly identified being traded in Brazil as *cação* (Carvalho *et al.*, 2015; Staffen *et al.*, 2017; Almerón-Souza *et al.*, 2018; Cruz *et al.*, 2021; Merten-Cruz *et al.*, 2021). This hammerhead shark case further highlights the importance of the application of molecular markers towards shark conservation efforts. The present study applied molecular markers to the problem of shark trade identification while species identifying meat traded in a non-coastal region of Brazil. There is a need for similar studies to be replicated in other non-coastal regions of the country to further understand the *cação* trade in locations where elasmobranch consumption is not habitual.

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