Application of DNA mini-barcoding reveals illegal trade in endangered shark products in southern Africa

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In recent decades, a combination of increasing demand and economic globalisation has created a global market for elasmobranch products, especially the highly prized shark fins for Asian markets. Morphological species identification, as well as traditional cytochrome c oxidase subunit I (COI) barcoding of shark fins and other products, become challenging when in a processed state (such as dried or bleached shark fins). Here a mini-barcoding multiplex assay was applied to determine the species of origin in case studies from southern Africa involving confiscated shark fins in different states of processing. This highlights that the illegal shark fin trade in southern Africa to a large extent comprises threatened species. Matching of sequences of the confiscated fins against public databases revealed several threatened species, including the CITES-listed species Carcharhinus longimanus, Isurus oxyrinchus, Rhynchobatus djiddensis and Sphyra lewini. The findings highlight the need for improved trade monitoring, such as to eliminate illegal trade in shark fins, which can in part be achieved through more widespread genetic sampling of internationally traded products. However, a major limitation to DNA barcoding in general lies in the lack of curated voucher specimens available on public databases. To facilitate the application of molecular methods in a more comprehensive evaluation of elasmobranch trade regionally, a concerted effort to create reliable curated sequence data is recommended.

Keywords: Carcharhinus, case studies, COI gene, elasmobranchs, multiplex assay, Rhynchobatus djiddensis, shark fin trade, wildlife trade monitoring

Introduction

Over the past few decades there has been overexploitation of sharks on a global scale, primarily to supply international markets with products such as meat, skin, cartilage, liver and teeth (Clarke et al. 2006; Lack and Sant 2009; Dulvy et al. 2014). One of the most prominent of these markets is the shark fin trade whereby the fins of sharks and shark-like rays (such as wedgefishes and guitarfishes) are used for shark fin soup. This dish is a delicacy in some Asian countries and particularly in Hong Kong, which is considered a major fin trade hub (Fields et al. 2018; Cardeñosa et al. 2020). Worldwide, the main species targeted for the shark fin industry include blue shark Prionace glauca, shortfin mako shark Isurus oxyrinchus, silky shark Carcharhinus falciformis, dusky shark C. obscurus, sandbar shark C. plumbeus, tiger shark Galeocerdo cuvier, bull shark C. leucas, scalloped, smooth and great hammerhead sharks Sphyra lewini, S. zygaena and S. mokarran, common, bigeye and pelagic thresher sharks Alopias vulpinus, A. superciliosus and A. pelagicus, oceanic whitetip shark C. longimanus, and more recently also shark-like rays of the families Rhinidae (wedgefishes) and Rhinobatidae (guitarfishes) (Amaral et al. 2017; Fields et al. 2018). These species are targeted directly or caught as incidental bycatch (Worm et al. 2013; Oliver et al. 2015) and used to supply a market that is largely unmonitored and unregulated. Therefore, more than half of the chondrichthyan species that enter the fin trade are under threat (Dulvy et al. 2014).

Sharks and other chondrichthyan species are vulnerable to overexploitation owing to their K-selected life-history characteristics, such as slow growth, late attainment of sexual maturity, low fecundity and long gestation periods (Dulvy et al. 2014; Hutchinson et al. 2015). Consequently, there is evidence of widespread shark and ray population declines (Davidson et al. 2016), and as of 2021, at least...
32% of all shark and ray species globally are listed as threatened with high, very high or extremely high extinction risk (Vulnerable, Endangered or Critically Endangered, respectively) (Dulvy et al. 2021), according to the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (IUCN 2021). Even though a recent survey showed that at least 9% of the current global catch of sharks is biologically sustainable (Simpfendorfer and Dulvy 2017), mounting evidence suggests that apex shark populations are more vulnerable to exploitation than previously thought and ongoing declines are of major concern (Roff et al. 2018; MacNeil et al. 2020). The monitoring and sustainable use of sharks is especially important as they are among the most evolutionarily distinct fish lineages and play important structural and functional roles as apex predators or mesopredators, thus helping to maintain stable and functional marine ecosystems (Stevens et al. 2000; Heithaus et al. 2012).

