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Shark Finning and the Molecular Identification of Shark Species: Review and Perspectives

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Abstract

Worshipped and revered in several Indo-Pacific countries, and mostly feared among the western world, sharks are an old group of vertebrates dating back to the Devonian-Silurian boundary (~400Ma). Constantly represented as human predator by occidental movies, sharks have a spiritual appeal for several Indo-Pacific cultures. In contrast with this spiritual significance, in China sharks are a fierce animal believed to give strength and health for those whom consume their fins. They are also considered as a signal of prosperity and wealth. Fished for their meat and fins, several species are considered under high threat and are now facing extinction, with about 93% of nominal species included on the IUCN Red List. Mainly relying on the inefficiency of law enforcement authorities, the shark finning industry is a growing business with global scale actors and consequences. Understand the relation between spiritual beliefs, wealth and vitality, and the consumption of shark fins and meat is needed to precisely delineate the shark finning problem and to the development of efficient management and conservation policies. Molecular methods provide a valuable option for the identification of shark meat and body parts such as fins, although it still not consensual which one is the most appropriate.

Keywords: Sharks; Finning; Perspectives; Population genetics; Species identification; Forensics

Introduction

The Elasmobranchii is considered one of the most ancient and successful vertebrate lineages, been the most diverse clade of large predatory fishes with about 1200 species spread all over the world's seas [1,2], and including ocean- and freshwater-dwelling fishes, such as sharks, skates and rays. Sharks species play a crucial ecological role by acting as primary predators [3] and occupying an important position in marine ecosystems [4].

In the last decades, several studies prompted the accelerated depletion of natural stocks of several shark species in a global scale. Population declines ranging from 50% to almost extinction (about 99%) have been reported by several authors [5-10]. Constantly associated to uncontrolled exploitation of wild stocks the observed population decline is also due to some restrained biological features of sharks such as a slow growth rate, late sexual maturity associated with low fecundity levels and a high longevity [11].

Fished for their meat and fins, several shark species are considered under high threat and are now facing extinction [12], with about 93% of nominal species included on the IUCN Red List. Fourteen of these species figure as major targets for the shark finning industry: the blue shark Prionace glauca, the shortfin mako Isurus oxyryhnchus, the silky shark *Carcharhinus falciformis*, the dusky shark *Carcharhinus obscurus*, the sandbar shark *Carcharhinus plumbeus*, the tiger shark Galeocerdo cuvier, the scalloped hammerhead shark *Sphyrna lewini*, the smooth hammerhead shark *Sphyrna zygaena*, the great hammerhead shark *Sphyrna mokarran*, the common thresher shark *Alopias vulpinus*, the bigeye thresher shark *Alopias superciliosus*, the pelagic thresher shark *Alopias pelagicus*, the bull shark *Carcharhinus leucas*, and the oceanic whitetip shark *Carcharhinus logimanus*.

Shark finning is the fishing practice where sharks have their fins removed prior to the body being discarded [13], sometimes while they are still alive. Mainly guided by an association of traditional culture, spiritual beliefs, and social reasons, it is a banned fishing practice in several countries all around the world. Albeit illegal, it still remains as a lucrative option since high values are obtained with the fins. A mix of cultural and social behaviours allied with the needs of replenishment of a growing market made the shark finning a very profitable activity. Mainly relying on the inefficiency of law enforcement authorities, the shark finning is a growing business with widespread actors and consequences. The present manuscript reviews some of the published literature on shark finning and molecular identification of sharks, and delineates how the molecular approach could help on the implementation of management and conservation policies by law enforcement authorities.

