

DNA barcoding reveals the diversity of sharks in Guyana coastal markets

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A fundamental challenge for both sustainable fisheries and biodiversity protection in the Neotropics is the accurate determination of species identity. The biodiversity of the coastal sharks of Guyana is poorly understood, but these species are subject to both artisanal fishing as well as harvesting by industrialized offshore fleets. To determine what species of sharks are frequently caught and consumed along the coastline of Guyana, we used DNA barcoding to identify market specimens. We sequenced the mitochondrial *coI* gene for 132 samples collected from six markets, and compared our sequences to those available in the Barcode of Life Database (BOLD) and GenBank. Nearly 30% of the total sample diversity was represented by two species of Hammerhead Sharks (*Sphyrna mokarran* and *S. lewini*), both listed as Endangered by the International Union for Conservation of Nature (IUCN). Other significant portions of the samples included Sharpnose Sharks (23% - *Rhizoprionodon* spp.), considered Vulnerable in Brazilian waters due to unregulated gillnet fisheries, and the Smalltail Shark (17% - *Carcharhinus porosus*). We found that barcoding provides efficient and accurate identification of market specimens in Guyana, making this study the first in over thirty years to address Guyana's coastal shark biodiversity.

Keywords: BOLD, Carcharhinidae, Elasmobranch, Guianas, Sphyrnidae.

Um desafio fundamental para a pesca sustentável e a proteção da biodiversidade nos neotrópicos é a identificação precisa das espécies. A biodiversidade dos tubarões costeiros da Guiana é pouco compreendida, porém essas espécies estão sujeitas tanto à pesca artesanal quanto à pesca industrializada não costeira. Para determinar quais espécies de tubarões são frequentemente capturadas e consumidas ao longo do litoral da Guiana, utilizamos DNA barcoding para identificar espécimes comumente encontrados e adquiridos em mercados. Nós sequenciamos o gene mitocondrial *coI* para 132 espécimes adquiridos de seis mercados e comparamos estas sequências com as disponíveis no Barcode of Life Database (BOLD) e GenBank. Quase 30% da diversidade total amostrada foi constituída por duas espécies de tubarões martelo (*Sphyrna mokarran* e *S. lewini*), ambas listadas como espécies ameaçadas pela UICN. Outras porções significativas da amostragem incluem Cações-Frango (23% - *Rhizoprionodon* spp.), considerados vulneráveis em águas brasileiras, devido a pesca de arrasto não regulamentada, e o Cação-azeiteiro (17% - *Carcharhinus porosus*). Descobrimos que o barcoding é uma forma identificação eficiente e precisa para espécimes de mercado na Guiana, tornando este estudo o pioneiro na documentação da biodiversidade dos tubarões costeiros da Guiana.

Palavras-chave: BOLD, Carcharhinidae, Elasmobrânquios, Guianas, Sphyrnidae.

Introduction

Many sharks, relative to teleost fishes, have delayed reproductive maturity and relatively few, if precocial, young. These reproductive tendencies make shark populations susceptible to overfishing, as large, mature adults are typically the individuals targeted by fisheries (Dulvy, Forrest, 2010). Particularly devastating to sharks is the large demand in Asian markets for shark fins, which are sometimes removed from live animals before the carcass is landed in markets. These fins, once frozen, dried, and distributed to consumers are

extremely difficult to identify to species - making the effect of the shark-finning industry difficult to track and monitor (Clarke *et al.*, 2007).

The biodiversity of the Neotropics and the waters of the Guiana Shield drainages are particularly rich. However, neither the marine fish resources nor coastal fisheries of Guyana are well-understood, compared to the country's freshwater ichthyofauna. For example, a recent IUCN report on conservation of chondrichthyans in the Atlantic and Caribbean does not even mention Guyana, Suriname, or French Guiana (Carlson *et al.*, 2012). Guyanese artisanal