For many commercially important shark species, catches are unregulated and seldom recorded to species level, preventing the development of effective shark management strategies (Barker and Schlussel 2005). Further lack of species-specific data (e.g. catch rate, annual landings and bycatch/discard level) stems from misidentified species or elasmobranchs that have been discarded at sea, and because fisheries report only retained (landed) catches (Lack and Sant 2009; FAO 2014). For multi-species fisheries, species identification during port inspections is highly challenging if using traditional morphological or taxonomic tools, as carcasses are usually processed at sea, where key distinguishing morphological features such as heads and fins of specimens are often removed (Abercrombie et al. 2005; Mendonça et al. 2010; Gulak et al. 2017). Additionally, morphological features are frequently similar between species—such as for carcharinids like the common blacktip C. limbatis and the Australian blacktip C. tilstoni (Tillet et al. 2012)—making discriminant species identification difficult. In the case of morphologically similar species, catch data are often aggregated for several species, thus making catch and landings data of low resolution (Dulvy et al. 2000; Barausse et al. 2014; Williams 2017). Consequently, aggregated data may conceal trends within individual species, with the decrease of one species being compensated for by increases in others (Dulvy et al. 2000). Additionally, data from scientific surveys may also be confounded by misidentification where species lack an unambiguous phenotype-based identification method (Marino et al. 2017).

Molecular-based methods have regularly been used over the last decade as alternatives to morphological identification (Amaral et al. 2017). These molecular techniques include DNA barcoding and sequence-based identification methods (Ward et al. 2008; Bineesh et al. 2017). Importantly, this method has also been effective in revealing the mislabelling of shark products, as well as identifying threatened species in the shark fin trade and trade of other shark products (Liu et al. 2013; Moore et al. 2014; Cardeñosa et al. 2017; Steinke et al. 2017; Hobbs et al. 2019). Shark fins in the trade can, however, be found in numerous stages of processing, some of which can reduce the efficacy of the standard COI barcoding approach. Wet fins are those that have been removed from a recently harvested shark (not dried or processed further) and still contain skin (Abercrombie et al. 2018). Most fins entering the international trade are dried but unprocessed and are rigid, still containing both skin and cartilage. Both wet and dried, unprocessed fins generally contain genomic DNA of sufficient quality that can be amplified using PCR (Abercrombie et al. 2018). However, fins can also be processed, dried and chemically treated to remove the skin, and these processed fins are typically a yellow or golden colour. Processed fins often contain degraded genomic DNA, meaning that the DNA has broken down into very small DNA fragments, often incompatible with the use of standard genetic identification techniques that require non-degraded DNA (Abercrombie et al. 2018). To overcome this problem, a DNA mini-barcode assay was developed by Cardeñosa et al. (2017), whereby shorter COI gene fragments are amplified simultaneously in a single multiplex-PCR and one to two downstream DNA sequencing reactions, to achieve the genetic identification of species when dealing with processed shark fins. This method has been successfully applied in several cases, thereby leading to successful species identification despite the shorter information content of the generated sequences (Hellberg et al. 2019; Cardeñosa et al. 2020).

In South African fisheries, the misidentification of shark species in fisheries operations is a major concern (da Silva et al. 2015). The five commercially valuable inshore species that are commonly targeted in South Africa include the common smoothhound Mustelus mustelus, whitespotted smoothhound M. palumbs, tope shark Galeorhinus galeus, copper shark C. brachyurus and dusky shark C. obscurus (da Silva and Bürgener 2007; da Silva et al. 2015; 2018). When shark carcasses arrive at processing facilities, the fins are removed, after which the sharks are filleted and skinned. Most of the meat of processed demersal sharks is exported to Australia, primarily for the fish and chips trade, while fins are dried and exported to Hong Kong, particularly in the case of species with more-valuable fins (da Silva and Bürgener 2007). Under the Marine Living Resources Act (MLRA, Act No. 18 of 1998: RSA 1998), shark finning (the process of removing the fins and then discarding the carcass) is prohibited in South Africa. However, fins detached from carcasses of sharks that are caught in international waters may be landed in South Africa.

