Genetic identification of sharks traded and consumed in Australia

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This thesis is written in the form of a manuscript for submission to Fisheries Research, with the following exceptions: 1.5 space text, figures and figure captions are integrated into the text, a table of contents has been included and the introduction, methods, results and discussion are extended.

Declaration

I wish to acknowledge the following assistance in the research detailed in this report:

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All other research described in this report is my own original work.

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Abstract

Shark species worldwide are under threat mainly from overharvesting either as bycatch or in targeted fisheries. In Australia shark flesh is mostly sold under the name 'flake' and distributed around the country. The Australian Fish Names Standard state that sharks sold under the name flake must be either Australian Gummy shark (*Mustelus antarcticus)* or New Zealand rig (*Mustelus lenticulatus),* however labelling is not mandatory. In this study we hypothesized that many sharks sold as flake did not qualify under the naming standard, and that threatened species were being traded. We used DNA barcoding to identify species from 91 samples obtained from 29 seafood retailers across the country. To determine species composition we used Sanger sequencing of two genes, the Cytochrome Oxidase subunit one gene (CO1) and the 12S mitochondrial RNA gene (12S). We identified 67 cases (78.8%) of mislabelling and 16 cases (35.6%) of threatened species being traded, including the Scalloped Hammerhead (*Sphryna lewini)* and School Shark (*Galeorhinus galeus)*. Our results showed that mislabelling of shark flesh is occurring in Australia, and that threatened species make up a sizeable portion of the species sold. These data demonstrate the need to improve labelling standards to work towards minimizing our impact on threatened shark species.

Keywords: Mislabelling, seafood, DNA barcoding, threatened species

1. Introduction

1.1 Conservation and Sustainability Issues for Sharks

Shark and ray populations worldwide are in rapid decline, as the global demand for meat and fins puts increasing pressure on fisheries to exploit populations throughout their distribution. One quarter of all shark species globally are listed as under threat after dramatic population declines in the last decade due to overharvesting either as bycatch or in targeted fisheries (Clarke et al. 2006; Dulvy et al. 2014; IUCN 2019). It is estimated that approximately 750 000 metric tonnes of chondrichthyans are listed in catch reports around the world each year (Okes and Sant 2019). Illegal, unreported and unregulated fishing (IUU) is estimated to account for a further 11-26 billion tonnes per year (Agnew et al. 2009). As a result, consistent, fisheries-driven declines of chondrichthyans are observed in oceans around the world (Dulvy et al. 2014; Dulvy et al. 2017).

While many international regulations and national laws are placed on fishing industries, aimed at discouraging illegal catch of threatened species, these laws are often unmonitored and unenforced resulting in a misrepresentation of species in statistical reports (Dudgeon et al. 2012; Dent and Clarke 2015; Simpfendorfer and Dulvy 2017; Feitosa et al. 2018). In many fishing ports around the world, catches of endangered species protected by national or international legislation continue to be detected (Holmes et al. 2009; McClenachan et al. 2016; Shea and To 2017; Feitosa et al. 2018). Threatened species are at significant risk of overexploitation, particularly in countries where sustainable management is outcompeted by market demand or governments driven by limited resources (McClenachan et al. 2016; Dulvy et al. 2017; Simpfendorfer and Dulvy 2017). While the number of countries enforcing management initiatives informed by scientific assessments of population size are increasing, limits on shark fishing remain largely inadequate at local, national and international scales (Dulvy et al. 2017).

Regional estimates of illegal and unreported fishing average 18% from 2000-2003, with some regions substantially higher (eg. 37% for the Eastern Central Atlantic region; Agnew et al. 2009). Bycatch is also a significant problem driving a need for more focus from governments on the urgency of implementing bycatch-reducing fishing practices (Zeeberg et al. 2006; Simpfendorfer and Dulvy 2017). The impacts are further exacerbated by unreliable statistics in governmental reports, product mislabelling and under-reported catch sizes (Xiong et al. 2017; Dulvy et al. 2017; Shea and To 2017; Pazartzi et al. 2018; Hellberg et al. 2019; Hobbs et al. 2019). For these reasons it is therefore increasingly difficult to estimate the impacts of fishing and manage for sustainability.