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fisheries make use of several different gear types, including nylon gillnets and fyke nets (locally called ‘Chinese’ seines) set within close proximity to shore, as well as monofilament driftnets set further from shore in deeper waters (< 40 m in depth, 12-60 km off the coastline). The driftnet fishery is directed at both demersal and pelagic species, by two sets of fishers, those who make day-trips and those who go on longer, 10-12-day trips. Survey data on shark species in Guyana are sparse, with studies by Brown (1942) and Mitchell, Lowe-McConnell (1960) reporting high elasmobranch abundances in longline (cadell) fisheries, and recommending establishment of a directed fishery for sharks (Rathjen *et al.*, 1969). This directed ‘cottage’ fishery was established in the early 1980s, and shark carcasses are reported to have been purchased by exporters before even being landed in markets, perhaps driven by increasing demand for shark fins to Asia (Maison, 1998). While most of the meat products from the shark fishery are consumed locally (as salted fish), fins and vertebrae are exported. The proportion of the catch which is exported *vs.* locally-consumed is difficult to evaluate (Maison, 1998; Shing, 1999).

Over the past decade, the need for rapid species identification, particularly to monitor morphologically cryptic species, has increased concomitantly with the need to survey biodiversity in regions of elevated risk from human development. DNA barcoding using universal primers for the cytochrome oxidase I mitochondrial gene (*col1*), allows for rapid and reproducible assessment of species identification, both in the field and in consumer markets. The success and accessibility of barcoding technology has led to a wealth of publicly-available sequences in the Barcode of Life Data System (BOLD; Ratnasingham, Hebert, 2007). Several

studies have used the BOLD database to identify seafood products, including shark fins (Wong, Hammer, 2008; Holmes *et al.*, 2009; Fields *et al.*, 2015). In Guyana, sharks caught in offshore driftnet fisheries are landed in markets without their heads (Fig. 1), leaving the relative placement and size of the fins as the key features for species identification. In order to manage coastal resources more efficiently and sustainably, a catalogue of coastal fish biodiversity is needed as a foundation.

Understanding what species are present in a region is the first step in both conserving aquatic biodiversity and promoting sustainable fisheries. A DNA barcoding approach was used to survey the shark species landed across six coastal Guyanese fish markets. The objectives of this study were to: (1) use DNA barcoding to determine the diversity of species landed, and (2) determine what proportion of collected samples are species-at-risk based on the classification of the International Union for Conservation of Nature (IUCN).

Material and Methods

Sampling sites. Shark tissue samples ($n = 144$; muscle or fin clips in 95% ethanol) used in this study were collected from six fish markets, spanning the populated Guyana coastline (Meadow Bank and Mahaica markets, Demerara-Mahaica Region; Parika market, Essequibo Islands-West Demerara Region; Berbice and Rosignol markets, Mahaica-Berbice Region; Albion market, East Berbice-Corantyne Region; Fig. 1). Shark carcasses landed in these markets are typically decapitated and gutted, and have undergone moderate to significant exposure to sun and the elements, typically with little refrigeration. Local names for each carcass sampled were also recorded.