In Mozambique, shark fins have been exported for at least two decades, through several companies licenced to export them (Pierce et al. 2008). However, in legal instruments introduced recently in Mozambique—such as the biodiversity law (no. 5/2017, de 11 de Maio), the CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) regulation (Decreto 34/2016, de 25 de Agosto)
Case study 1 — The first case study involved 109 pieces of shark fins confiscated by the Mozambique Customs Authority in Maputo, Mozambique, on 12 December 2018 and 11 January 2019, and believed to originate from two different locations along the Mozambican coast, hereinafter referred to as Location A and Location B. The National Institute of Fishery Research (Instituto Nacional de Investigação Pesqueira, IIP), Mozambique, requested DNA analysis of the shark fin samples to confirm species identification. These samples comprised fin pieces that were cut into irregular shapes and were of different colours and forms (Figure 1). The samples were extremely desiccated and had apparently been treated with chemicals, which were visible upon inspection. DNA was successfully extracted from a total of 89 samples for further analysis: 43 which were visible upon inspection. DNA was successfully desiccated and had apparently been treated with chemicals, colours and forms (Figure 1). 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cycle of initial denaturation at 95 °C for 2 min; (ii) 35 cycles of denaturation at 94 °C for 1 min, annealing at 54 °C for 1 min, elongation at 72 °C for 1 min; and (iii) a final elongation of one cycle at 72 °C for 10 min.

The PCR amplicons were visualized on a 3% (w/v) agarose electrophoresis gel for confirmation of successful amplification of the three amplicons (Shark150, Shark474, and Full COI). For standard Sanger sequencing chemistry (BigDye® Terminator 3.1 Cycle Sequencing Kit, Life Technologies, South Africa), the M13F primer (5'-TCAACCAACCATGACCGTGAGCAGCTAGCG-3') and the recommended PCR protocol outlined in Ward et al. (2005). Amplification success was confirmed for all 24 samples. The IUCN Red List status of the identified species include the following categories: Critically Endangered (R. djiddensis, Carcharhinus longimanus and S. lewini), Endangered (A. amblyrhynchos and I. oxyrinchus), Vulnerable (Hemipristis elongata, Carcharodon carcharias, Carcharhinus albimarginatus, C. amboinensis, C. leucas and C. brachyurus) and Near Threatened (P. glauca and C. cuvier) (IUCN 2021). Five species were identified in multiple samples, namely S. lewini, I. oxyrinchus, C. longimanus, C. cuvier and R. djiddensis, which could be an indication for the targeting of species containing fins with a higher market value.

Application of the mini-barcoding assay to the second case study demonstrated that all samples amplified for the 150-bp and the 200-bp fragment, and one sample for the 650-bp fragment. After comparing specimen sequences to reference sequences on the BOLD and NCBI GenBank databases, it was concluded that, for the 200-bp sequences, species-level identification could not be made but confirmed them to be from the genus Mustelus. Top hits consisted of M. manazo, M. asterias and M. palumbes; however, M. manazo and M. asterias do not occur in South African waters so it is more likely that the specimens are whitespotted smoothhound M. palumbes. This indicates that the 200-bp COI fragment alone is not sufficient to identify Mustelus species to species level. Therefore, in cases involving closely related species such as within the genus Mustelus, sequence data from both the 150-bp and the 200-bp fragment should be generated to potentially provide species-level identification.

In the third case study, which involved confiscated shark fins at OR Tambo International Airport, all samples amplified successfully for the 150-bp gene fragment, eight samples for the 200-bp, and two also for the 650-bp fragment. A total of six shark species were identified from the 10 fins sampled:

Table 1: Five mitochondrial COI primer names, sequences and volumes (initial concentration = 10 μM) used in the mini-barcoding multiplex PCR of 15 μl (adapted from Cardeñosa et al. [2017]), amplifying three COI gene regions (150 bp, 200 bp, and 650 bp)

| Primer name | Primer sequence (5’–3’) | Reference | Volume (μl) |
|-------------|------------------------|-----------|-------------|
| VF2_tl      | TGTAACACCGAGGGCCAGCTCAACCAAACCAAGACATTGGCCAC | Ward et al. (2005) | 0.9000 [0.6 μM] |
| FishR1_tl   | CAGGAAAACAGCTTAGACACTTCAAGGGTGACCACAAGAATCAGA | Ward et al. (2005) | 0.4500 [0.3 μM] |
| FishR2_tl   | CAGGAAAACAGCTTAGACACTTCAAGGGTGACCACAAGAATCAGA | Ward et al. (2005) | 0.4500 [0.3 μM] |
| Shark150R   | AGAGATACAAAGCCGTTGGGG | Fields et al. (2015) | 0.2250 [0.15 μM] |
| Shark474F   | CHATTATCCAAATATCAACACC | Cardeñosa et al. (2017) | 0.1125 [0.075 μM] |
C. leucas, C. limbatus and C. amblyrhynchoideis, were represented more than once. Of the nine fin-clip samples from the case studies that were tested using traditional COI barcoding amplification (FishF1 and FishR1 primers), only two amplified successfully for the full 655-bp region. However, using the multiplex assay, all nine samples amplified successfully for both the 150-bp and the 200-bp fragment, as well as two samples for the 650-bp fragment. These results confirm the limitations of using only the traditional COI barcoding primers when samples are processed, as was the case in the current study.