Accurate data on the seafood trade is critical to manage for the sustainability of the industry. Reliable catch data is often impeded by malpractice and negligence, as well as mismanagement

within local industries (Clarke et al. 2006; Dulvy et al. 2017; Feitosa et al. 2018). Many regulations are currently in place to address the issue of sustainability in trade practice, for example World Conservation Monitoring Centre's Convention on International Trade in Endangered Species of Wild Fauna and Flora [CITES] Trade Database – United Nations Environment Program (Baker 2008; Gerson et al. 2008). However, legislation allow for loopholes in labelling where threatened species trade can continue unmonitored. This issue is highlighted with only 62% of the total global recorded catch of sharks and rays recorded to a taxonomic level in 2017, and 32% of this recorded to species level (Okes and Sant, 2019).

1.2 Molecular Approaches to Species Identification

Molecular tools allow for a more thorough understanding of the trade, and genetic analyses of products to determine species composition and origin is a fast-growing area of molecular ecology. Morphological identification of species is often impeded by the removal of distinguishing features such as heads and fins, making species identification unreliable (Feitosa et al. 2018). The use of DNA barcoding and forensic genetic techniques to monitor the wildlife trade is considered critically important (Baker 2008; Eaton et al. 2010).

DNA barcoding uses databases of species-specific sequences to match sequences of unknown origin. The mtDNA gene cytochrome *c* oxidase 1 (CO1) is widely considered a reliable genetic marker for effectively testing species of origin in the wildlife trade, being largely invariant within species and showing sufficient variation among species (Dawnay et al. 2007; Baker 2008; Ogden 2008; Dudgeon et al. 2012). Universal primers for identification of fish using CO1 were developed by Ward et al. (2005) and have been used to effectively distinguish shark species (eg. Almeron-Souza et al. 2018; Feitosa et al. 2018; Pazartzi et al. 2018).

DNA barcoding can also be applied when DNA has been degraded, for example in dried shark fins. The use of mini-CO1 sequences or the rRNA region, where sequence length is approximately less than 200 bp, is frequently being incorporated into forensic studies analysing shark tissue and identifying shark fin (eg. Hobbs et al. 2019). Universal mini-primers have also been effectively identifying fish species from marine eDNA samples (eg. Miya et al. 2015). These universal primers target the region of the 12S RNA gene and can be used to identify fish species to taxonomic family, genus and species from eDNA collected in aquarium and seawater (Miya et al. 2015).
Environmental DNA (eDNA) refers to genetic material left behind by organisms passing through an environment, including dead skin cells, metabolic waste and damaged tissue (Miya et al. 2015). This technique is increasingly used as a minimally invasive detection tool for rare or threatened

species, where visual monitoring systems are unreliable and resource-inefficient (Miya et al. 2015; Bakker et al. 2017).

Metabarcoding, where multiple DNA sequences can be barcoded, is increasingly used in monitoring marine ecosystems from water samples (eg. Bakker et al. 2017; Boussarie et al. 2018). In the case of elasmobranches, metabarcoding using eDNA has been used to identify shark diversity from natural seawater samples (Miya et al. 2015; Bakker et al. 2017; Boussarie et al. 2018). The presence of species at a sampled location is able to be determined with higher accuracy than traditional visual monitoring techniques such as underwater visual censuses (UVCs) and baited remote underwater video stations (BRUVSs) (Boussarie et al. 2018). With the growing need for more reliable monitoring tools fisheries, metabarcoding techniques have the potential for use as forensic tools for tracking threatened or endangered marine species. Metabarcoding eDNA has already been used to track the presence of invasive species in the ballast water of fishing boats (Gerhard and Gunsch 2019), and has further potential for being used to monitor species processed on the boat from DNA collected in ballast water.

Metabarcoding enables increased utility for large-scale and indirect monitoring, and further has potential as an approach for large-scale monitoring of the shark trade (Bakker et al. 2017; Boussarie et al. 2018). There are substantial difficulties involved with obtaining tissue samples from specimens on the markets, particularly when many trading ports pass thousands of sharks through their docks each day. Sampling the refuse water left behind in seafood markets, or in the ballast of fishing boats would allow for a more comprehensive, resource-efficient and less-invasive analysis of sharks processed in trading ports (Bakker et al. 2017; Boussarie et al. 2018).