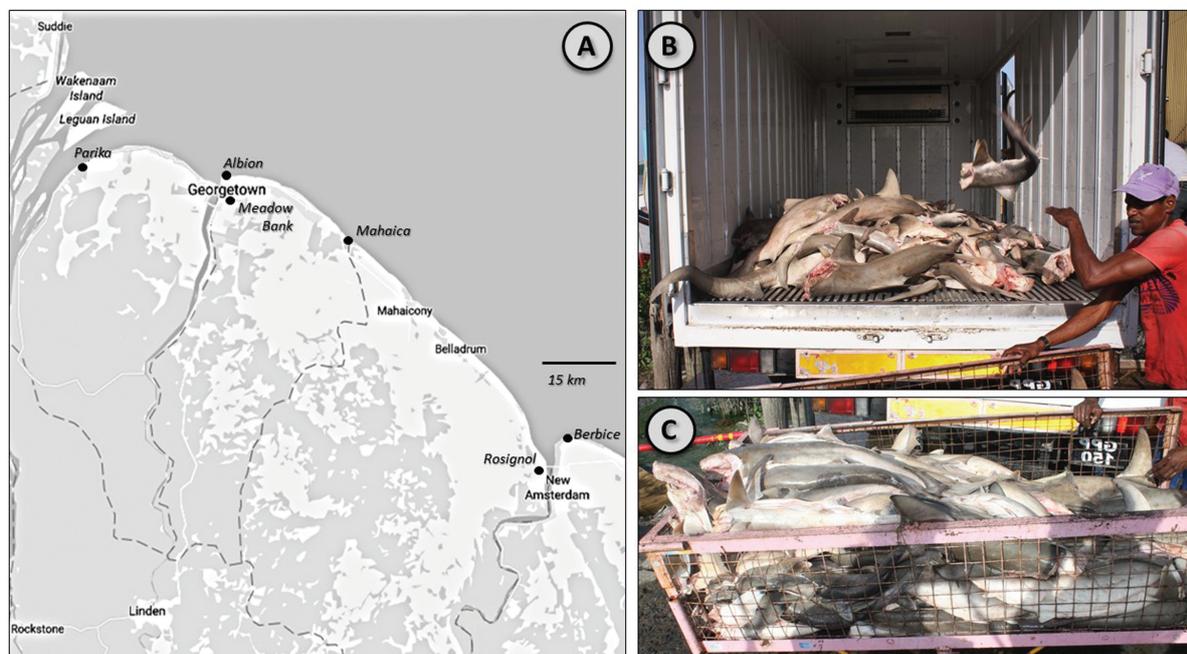


Fig. 1. Inset a. Map of coastal Guyanese fishing towns. Towns in italics represent locations of fish markets sampled for this study. Insets b. and c. Photographs of decapitated sharks landed at Meadow Bank market.

DNA extraction, PCR, and sequence acquisition. Whole-genomic DNA was extracted from each sample using the DNeasy blood and tissue kit, following manufacturer's instructions (Qiagen, Inc., Valencia CA, USA). An approximately 500 bp fragment of the mitochondrial cytochrome oxidase 1 (*col*) gene was amplified using a primer cocktail from Ward *et al.* (2008). Amplification reactions for *col* were performed in 25.0 μ L reactions, with 1x Taq buffer (with 60% v/v KCl, 40% $(\text{NH}_4)_2\text{SO}_4$), 1.5 mM of MgCl_2 , 0.5 μ L of dNTPs (10mM), 1U Taq polymerase

(ThermoScientific, Waltham, MA), 2.0 μ L DNA, 0.2 μ M each of primer: 'FishF1-M13,' 'FishF2-M13,' 'FishR1-M13,' and 'FishR2-M13,' with the remaining volume composed of molecular H_2O . Thermal cyclers conditions were as follows: initial denaturation step of 95 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, 54 °C for 45 s, 68 °C for 45 s, and a final extension phase of 72 °C for 10 min. A newly-designed sequencing primer and previously-published primers (Tab. 1) were used to sanger-sequence the amplified *col* fragment at the Centre for Applied Genomics (TCAG, Toronto, Canada).

Tab. 1. Primers used in this study. *M13 tail sequences are between brackets.

Primer	Sequence (5'-3')*	Reference
FISHF1_M13	(TGTAACACGACGGCCAGT)CAACCAACCACAAAGACATTGGCAC	Ward <i>et al.</i> (2005)
FISHF2_M13	(TGTAACACGACGGCCAGT)CGACTAATCATAAAGATATCGGCAC	Ward <i>et al.</i> (2005)
FISHR1_M13	(CAGGAAACAGCTATGAC)TAGACTTCTGGGTGGCCAAAGAATCA	Ward <i>et al.</i> (2005)
FISHR2_M13	(CAGGAAACAGCTATGAC)ACTTCAGGGTGACCGAAGAATCAGAA	Ward <i>et al.</i> (2005)
Shark_INTFd	GCCAYGCHTTTGTATAATCTT	This study

Alignment and analysis. Sequence chromatograms were edited in Geneious v6 (Kearse *et al.*, 2012) for the 132 samples that were successfully sequenced. Two approaches were used to determine species identity from this project's pool of sequences. First, the Barcode of Life Data System (BOLD) Batch ID engine was used to determine the species identity for each sample, individually (Ward *et al.*, 2008) as BOLD is the largest repository of *col* barcode sequences. Second, GenBank's Basic Local Alignment Search Tool (BLAST) was used to examine similarities between each of the samples and the sequences databased in GenBank. The use of both GenBank and BOLD improved species identification metrics by maximizing the amount of *col* sequences for comparison. Both BOLD and GenBank BLAST search results display sequences which most closely match the input query, in order of highest similarity. In cases where samples matched to more than one species, rather than consider the top sequence hits to the samples, the three species with highest similarity matches out of the first 100 matches were recorded (S1 - Available only as online supplementary file accessed with the online version of the article at <http://www.scielo.br/ni>). BOLD includes both published sequence data and data from 'private' sources, for which there are no publicly available information collection location or collection method. Private matches were avoided in tabulated results unless no public matches were found, as the lack of publicly-available data on these specimens makes them difficult to validate.