**Discussion**

Overall, the application of the mini-barcoding assay in case studies involving confiscated shark fin samples demonstrates that several CITES-listed and threatened elasmobranch species are traded through southern Africa. The multiplex assay was characterised by a high identification success rate compared with the traditional COI barcoding method for the processed (dried or chemically treated) samples tested here. Although there were some difficulties with species-level identification, this was not totally unexpected as the mini-barcoding assay was specifically designed for CITES-listed species and was previously found not to be successful for all *Carcharhinus* species (Cardeñosa et al. 2017).

**Case study 1**

At the time of the confiscations in Mozambique (Locations A and B), approximately 40% of the samples tested were from CITES-listed species; however, based on updated and current (2021) CITES listings, over 80% of the samples tested represent species listed on CITES Appendix II, which are thereby subject to trade regulation, including...
**Carcharodon carcharias**, *Carcharhinus longimanus*, *I. oxyrinchus*, *R. djiddensis* and *S. lewini* (CITES 2021). Hammerhead sharks (*Sphyra* spp.) are among the top sources of shark fins as they have the finest quality fin needles (ceratotrichia) for consumption and have a high commercial value in the Asian shark fin trade (Abercrombie et al. 2005). *Sphyra lewini* is experiencing severe population declines throughout its distribution (Ferretti et al. 2008; Gallagher et al. 2014). In South Africa, a decline of 64% was observed for *S. lewini* populations over a 25-year period (1978–2003), with estimates based on catches in the bather protection nets along the coastline of KwaZulu-Natal Province (Dudley and Simpfendorfer 2006). The species was recently re-assessed as Critically Endangered (Rigby et al. 2019) on the IUCN Red List. Of greatest concern for *S. lewini*, in the context of this study, is its considerable contribution (46%) to the 89 sequenced fins that were confiscated in case study 1, indicative of the intense fishing pressure on this species.

In addition to its CITES listing that requires strict trade control, *C. longimanus* is required to be prohibited from capture in all fisheries within party states by virtue of its listing on Appendix I of the Convention on the Conservation of Migratory Species of Wild Animals (CMS) (CMS 2020) and also is prohibited from capture in tuna and tuna-like fisheries in the Indian Ocean, through a retention ban defined under Resolution 13/06 of the Indian Ocean Tuna Commission (IOTC 2013). Mozambique is a party state with respect to both the CMS and IOTC and therefore the commercial exploitation and trade of this species in Mozambican waters contravenes the regulations of numerous multilateral environmental agreements.

*Isurus oxyrinchus* was identified as constituting the second-largest portion of the samples in case study 1. This is concerning for an Endangered species, as a recent study showed fishing mortality rates were well above those previously reported for the species in the western North Atlantic Ocean (Byrne et al. 2017). According to Fields et al. (2018), 2.77% of samples from the main fin markets in Hong Kong (i.e. Sheung Wan and Sai Ying Pun) consisted of *I. oxyrinchus*. This species was recently listed in CITES Appendix II (CITES 2019).

Also noteworthy is that three of the samples confiscated in Mozambique were identified as *R. djiddensis*. This species belongs to the batoid family Rhinidae (wedgefishes), which are large benthopelagic shark-like rays (Giles et al. 2016). *Rhynchobatus djiddensis* is exploited by fisheries that are driven by the high value of their fins in international trade, and declines have been noted throughout their range (Moore 2017; Jabado 2018). A recent trend shows that the fins of wedgefishes are becoming more common in the shark fin trade (Fields et al. 2018). Declines of *R. djiddensis* have been observed in South Africa (Daly et al. 2021) and in Mozambique, where this species was previously reported to be abundant (Pierce et al. 2008; Hopkins 2011). In South Africa, *R. djiddensis* was caught as bycatch by demersal prawn trawlers operating on the Thukela Bank (located off central KwaZulu-Natal), until the fishery closed in 2002 (Jordaan et al. 2021). Most specimens caught were alive and were released, although subsequent survival is not known (Fennessy 1994). In Mozambique, *R. djiddensis* is caught as a target and bycatch species in the artisanal and small-scale commercial fisheries operating in Inhambane and Sofala provinces (Pierce et al. 2008) and has become one of the most-exported species according to fin inspection reports from the fishery sector (INIP 2020). Heavy exploitation of wedgefishes also used to occur in Tanzania by means of bottom-set gillnets, prawn trawlers and possibly also spearfishing (Barnett 1997); however, their numbers are declining and it is now considered by some to be rare (Schaeffer 2004). Additionally, wedgefishes are targeted by foreign vessels off eastern Africa (offshore of Mozambique, Tanzania and Madagascar) (Kyne et al. 2020). Thus, for *R. djiddensis* the threat to the population seems not to be within South Africa, where this species is protected, but rather in neighbouring countries where the species is under severe threat of exploitation (Kyne et al. 2020; Daly et al. 2021). Recently, species in the family Rhinidae have shown severe population declines globally, resulting in 9 of the 10 species (90%) being assessed as Critically Endangered on the IUCN Red List (Kyne et al. 2020), including *R. djiddensis* (Kyne et al. 2019). All 10 rhinid species were also recently included in CITES Appendix II (CITES 2019).