1.3 Shark Trade in Australia

In Australia, over an estimated 5 000 tonnes of shark are landed each year (Woodhams and Hart, 2018). Targeted fisheries occur in both the northern and southern states, and sharks are often sold at local fish markets and in takeaway shops. The Australian Fish Names Standard (AFNS) aims to provide a standard name for all fish species traded within Australia, and as such has a designated name associated with each species. However, labelling under the standard is only voluntary with minimal regulation to ensure species are accurately identified on the market, and that labels comply with regulations. Developed by the Australian Fish Names Committee under the Fisheries Research and Development Corporation (FRDC), the names standard has been officially recognised as the Australian Standard since 2007. The label Flake is designated to two shark species the Australian Gummy Shark (*Mustelus antarcticus)* and New Zealand Rig (*Mustelus lenticulatus). Mustelus*

antarcticus is targeted in the Commonwealth Southern and Eastern Scalefish and Shark Fishery (SESSF), which covers the southern coastlines of Australia from New South Wales to Western Australia, and with an average annual catch of around 1,700t from 2016-18 (Woodhams et al. 2018; Patterson et al. 2019). Current catch of *M.antarcticus* in Australia is considered sustainable under management plans, however sharks are caught in fisheries all around the country with unknown impacts (Simpfendorfer and Dulvy, 2017; Simpfendorfer et al. 2019). Trade of threatened species in Australia occurs under the *Environment Protection and Biodiversity Conversation Act 1999* (EPBC) where species such as the School Shark (*Galeorhinus galeus)* and Scalloped Hammerhead Shark (*Sphryna lewini)* are listed as 'Conservation Dependent' and still allowable as catch in commercial fisheries under certain conditions. Stronger labelling requirements can encourage the maintenance of species-specific catch data and contribute to monitoring of threatened populations (Liu et al. 2013; Barbuto et al. 2010; Almeron-Souza et al. 2018; Bunholi et al. 2018; Pazartzi et al. 2019)

Study scope and aims

As shark populations continue to decline, monitoring fisheries and encouraging their sustainable management is increasingly essential. Mislabelling on the market has been demonstrated in countries around the world, but the extent of mislabelling in Australia has received little research and is needed to better inform management plans and understand the impact of fisheries on sharks in Australian waters. In this study, we used DNA barcoding of the CO1 and 12S RNA genes to identify species of shark sold by wholesalers, fish markets and takeaway shops around Australia. We used these data to describe mislabelling under the AFNS guidelines and further assess potential impacts on threatened species. Secondly, we trialled a metabarcoding approach using the 12S RNA for its potential as a cost-effective approach to identification.

2. Materials and Methods

2.1. DNA identification of shark tissue

2.1.1 Sample collection and storage

Between February and June 2019, a total of 91 shark meat products were collected from 29 different retailers (Table 1). All products were obtained fresh or uncooked with 25 samples collected from small-scale takeaway stores, and the final 66 obtained from fish markets or wholesalers. Repeat sampling from retailers was limited to a maximum of four tissue samples from

each vendor on the same day. Each sample was sourced from a different fillet, but obtained from the same product display. Each fillet was counted as a single sample. Sampling was generally limited by availability of samples at the time of collection. If samples could not be obtained, notes were still taken on the species the vendors typically sell. Sampling was also limited by ability to visit locations, with many samples at the main cities collected over the same day.

Small tissue samples from each product were preserved in 2ml labelled tubes filled with 95% ethanol and stored at 25°C (room temperature). Details from each sample were recorded including retailer, location, date, label name and product identification from vendor (eg. if the product was labelled as 'flake' the vendor was asked if they could identify the species present).

Table 1. Location of sample collections, and the number of samples (including repeats) collected at each major city.