Brief Cultural Background

Worshipped and revered in several Indo-Pacific countries, and mostly feared among the western world, sharks are a controversial group of old vertebrates. Constantly represented as human predators in occidental movies, sharks indeed have a spiritual appeal for several Indo-Pacific cultures [14]. Regarded as mythological deities, sharks are worshipped in Japan [14] and Fiji Islands, while in Vietnam the whale shark (*Rynchodon typus*) is revered, with sacred burial rituals given to its body remains [15]. In Hawaiian culture, sharks also have spiritual significance since they are regarded as similar to high royalty members [15-17], while in China, in contrast with this spiritual significance, sharks are regarded as a fierce animal, believed to give strength and health for who consume their fins. The consumption of shark fins is also considered as a symbol of prosperity and wealth.

Considered a part of the Chinese culture at least since the Sung dynasty (AD 960-1279), shark fin soup is a traditional dish served for the Japanese imperial lineages [18], since the risk and difficulty associated to its capture is regarded as a tribute to the emperor and its lineage [19,20]. Shark fins are also regarded as aphrodisiac and tonic [20], related with the traditional belief that eating them could bring health benefits. An additional social parameter should be considered since in China, seafood consumption is associated with the concept of wealth and prosperity [15]. As presented by Cheung and Chang [21], the consumption of shark fin soup can be regarded as a cultural product since serving seafood and especially the shark fin soup is commonly used to reinforce social position and respect among Chinese people [22].

Ranging from U$10 up to U$180 per bowl, depending on the species and the amount of fins used, Chinese consumers consider the species exclusiveness and some properties of the fin such as its color, thickness, and texture of the fin rays [20] when buying them for soup preparation. At the end of the line, these features directly influence which shark species are the most desirable for consumption and also the most exploited ones. This is the case for the hammerhead sharks of the genus Sphyrna, and carcharhinids such as *Carcharhinus longimanus*, *Carcharhinus leucas*, *Carcharhinus falciformis*, *Carcharhinus plumbeus*, for the threshers *Alopias superciliosus*, *Alopias pelagicus*, and *Alopias vulpinus*, the *mako shark Isurus oxyrinchus*, the basking shark *Cetorhinus maximus*, and also for the whale shark *Rhincodon typus* and for the great white *Carcharodon carcharias*.

Molecular Markers and Shark Species Identification

Despite the high values associated with the shark fins market, and the fact that it is widely accepted as the major factor for the shark populations decline, international managers still consider sharks as a by catch rather than a group of species which indeed require management from international authorities [13,20,23-25].

One of the most critical problems faced by law enforcement authorities on the control and management of oceanic sharks is the large absence of data [26]. Mainly due to species identifications issues, the under report of shark catches in fishery statistics is common. Clarke et al. [26] observed that shark species identification is often unreliable with more acceptable results limited to a few geographical locations, such as for the western North Atlantic, Japan, New Zealand, and several Pacific islands [8-9,25,27-32].

Obstacles on species identification are a global issue and the development and use of genetic approaches to achieve reliable species identification is globally disseminated [33-36]. Several recent studies addressed specifically the problem related with the identification of shark species using molecular approaches [37-43].

During the last decades several molecular identification techniques have been proposed to deal with shark species delimitation problems. Methods such as protein electrophoresis [41,44-46], restriction length polymorphisms (RFLPs) [39,42], PCR methods [40,47-53], species identification using insertion-deletion regions (indels) [54], and the nucleotide sequencing approaches mainly focused on mitochondrial genes and commonly using the DNA barcoding methodology, such as presented by several studies [55-70]. Pank et al. [47] used the nuclear ITS 2 regions to identify two *Carcharhinus species* (*C. plumbeus* and *C. obscurus*). The same methodology was later used by several authors which expanded it with the addition of several new species [43,52,71]. Abercrombie et al. [51] used the same method but with distinct primers for the identification of three large hammerhead sharks (*Sphyrna lewini*, *S. zygaena*, and *S. mokarran*), and confirmed the commerce of these species. Clark et al. [13] approached the shark fins and meat trade in Asian markets using a statistical approach based on the molecular identification of shark species by multiplex PCR methods. Some other studies using the 5S rRNA for shark species identification were also produced during the last decade. Pinhal et al. [72] used a 5S rRNA analysis on the identification of eight shark species (*Alopias superciliosus*, *Sphyrna lewini*, *Isurus oxyrynchus*, *Carcharhinus leucas*, Carcharhinus obscurus, *Carcharhinus limbatus*, *Carcharhinus acronotus*, and *Galeocerdo cuvier*). Pinhal et al. [73] expanded their previous analysis for the successful identification of two Rhizoprionodon species (*R. lalandii* and *R. porosus*). Morgan et al. [74] proposed a real-time qPCR approach on the identification of three closely related carcharhinid species (*Carcharhinus limbatus*, *C. tilstoni*, and *C. amblyrhynchoides*) based on the mitochondrial ND4 gene.