The remaining 24 samples from case study 1 that could not be identified to species level were all identified to genus level (*Carcharhinus* spp.). The genomic DNA obtained from these samples was degraded because the samples were dried and apparently treated with chemicals.

In terms of the different locations, for Location A there was a greater diversity of species, with 10 different elasmobranch species identified. Three of these were represented more than once, namely *S. lewini*, *R. djiddensis* (both Critically Endangered) and *G. cuvier* (Near Threatened), while 23 samples were identified as belonging to the genus *Carcharhinus*. While incidental bycatch cannot be excluded, the findings are more likely due to the targeting of larger shark species and particularly those known to have higher-value fins. For Location B, four species were identified, *S. lewini*, *C. longimanus* (Critically Endangered), *I. oxyrinchus* (Endangered) and *P. glauca* (Near Threatened), the first three of which are CITES-listed. Both *S. lewini* and *I. oxyrinchus* are common in trade because of their high fin value (Abercrombie et al. 2005; Fields et al. 2018). Overall, the findings of this case study indicate the continuous exploitation of elasmobranch species listed by CITES or regarded as threatened by the IUCN (i.e. Critically Endangered, Endangered and Vulnerable), as well as (and possibly specifically for) the trade in their fins. Additionally, the fact that most of these fin samples were disguised into smaller pieces suggests that deliberate attempts were made to prevent identification of the species, highlighting the importance of molecular species identification—in addition to visual identification—for improved law enforcement with regard to the illicit shark fin trade.

**Case study 2**

Shark fins confiscated from Cape Town Harbour were morphologically identified as *M. mustelus*; however, based on COI sequencing (200-bp fragment), they were most
likely *M. palumbes*. This conclusion was drawn since the other *Mustelus* species with high sequence similarity do not occur in South Africa. For future case studies involving closely related species such as *Mustelus* spp., additional COI or other gene-region sequences should therefore be included if possible. The results highlight the problem of morphological misidentification, which in this case could be attributed to the fact that the samples were from juvenile specimens. Some identification features are not yet developed or visible in juveniles, making morphological identification more difficult and less accurate. For instance, spot patterns in *M. mustelus* can range from the absence of markings to the presence of large black spots, with the spots increasing in number with age (da Silva et al. 2018). *Mustelus palumbes*, by contrast, is covered with numerous small white spots (Compagno 1984; Farrell et al. 2009; da Silva et al. 2018). *Mustelus palumbes* is currently classified as Least Concern on the IUCN Red List and is endemic to southern Africa (Namibia, South Africa and Mozambique: Pollom et al. 2020). *Mustelus mustelus* and *M. palumbes* are both common species caught in the suite of commercial fisheries targeting inshore species in South African waters, including the demersal shark longline fishery, the commercial linefishery and the inshore trawl fishery (DAFF 2012). However, since the two species occur at different depths, the overall majority of aggregated *Mustelus* reported in the inshore trawl fishery is likely to be *M. mustelus*. In addition, a recent stock assessment for *M. mustelus* showed that they were not currently overexploited but that the stock is fished at unsustainable levels (da Silva et al. 2019).