Location	Wholesale/Fish Market	Takeaway	Total
Perth/ Western Australia (WA)	12	-	12
Adelaide/ South Australia (SA)	1	4	5
Melbourne/ Victoria (VIC)	16	4	20
Canberra/ Australian Capital Territory (ACT)	15	-	15
Sydney & South Coast/ New South Wales (NSW)	12	2	14
Brisbane/ Queensland (QLD)	10	15	25

2.1.2 Molecular analysis and species identification

Genomic DNA was extracted using the spin column Isolate II Genomic DNA kit from Bioline (*Bioline Meridian Bioscience,* Australia) following the protocol guidelines with some modifications. The modifications included an extended lysing period (12-24 hours) of 23-25mg of tissue from each sample, incubating in a rotating incubator set to 400rpm.

Two pairs universal primers were chosen for amplification of approximately 655bp from the mtDNA CO1 gene (Ward et al. 2005) (Table 2). The polymerase chain reaction (PCR) cycling

conditions for the amplification of the CO1 gene was the same for each primer pair with an initial denaturation at 95°C for 10 min, followed by 45 cycles at 94°C for 30 s, 54°C for 30 s, 72°C for 1 min, and a final elongation phase of 72°C for 10 min. Preparation of a 30ul reaction volume included 15ul of 2X Amplitaq Gold 360 Master Mix (*Life Technologies Australia,* Australia*),* 1.5ul of each primer (10uM), 9ul of sterile distilled H_20 and 3ul of DNA.

A primer pair targeting the 12S RNA gene region was also chosen to amplify approximately 171bp (Taberlet et al. 2018). The PCR cycling conditions were as follows: initial denaturation at 95°C for 10 min, followed by 35 cycles at 95°C for 30 s, 59°C for 30 s, 72°C for 1 min, and a final elongation phase of 72°C for 7 min. Preparation of a 30ul reaction volume included 15ul of 2X Amplitaq Gold 360 Master Mix (*Life Technologies Australia,* Australia*),* 1.5ul of each primer (10uM), 9ul of sterile distilled H_20 and 3ul of DNA. PCR products were visualized on 2% agarose gel stained with SybrGreen. Fragments were tested against an Invitrogen 100bp DNA Ladder (*Thermo Fischer Scientific,* US*).* Negative (milli-q water) and positive (*Negaprion brevirostris* DNA) controls were included in all runs.

PCR purification and sanger sequencing in the forward direction was conducted by Macrogen (Seoul, South Korea). Sequences were aligned and trimmed using MEGA 7 software (Kumar et al. 2015). Sequences were initially checked against the Genbank BLASTn nucleotide collection, and then cross-referenced with the Barcode of Life Data System (BOLD) (Ratnasingham and Herbert, 2007). Sequences were assessed by similarity to the target sequence in the database (Per Identity) and percentage of coverage over the target sequence (Query Cover). E-values were also used to assess the likelihood of matches by chance in the database. Species were considered identified when Max Score was highest, Percentage Similarity (Per. Identity) and Query Cover was greater than 92%, and the E-value was less than 0.00001 (Appendix A). In instances where all three identifying categories were identical to multiple species in the same genus, only a genus ID was assigned. Species identified were then researched on International Union for Conservation of Nature (IUCN) Red List of Threatened Species (2019) and the listed conservation status of each species was recorded.

Table 2. PCR primers used for shark identification.

Target gene	Primer name	Primer sequence (5'-3')	Primer length (bp)	Amplicon length (bp)	Reference
CO1	FishF1	5′-TCAACCAACCACAAAGACATTGGCAC-3′	26	655	Ward et al. 2005
	FishR1	5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′	26	655	Ward et al. 2005
	FishF2	5'-TCGACTAATCATAAAGATATCGGCAC-3'	26	655	Ward et al. 2005
	FishR2	5'-ACTTCAGGGTGACCGAAGAATCAGAA-3'	26	655	Ward et al. 2005
12S	Elas02-F	5'-GTTGGTAAATCTCGTGCCAGC-3'	21	171	Taberlet et al. 2018
	Elas02-R	5'-CATAGTAGGGTATCTAATCCTAGTTT-3'	26	171	Taberlet et al. 2018

2.1.2 Phylogenetic analysis

Phylogenetic analyses were performed using the neighbour-joining (NJ) method in MEGA 7 (Kumar et al. 2015) to observe accuracy of species assignments. Species CO1 sequences were downloaded from BOLD and included in the phylogenetic analysis for CO1 as reference material. To evaluate the robustness of the branches of the NJ tree, 1000 bootstrap replications were run under the maximum composite likelihood approach.