To visualize where Guyana shark samples fell within an all-carcharhiniform gene tree, all 1691 carcharhiniform *col* sequences were downloaded from GenBank using PhyLoTA (Sanderson *et al.*, 2008), aligned to the sample sequences using the MUSCLE algorithm (Edgar, 2004) in Geneious, and then clustered using maximum likelihood in RaxML v8 (Stamatakis, 2006) under the GTR+I+G model of evolution, with 100 thorough bootstrap replicates. The suitability of this GTR+I+G substitution model was selected over 56 other models using PartitionFinder (Lanfear *et al.*, 2012). The

phylogeny was rooted to a clade of Hemigaleidae + Triakidae species, following the molecular phylogenetic relationships reported by Vélez-Zuazo, Agnarsson (2011, S2 - Available only as online supplementary file accessed with the online version of the article at <http://www.scielo.br/ni>).

Results

Despite the poor quality of the market samples, *col* fragments from 132 out of 144 samples (92% of the total sample size) were successfully amplified and analyzed. Samples which were unable to be amplified likely experienced DNA degradation because of intense sunlight and prolonged heat exposure. Attempts were made to amplify these samples with a variety of primers, but consistent failure implicates poor DNA quality, rather than poor primer complementarity.

Identification with BOLD and BLAST. All thirteen species of sharks found in Guyanese fish markets were carcharhiniforms, belonging to the families Sphyrnidae (Hammerhead Sharks) and Carcharhinidae (Requiem Sharks) (Tab. 2; S1 - Available only as online supplementary file accessed with the online version of the article at <http://www.scielo.br/ni>). Hammerhead diversity included four species: Scalloped Hammerhead Sharks [*Sphyrna lewini* (Griffith & Smith, 1834)], Great Hammerhead Sharks [*S. mokarran* (Rüppell, 1837)], Golden or Smalleye Hammerhead Sharks [*S. tudes* (Valenciennes, 1822)], and Bonnethead Sharks [*S. tiburo* (Linnaeus, 1758)]. We also provide photographic evidence for the presence of *S. media* Springer, 1940, the Scoophead Shark, although since sequence data for this shark is not available in either BOLD or BLAST databases, we cannot yet confirm its presence in Guyana waters with DNA barcoding. Carcharhinid diversity numbered nine species, with the most abundant being Sharpnose Sharks [*Rhizoprionodon lalandii* (Valenciennes, 1839) and *R. porosus* (Poey, 1861)] and Smalltail Sharks [*Carcharhinus porosus* (Ranzani, 1839)] (Tab. 2).

In general, high percentage similarity (98-100%) was found between the sample sequences and the matches from BOLD and Genbank (S1- Available only as online supplementary file accessed with the online version of the article at <http://www.scielo.br/ni>). Results from GenBank tended to have lower similarity of queried samples to database sequences (92-99% similarity) compared to BOLD results (S1). The BOLD system uses BLAST's search algorithm, so it is likely that the difference in percent similarity is either due to the difference in number of specific catalogued *col* sequences between the databases, or because most sequences in BOLD are at the 5' end of the *col* gene, and therefore might have different sequence overlap compared to GenBank (Wong, Hanner, 2008). While many of the samples were >99% similar to database

sequences in either BOLD or Genbank, sample #11804 was an interesting exception. This sample showed 94.7% similarity to *S. tudes* according to BOLD, and 95% similar to sequences of *S. tiburo* according to GenBank. Photographs of this specimen from the markets (Fig. 2), one of the few animals sampled which was not decapitated, suggests that this specimen may be *S. media* (IUCN status: Data Deficient, Casper, Burgess, 2006), a species not represented in BOLD or GenBank. This specimen is putatively identified as *S. media* because it has a short and broad, wedge-shaped cephalofoil, with a convex medial notch. In addition, this specimen has the origin of its first dorsal fin over the inner margins of the pectoral fins, and the trailing edge of the second dorsal fin not reaching the caudal fin, all characters which suggest *S. media*.

Tab. 2 Shark species in Guyanese fish markets as identified through DNA barcoding.