**Case study 3**

Shark fins confiscated from OR Tambo International Airport were all identified to species level using the mini-barcoding approach (Table 2). Samples were assigned to six species in the genus *Carcharhinus* but none of them matched the original morphological identification of either *C. amblyrhynchos* or *Sphyra* spp. In a recent study conducted in 2014–2015, *C. leucas*, *C. limbatus* and *C. brevipinna* were three of eight species that each comprised more than 1% of the fin trimmings from an assessment of a retail market (Sheung Wan and Sai Ying Pun fin market) in Hong Kong (Fields et al. 2018). In the same study, *C. amblyrhynchos*, *C. amboinensis* and *C. plumbeus* are also mentioned as being sought after, specifically for the shark fin trade. A few *C. amblyrhynchos* samples (0.13%) were identified from the 2014–2015 trimmings, while 54 samples of *C. amboinensis* (1.13%) were identified (Fields et al. 2018). This is concerning as *C. amboinensis* seems to be highly structured genetically, making coastal populations even more vulnerable to localised overexploitation (Chapman et al. 2015).

Previously, *C. plumbeus* was commonly found in the Hong Kong shark fin auction trade, making up 2–3% of the fins auctioned (Clarke et al. 2006). However, in the recent study by Fields et al. (2018), *C. plumbeus* was rarely encountered, with only 11 samples (0.23%) identified from the trimmings collected during 2014–2015. Fisheries located on the coast of Western Australia and on the Atlantic coast of the United States were supplying large amounts of *C. plumbeus* from 1999–2001 (McAuley and Rowland 2012). Subsequently, significant population declines of this species led to large reductions in catch limits (McAuley and Rowland 2012). Thus, the current study further confirms that the above-mentioned species are of some importance for the shark fin trade and market in Hong Kong. These results also highlight that those policies aimed at mitigating the vulnerability to extinction of certain shark species need to be comprehensive and coordinated at the global level.

**Conclusions**

The above case studies involving confiscated shark fins demonstrate that the mini-barcoding multiplex assay can elucidate species-level identification for many threatened southern African shark and ray species, although for closely related species (such as *Mustelus* spp. and some *Carcharhinus* spp.) it is not always successful in identification to species level, and hence alternate COI or other gene fragments should also be analysed.

One important limitation is the lack of voucher information for many species, not just for the study region, but also globally. Studies have previously reported on the prevalence of misidentifications in databases such as NCBI and BOLD, which severely hampers accurate species identification in different taxa (Meiklejohn et al. 2019; Wannell et al. 2020). In elasmobranchs, levels of species misidentification based on morphology are high owing to the occurrence of cryptic species and the overlap of morphological traits between species. Additionally, taxonomic revisions commonly render sequence database depositions outdated and therefore require ongoing curation (Wannell et al. 2020). Complete curated data sources are undoubtedly the most important aspect for correct species identification of confiscated material, irrespective of the barcoding methodology used and state of processing (Fernandes et al. 2020). To facilitate the use of this barcoding assay in support of law enforcement, greater effort should be directed to the collection and curation of voucher DNA barcode sequences.

Although this study may not be representative of all elasmobranch species being traded through southern Africa, it confirms that several threatened species are targeted and exploited. Of great concern is the large percentage of confiscated shark fins from CITES-listed and threatened species (*S. lewini, I. oxyrinchus, C. longimanus* and *R. djiddensis*), including for illicit trade. Based on one of the case studies, South Africa possibly acts as an intermediate transportation zone (for example, between other western Indian Ocean countries and Hong Kong, in this case) for the export of shark fins. This highlights the importance of monitoring and enforcement of existing regulations and coordination among countries, which could to a certain extent be achieved through improved implementation and stricter enforcement of CITES trade controls.

**Acknowledgements** — We thank collaborators and field scientists for assisting with the collection of the case study samples vital to this research: the former South African Department of the Environment, Forestry and Fisheries (DEFF), Eugene Swart (of the former Department of Environmental Affairs), the Mozambique National Fisheries Research Institute (Instituto Nacional de
Investigaçao Pesqueira), the Mozambique Customs Authority, the Mozambique National Administration for Conservation Areas (Administração Nacional das Áreas de Conservação), and the Mozambique National Institute of Fish Inspection (Instituto Nacional de Inspeção do Pescado). The Central Analytical Facilities at Stellenbosch University are acknowledged for providing capillary electrophoresis. Funding that enabled this research was provided by the National Research Foundation (NRF) of South Africa. Aspects of this project were also funded by the Shark Conservation Fund, a philanthropic collaborative for pooling expertise and resources to meet the threats facing the world’s sharks and rays. The Shark Conservation Fund is a project of the Rockefeller Philanthropy Advisors.

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