2.2 Testing primers with environmental DNA

In order to test the versatility of 12S primers for metabarcoding eDNA from elasmobranchs, we sampled seawater from three tanks in the SeaLife Sydney Aquarium, Sydney Australia. This aquarium was chosen because of the diverse elasmobranch communities within a variety of tanks. The three selected tanks; Shark Valley OC1 (water volume = 1 750 000L), Dugong Island OC2 (water volume = 1 750 000L) and Day and Night on the Reef OC4 (water volume = 1 450 000L)

tanks harbor diverse Australian elasmobranchs (sharks and rays) from benthic to pelagic species living in waters and shallow coastal deep waters.

2.2.2 Water sampling and DNA extraction

All filtering equipment was exposed overnight to a 10% bleach solution before use. For aquarium samples, approximately 52L of seawater was collected from the aquarium; 31L from the surface and 21L from the tank floor. Samples were collected in sterile plastic bags handled by myself or working aquarium divers in the tank. The sampling was conducted between 8.00 and 14.00 during feeding and maintenance dives over three days (7, 9 and 15 May 2019). The sampled water was sealed in the bags and transported in two 33L eskies filled 1/3 with ice and immediately brought to the laboratory. Filtering began within two hours of collection.

Thirteen to fifteen 1-2L lots of seawater from each tank were vacuum-filtered using Sentino Microbiology Pumps with 47mm x 500mL reusable magnetic filter funnels (polyphenylsulfone plastic; Pall Corporation*).* Samples were filtered on 0.45 um filter membranes. Samples of 1L were filtered from tanks OC4 and OC2, and samples of 1-2L were filtered from tank OC1 (nine 2L samples and six 1L samples). Milli-Q water was used as a negative control and filtered identically alongside the samples (1L for OC4 and OC2; 2L for OC1) to monitor contamination throughout filtering and DNA extraction.

DNA was extracted from the filters using the DNeasy PowerWater kit (*Qiagen*, Victoria, Australia) following the included protocol up until step 10. Alterations to the protocol involved vortexing for 10minutes instead of 5 minutes at step 7. After step 10, the samples were placed in the QIACube (classic) DNA extraction robot (*Qiagen*, 2007) following the PowerWater IRS digital protocol.

2.3.3 Molecular analysis

In addition to the PCR conditions described for the 12S RNA primers given above, a quantitative PCR (qPCR) was conducted. Two different DNA concentrations were used, undiluted and diluted 1:10 with water. PCR products were visualized on 2% agarose gel stained with SybrGreen and tested against an Invitrogen 100bp DNA Ladder (*Thermo Fischer Scientific)* including positive and negative controls. Amplicons could not be visualized for any PCR reactions.