Among the nucleotide sequencing methods, Heist and Gold [39] used mitochondrial DNA sequencing on the identification of eleven species of *Carcharhiniformes*. Douady et al. [75] also used the mitochondrial DNA to examine the phylogenetic relationships of shark orders, and Greig et al. [55] used the same approach to identify thirty-five shark species from the North Atlantic. Rodrigues-Filho et al. [76] used mitochondrial DNA on the identification of eleven shark species exploited by fisheries in Brazil. Naylor et al. [61] presented a sequence-based approach using the mitochondrial NADH2 gene on the identification of 574 shark species from all around the world, while in the same year Caballero et al. [59] proposed a mix of new and previously published PCR multiplex on the identification of shark landings on the eastern tropical Pacific. Fields et al. [67] validated a mini-barcoding essay for use on degraded material such as processed shark fins, from where they identified seven of the eight CITES listed shark species (the *porbeagle*, *Lamna nasus*, oceanic whitetip, *Carcharhinus longimanus*, the scalloped hammerhead Sphyrna lewini, the smooth hammerhead, *S. zygaena*, and the great hammerhead *S. mokarran*).

Still using nucleotide sequencing methods but now in a DNA barcoding context [77], several authors used the first 650bp of the mitochondrial COI gene on shark species identification. One of the first DNA barcoding studies on sharks, Ward et al. [78] used mitochondrial COI barcoding sequences on the identification of sixty-one distinct shark species. Moura et al. [56] also used DNA barcoding methods on the identification of northeastern Atlantic deep-water sharks, discussing the use of the barcoding methodology as a tool for the assessment and implementation of management policies. Ward et al. [79] used COI sequences on the identification of 123 shark species, being successful for the vast majority of them. Wong et al. [57], although analyzing the barcoding region, proposed a character-based approach on the identification of 74 shark species, while Holmes et al. [44] focusing on dried fins retained by law enforcement authorities from illegal fisheries, identified and quantified the relative abundance of 20 shark species.

In the present decade, DNA barcoding remains a very popular tool. Barbuto et al. [58], used the classical DNA barcoding approach on the successful identification of frauds related with shark products sold as the species *Mustelus mustelus* and *Mustelus asterias* in Italy. Nicolè et al. [60] also used the methodology together with some secondary markers on seafood products identification, with a high success rate. Carvalho and Freitas [62] used the barcoding methods on the identification of shark fins from illegal fisheries retained by the Brazilian authorities, and successfully identified the species *Prionace glauca*, *Sphyrna zygaena*, and *Isurus oxyrinchus*. Liu et al. [63] analyzed the species composition of shark meat from fish markets in Taiwan, pointing the species Alopias pelagicus, *Carcharhinus falciformis*, *Prionace glauca*, and *Isurus oxyrinchus* as the most prevalent species on the Taiwan fin trade, while some CITES species were also found such as the great white *Carcharodon carcharias*, the oceanic whitetip shark *Carcharhinus longimanus*, and two hammerhead sharks *Sphyrna zygaena* and *Sphyrna lewini*. Espinoza et al. [68] presented the Mexican first efforts to combat the shark fin trade on the Mexican Pacific waters. The authors used DNA barcoding on the identification of six shark species (cf. *Prionace glauca*, *Carcharhinus falciformis*, *Carcharhinus limbatus*, *Alopias pelagicus*, *Mustelus henlei*, and *Rhizoprionodon longurio*) from confiscated samples provided by the Mexican Government Agency from exportation vessels at Mazatlán and Manzanillo ports. Sembiring et al. [65] and Prehadi et al. [66] successfully identified shark landings from Java Island, Indonesia using a molecular approach and discussed the diversity decline observed for the Indonesian sharks, while Bineesh et al. [69] used the same approach identifying sharks from the Indian commercial fishery. Recently Steinke et al. 2017 used a DNA barcoding approach coupled with a secondary barcoding marker, the 16S rRNA, to identify dried fins and gill plates from Canadá, China, and Sri-Lanka, founding twelve species cited or approved to be listed by CITES, with more than half of the identified species included within the IUCN Red List categories “Endangered” and “Vulnerable”.

