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The fishing and illegal trade of the angelshark: DNA barcoding against misleading identifications

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ABSTRACT

Morphological identification in the field can be extremely difficult considering fragmentation of species for trade or high similarity between congeneric species. In this context, the shark group belonging to the genus *Squatina* is composed of three species distributed in the southern part of the western Atlantic. These three species are classified in the IUCN Red List as endangered, and they are currently protected under Brazilian law, which prohibits fishing and trade. Molecular genetic tools are now used for practical taxonomic identification, particularly in cases where morphological observation is prevented, e.g., during fish processing. Consequently, DNA barcoding was used in the present study to track potential crimes against the landing and trade of endangered species along the São Paulo coastline, in particular *Squatina guggenheim* (n = 75) and *S. occulta* (n = 5), as well as the Brazilian guitarfish *Pseudobatos horkelii* (n = 5). DNA barcoding revealed the continuous fishing and trafficking of these protected species, thus giving clear evidence that the current conservation models and methods of monitoring are not working.

1. Introduction

The Elasmobranchii subclass of Chondrichthyes currently consists of 509 shark species and 630 rays (Weigmann, 2016). However, this biodiversity should be greater owing to the continuous description of new species in recent years (Borsa, 2016). Out of this total, more than 90% is listed on the IUCN Red List with about 16.5% in the threatened categories and at least 40% listed as "Data Deficient" (IUCN, 2017). Such statistics call for the immediate collection of biological data toward formulating and implementing novel plans for the conservation of these species.

Several characteristics make this group especially susceptible to overexploitation, such as longevity, late sexual maturation, low fecundity and long gestation periods (Stevens et al., 2000; Dulvy et al., 2008; Field et al., 2009). Consequently, many Elasmobranch species are impacted by artisanal fisheries, as well as recreational and industrial fishing for meat, fins, liver oil, or cartilage (Vannuccini, 1999). Early on, the lower commercial values of these species tended to limit direct exploitation (Walker, 1999; Molina and Cooke, 2012). Nowadays, however, estimates indicate that about 100 million sharks are caught annually, even when excluding illegal, unreported and unregulated catches (Liu et al., 2013).

Even with growing awareness of the vulnerability of most species of sharks and rays to exploitation (Castro, 1987; Camhi, 1998), attempts to manage species of this group have largely failed, essentially by the lack of basic information on catches, landings, and commercial sales. The correct management of any species, including Elasmobranch, is based on precise identification (Oliver et al., 2015; Davidson et al., 2016). In this case, however, morphological identification is made very difficult by the common fishing practice of cutting off the head, fins and tail, which are deposited at sea, to increase the space needed for storage and to preserve the meat longer (De Franco et al., 2012).

Among exploited sharks, angelsharks comprise the second most threatened Elasmobranch family globally, and they stand out in many regions of the world (Dulvy et al., 2014; Meyers et al., 2017). The angelshark genus *Squatina* (Squatinidae) comprises 22 extant,

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morphologically homogeneous, benthic species (Vooren and Da Silva, 1992; Compagno, 2005; Castro-Aguirre et al., 2006; Last and White, 2008), which inhabit continental shelves and upper slopes down to 500 m (Compagno, 2005). They are moderately sized (total length about 1–2 m) and globally distributed in temperate to tropical seas (Compagno, 2005; Last and White, 2008). While some species occur over a wide geographic range, most are restricted to a smaller area (Compagno, 2005). Among the species distributed in the coastal waters of southeastern Brazil and Patagonian central Argentina (Cousseau and Figueroa, 2001; Vooren and Klippel, 2005), the spiny angelshark *Squatina guggenheim*, the Argentine angelshark *S. argentina*, the Hidden angelshark *S. occulta* and the Atlantic angelshark *S. dumeril* are frequently landed and marketed, despite conservation laws controlling local fisheries. The morphological identification of such phylogenetically closely related species is particularly difficult, and Vaz and de Carvalho (2013) have only recently reviewed the taxonomy of the genus and validated three of these species, especially samples with morphological traits related to angelsharks.

Given these results, the development of molecular tools applied to species identification has gained in importance, especially for the quantification of exploited natural populations, evaluation and inspection of the trade of species under government protection, and the certification of processed products, adding value to their commercialization (Ogden, 2008; Migone and Howlett, 2012; Maralit et al., 2013; Helyar et al., 2014). Studies using genetic identification of fishery products are appearing in the literature with more frequency, and some of them have been directed to the shark group (e.g., Clarke et al., 2006; Sebastian et al., 2008; De Franco et al., 2012; Maduna et al., 2017).

In view of the capture and commercialization of Elasmobranch species worldwide, even legally protected species, the usual method of fish processing that prevents morphological identification, and the natural difficulties encountered when distinguishing a large number of species, the present study aimed to use DNA barcoding to track potential crimes against the landing and trade of endangered species along the São Paulo coastline.