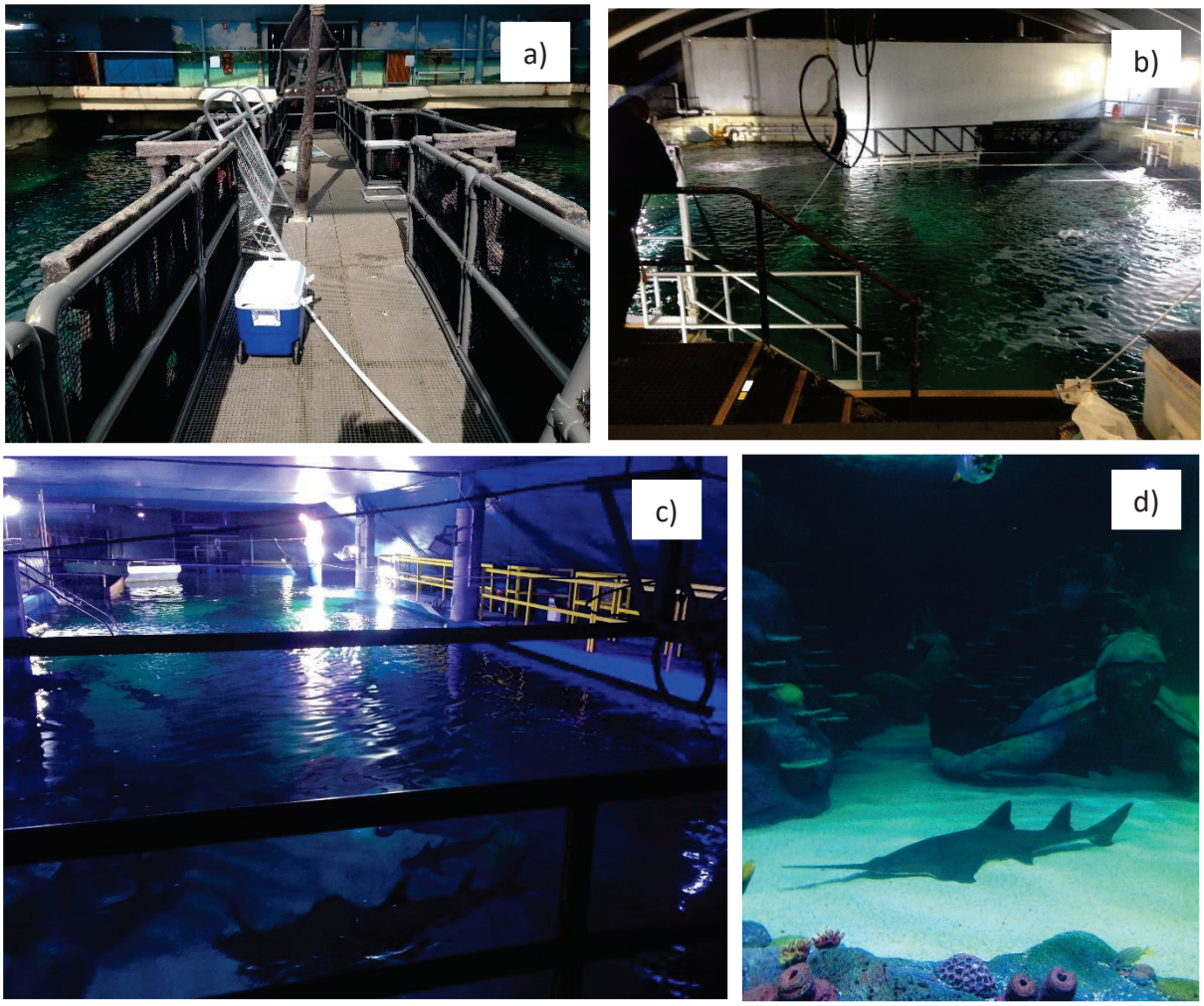


Figure 1. *(a-d)* Three tanks used for water sampling at Sealife Sydney Aquarium: *(a)* Dugong Island OC2 (volume = 1 750 000L); *(b)* Shark Valley OC1 (volume = 1 750 000); *(c-d)* Day and Night on the Reef OC4 (volume = 1 450 000L).

3. Results

3.1 DNA and sequencing evaluation

Out of the 91 shark samples, only 45 samples were successfully sequenced using the CO1 primers (Fish1: n = 25, Fish2: n = 20) (Appendix A). Sequences of good quality were obtained for 90 samples using primers for the 12S RNA region. Sequences available for the 12S RNA in BLAST were not sufficient to cover all species present in the samples, and matches were generally of lower probability (see E-values – Appendix A Table 3A). Species such as the School shark (*Galeorhinus galeus)* did not have a 12S sequence in the database, while some genera of *Carcharhinus* and *Mustelus* could not be identified to species level. In total 85 samples could be identified to family level using 12S, including 18 samples identified to species level (*Callorhinchus capensis, Deania*

calcea and *Carcharhinus leucas).* Cross-examination with the available CO1 sequences was required to determine the best species match. All CO1 sequences obtained had E-values close to 0.0, with >92% similarity to database records. Most comparisons in the BOLD database showed similar matching profiles.