Scientific name	Common name	Proportion specimens in total sample	IUCN status
<i>Rhizoprionodon lalandii</i>	Brazilian Sharpnose Shark	18.9%	Data Deficient
<i>Carcharhinus porosus</i>	Smalltail Shark	17.4%	Data Deficient
<i>Sphyrna lewini</i>	Scalloped Hammerhead	17.4%	Endangered
<i>Sphyrna mokarran</i>	Great Hammerhead	10.6%	Endangered
<i>Carcharhinus limbatus</i>	Blacktip Shark	8.3%	Near Threatened
<i>Galeocerdo cuvier</i>	Tiger Shark	6.8%	Near Threatened
<i>Sphyrna tudes</i>	Golden Hammerhead	6.0%	Vulnerable
<i>Carcharhinus acronotus</i>	Blacknose Shark	5.3%	Near Threatened
<i>Rhizoprionodon porosus</i>	Caribbean Sharpnose Shark	3.8%	Least Concern
<i>Sphyrna tiburo</i>	Bonnethead Shark	2.3%	Least Concern
<i>Carcharhinus leucas</i>	Bull Shark	0.8%	Near Threatened
<i>Carcharhinus falciformis</i>	Silky Shark	0.8%	Near Threatened
<i>Carcharhinus plumbeus</i>	Sandbar Shark	0.8%	Vulnerable
<i>Sphyrna media</i>	Scoophead Shark	0.8%	Data Deficient



Fig. 2. Putative Scoophead Shark (*Sphyrna media*) sampled at Meadow Bank. Insets **a.** and **b.** are the dorsal and lateral views.

In several cases, specimens showed very high similarity (99-100%) to database sequences from more than one species, whether BOLD or GenBank results were considered. Barcoding methods were unable to distinguish between the sister pairs *Rhizoprionodon porosus*/*R. terraenovae* (Richardson, 1837) and *Carcharhinus plumbeus* (Nardo, 1827)/*C. altimus* (Springer, 1950). Several previous studies have shown that several pairs of sister species, e.g. *C. plumbeus*/*C. altimus*, *C. limbatus* (Valenciennes, 1839)/*C. tilstoni* (Whitley, 1950), cannot be uniquely diagnosed using barcoding methods (Wong *et al.*, 2009; Spaet *et al.*, 2015).

Discussion

Most discussion of coastal elasmobranchs in the Guianas region have only involved reports from Venezuela, Suriname, and French Guiana and from offshore pelagic fisheries (Cervigón *et al.*, 1992; Tavares, Arocha, 2008). Few other sources document coastal Guyana shark species (Brown, 1942; Mitchell, Lowe-McConnell, 1960; Rathjen *et al.*, 1969; Maisson, 1998). This study agrees with previous authors, confirming the presence of the following species in Guyanese coastal waters: *C. acronotus* (Poey, 1860), *C. limbatus*, *C. porosus*, *R. lalandii*, *R. porosus*, *S. lewini*, and *S. tudes*. To this list add *C. leucas*, *C. plumbeus*, *Galeocerdo cuvier* (Péron & Lesueur, 1822), *S. media*, *S. mokarran*, and *S. tiburo* in Guyanese waters. Curiously, our study could not confirm presence of *Mustelus* species (*M. canis* and *M. higmani*) in Guyana coastal waters, which have been previously documented (Shing, 1999). There is conspicuous absence of lamnid species, such as Thresher Sharks (*Alopias* sp.) and Blue Sharks [*Prionace glauca* (Linnaeus, 1758)] in our sample, presumably since the artisanal Guyana driftnet fisheries do not fish far enough offshore to encounter lamnids frequently (Tavares, Arocha, 2008). Finally, while there are batoids reported from the Guianas [e.g. *Rhinoptera bonasus* (Mitchill, 1815), *Hypanus guttatus* (Bloch & Schneider, 1801), and *Fontitrygon geijskei* (Boeseman, 1948)] (Mitchell, Lowe-McConnell, 1960), they are not generally encountered by the driftnet fishery and are discarded at sea (authors, pers. comm.).