2. Material and methods

2.1. Sampling

The samples were obtained between 2015 and 2016 from industrial fishing boats that use bottom trawls and from regional fish distribution markets in the coastal regions of São Paulo, Brazil (Fig. 1). Muscle fragments were collected from 85 carcasses declared by fishermen and traders only as cação (shark) in the cities of Ubatuba (13), Santos (15), Praia Grande (1), Peruíbe (1) and Cananéia (55). Researchers were able to identify morphological traits and relate them to angelsharks. Collected material was preserved in 95% ethanol and stored at −20 °C at the Laboratório de Genética Pesqueira e Conservação (GenPesC) at the Universidade Federal de São Paulo (UNIFESP), Campus Baixada Santista.

2.2. DNA extraction, amplification and sequencing

Genomic DNA extraction was done with the NucleoSpin® Tissue kit (Macherey-Nagel), following the manufacturer's instructions. Amplification of the mitochondrial cytochrome oxidase *c* subunit I gene (COI) was performed using the enzyme Platinum® Taq DNA Polymerase (Thermo Fisher Scientific) with the following primers: Forward - FishF1: 5'-TCA ACC AAC CAC AAA GAC ATT GGC AC-3' and Reverse - FishR1: 5'-TAG ACT TCT GGG TGG CCA AAG AAT CA-3' (Ward et al., 2005). After amplification, PCR products were subjected to enzymatic purification using the ExoSAP-IT™ PCR Product Cleanup Reagent (Thermo Fisher Scientific). Sequencing followed the BigDye® Terminator v3.1 Cycle Sequencing kit protocol (Thermo Fisher Scientific), and the sequences were generated by the ABI PRISM® 3100 Genetic Analyzer (Thermo Fisher Scientific) automatic sequencing platform.

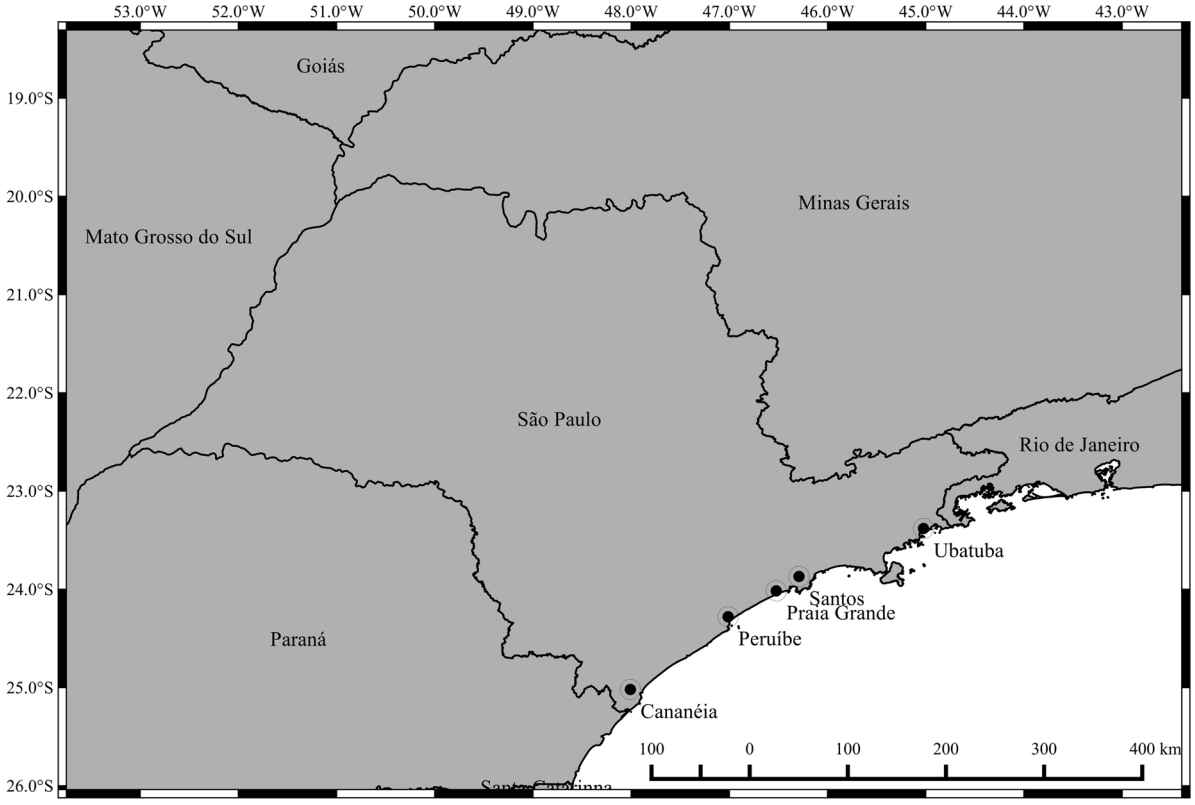

Fig. 1. Sampling by geographic location in the State of São Paulo, Brazil. Black dots represent the algelsharks sampling localities.