However, despite the large number of available studies using the DNA barcoding methods, its use for species identification is far to be consensual since some studies argue that a single and short DNA region is not as reliable for species identification as the traditional systematic approach is [80]. Abercrombie et al. [51] pointed that one the most economical and streamlined approach for shark species identification is the one presented by Pank et al. [47] and Shivji et al. [71]. Their approach uses a multiplex of species-specific primers to produce specific amplicons related with each screened species. Without any post-amplification processes such as enzymatic digestion or nucleotide sequencing, the method exhibits a short hands-on time and low cost, perfectly fitting on low budgets such as those observed on countries from where the resources for biological management and conservation actions are limited.

Following a forensic standard approach, Pereira et al. [81] and Carneiro et al. [82] proposed a forensic method for species identification using mitochondrial insertion-deletion regions. This approach was recently applied to shark species identification by [54] that used indel regions from the mitochondrial 16S rRNA on the identification of shark species, including several figured on the IUCN Red List, and also included between the most prevalent species targeted by the shark finning industries. As presented by Carneiro et al. [82], indels are a rare type of polymorphisms that are less prone to recurrent and back mutations, therefore reducing the chances of misidentification. The authors observed that a high level of species discrimination could be easily achieved by determining and combining the length of hypervariable regions with indel variants. Some advantages of the method relate to its usefulness on diverse low-cost genotyping platforms and reagents such as conventional agarose or polyacrylamide gels. The method also enables inter laboratory comparison and permits the identification of samples from admixtures, being appropriate for low quantity and/or degraded DNA samples.

As can be foreseen, although with several identification methods available, the wildlife species identification, including shark fins or body remains, still struggles to achieve methodological consensus among researchers. Although a large number of results and methods are available in the literature, several of them are not inter comparable; the constructed databases are often unavailable for scrutiny, and reliable public databases are still unavailable for the vast majority of shark species.

Conclusions

In summary, wildlife researchers and government authorities working on shark finning and shark species conservation still struggle with the lack of standards for procedures and analyses, a condition needed for an efficient translation between the scientific knowledge and the development of management and conservation policies for wildlife species, including sharks. Although all methods exhibit advantages and disadvantages, the forensic approaches tend to be a bit more intelligible for government authorities. The presented information could be easily discussed among wildlife researchers, law enforcement entities, and also by judicial authorities within a court environment, from where several commercial disputes take place.

Shark finning is far to be under control and to understand the relation between the cultural and social aspects, the dynamics of the international illegal fishery, and the consumption of shark fins is crucial to precisely delineate the problem due to the large role it plays in sharks exploitation, and also in the global decline of shark populations. The molecular methods brought a new perspective for sharks management and conservation actions, since they provide scientifically reliable tools for data collection and analysis. The new sequencing technologies allied with a more comprehensive population sampling are also important since they made possible the identification of raw and processed materials such as fins and all sort of body remains, and a more reliable population assignment, therefore enhancing law enforcement mechanisms of monitoring and control of illegal shark fisheries.

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