Of the 13 species of sharks recorded here, two are considered Endangered by the IUCN (Baum *et al.*, 2007; Denham *et al.*, 2007). Both endangered species are Hammerhead Sharks (Sphyrnidae), which comprise 37% of the sample. Hammerhead Sharks are particularly vulnerable to most common fishing practices (long-lines, driftnets, gillnets) even when bycatch is discarded (Carlson *et al.*, 2004; Morgan, Burgess, 2007). Fortuitously, even in markets in Guyana, where sharks are decapitated before landing, Hammerhead Sharks can be distinguished from other carcharhiniforms by the large size of their dorsal and pectoral fins, theoretically allowing managers to track how many of these animals are landed with some confidence. Hammerhead Shark species in Guyana are not considered by IUCN as a discrete population or unit, rather they are

grouped with populations from the Western Central Atlantic. Hammerhead Sharks are also presumably at risk in Guyana due to their susceptibility to entanglement gear used by other artisanal fisheries, as well as targeted specifically for their large fins, given that the Guyanese driftnet fishery export fins to Asian markets (Shing, 1999).

The most abundant carcharhinids in our dataset were Sharpnose Sharks, comprising over a quarter of the sampled specimens, which are classified as either ‘Data Deficient’ or of ‘Least Concern’ by the IUCN (Rosa *et al.*, 2004; Lessa *et al.*, 2006; Cortés, 2009). However, despite generally high fecundity, these small sharks are under some pressure from overfishing in Brazil, where *R. lalandii* is listed as locally ‘Vulnerable’ due to intense harvest of all size and age classes in artisanal gillnet fisheries (Rosa *et al.*, 2004; Motta *et al.*, 2005, 2007). *Rhizoprionodon lalandii* made up 83% of the samples from Guyanese fish markets. Given the ‘Vulnerable’ status of *R. lalandii* in Brazil, this might suggest that this species needs to be monitored more carefully in Guyana as well, given its prevalence in our sample. Alternatively, if *R. lalandii* populations in the Guianas are both stable and contiguous with Brazilian Sharpnose Shark populations, over harvested Brazilian populations could theoretically be replenished through immigration by coastal Guyana individuals (Mendonça *et al.*, 2011).

Several samples were equally similar to both *R. porosus* and *R. terraenovae* reference sequences according to both BOLD and GenBank. However, another explanation for this situation is that one or more specimens used for these databased reference sequences were misidentified, or represent hybrids (hybrids have been recognized in Requiem Sharks; Morgan *et al.*, 2012). Unfortunately, in the case of BOLD, some of the relevant reference sequences are “private,” with no corresponding voucher or metadata are publicly available at this time. This issue highlights the need for careful curation of voucher specimens and associated data with databased molecular sequences; without this information it is difficult for users of barcoding databases to confirm and correct erroneous reference sequences (Vilgalys, 2003). Vouchers allow matching of tissues and sequences to actual specimens, a critical factor for the validity and repeatability of taxonomic studies (Agerer *et al.*, 2000; Cerutti-Pereyra *et al.*, 2012).

Not all species are barcoded and available for comparison, as is the case with our putative sample of the Scoophead Shark, *S. media*. In addition, the quality and length of sequences cataloged on online databases is variable, and there are only partial sequences for some species. These limitations make it challenging for this study and others to catalog the diversity of life using barcoding methods. Barcoding studies should incorporate larger sequence reads and different regions of the mitochondrial genome (e.g. ND2, Control Region) to increase accuracy in species identification. For example, the *col* marker has been found to be of limited use for some closely-related species, particularly those within species complexes (Morgan *et al.*, 2012; Spaet *et al.*, 2015), and the

incorporation of other markers with different gene histories can help identify closely-related taxa.

Accurate taxonomy is critical for cataloging biodiversity. However, large animals are particularly difficult for museums to house and thus are under-represented in collections. In addition, in Guyana, sharks caught in both nearshore and offshore fisheries are decapitated prior to being landed in markets, making the identification of morphologically similar species, such as Requiem and Hammerhead Sharks (Carcharhiniformes) exceedingly difficult. The great morphological similarity among carcharhiniform species in the Caribbean makes it difficult for stocks to be monitored effectively without active participation by local managers trained in taxonomy, whether through fisheries-independent or dependent means (Iglésias *et al.*, 2010; Domingues *et al.*, 2013). Misidentification of landed species can lead to over- or under- estimations of fishery efforts and the effects on species-at-risk (Iglésias *et al.*, 2010).

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