3.2 Species identification

Overall, 12 species were successfully identified using CO1 primers, across eight genera (*Mustelus, Galeorhinus, Carcharhinus, Sphyrna, Pristiophorus, Orectolobus, Callorhinchus, Squalus*) and seven families (Triakidae, Carcharhinidae, Sphyrnidae, Pristiophoridae, Orectolobidae, Callorhinchidae, Squalidae) (Table 3). Two samples could only be classified to genus level (*Squalus spp)* based on a 95% query cover match for both *S.chloroculus* and *S.mitsukuri* on BLAST. However, the BOLD database showed a >94% match for *S.chloroculus* (Greeneye spurdog, IUCN: EN) singularly. The 12S primers successfully identified nine families (Triakidae, Carcharhinidae, Sphyrnidae, Pristiophoridae, Orectolobidae, Callorhinchidae, Squalidae, Squatinidae and Centrophoridae) (Table 4). In total, 90 sequences across CO1 and 12S were used to identify mislabelling, and 45 sequences across CO1 were used to identify threatened species.

The DNA barcoding identified clear discrepancies in labelling. In 67 cases (78.8%), mislabelling under the Australian Fish Names Standard (AFNS) was detected. In 53 cases (62.35%) mislabelling occurred where species labelled as Flake were neither *M. antarcticus* or *M. lenticulatus.* From 16 samples (35.6%) species listed under threatened categories on IUCN were identified from 45 CO1 sequences (Fig. 2). Notably, the Scalloped hammerhead (*Sphryna lewini)* is listed under CITES Appendix II and has been classified as IUCN endangered since 2009. The most commonly identified species from retailers was the common sawshark (*P. cirratus* n = 12), followed by School shark (*G. galeus* n = 5), Gummy shark (*M. antarcticus* n = 5) and Dusky whaler (*C. obscurus* n = 4).

The highest incidences of mislabelling occurred in Queensland and the Australian Capital Territory, with >90% of samples found to be mislabelled. The lowest incidences of mislabelling were found from samples from Victoria, with 33% of samples mislabelled. Eighty percent of the South Australian samples were mislabelled however this may be due to only four samples being identified through barcoding.

Table 3. Table providing a summary of 45 samples successfully identified using CO1 barcoding, including species common names listed under AFNS. Mislabelled cases under AFNS are highlighted in bold.

	Correct names				
Family	Scientific name	Common name	Labelled as	Sold in	Number
Carcharhinidae	*Carcharhinus obscurus*	Dusky Whaler	Bronze Whaler	WA	4
	Carcharhinus brevipinna	**Spinner shark**	**Flake**	QLD	3
	Carcharhinus leucas	**Bull shark**	**Flake**	ACT	1
Orectolobidae	***Orectolobus hutchinsi***	**Western wobbegong**	**Carpet Shark**	WA	4
	Orectolobus maculatus	Spotted wobbegong	**Boneless Fillet**	NSW	1
	Orectolobus halei	**Gulf wobbegong**	**Flake**	NSW	2
Triakidae	***Galeorhinus galeus***	**School shark/ Tope**	School Shark	VIC	4
			Flake	QLD	1
	Mustelus antarcticus	Gummy shark	Flake	SA	1
			Gummy Flake	VIC	4
Pristiophoridae	***Pristiophorus cirratus***	**Common sawshark**	**Flake**	ACT	7
			Gummy Shark	ACT	3
			Boneless Sweetfish	NSW	1
			Flake fillet	NSW	1
Callorhinchidae	***Callorhinchus capensis***	**Cape elephantfish**	**Flake**	QLD	1
Squalidae	***Squalus montalbani***	**Philippine spurdog**	**Flake**	VIC	2
	Squalus spp.	**-**	**Flake**	VIC	2
Sphyrnidae	***Sphyrna lewini***	**Scalloped hammerhead**	**Flake**	QLD	3

Table 4. Table providing a summary of 85 samples successfully identified using 12S barcoding, including species common names listed under AFNS. Mislabelled cases under AFNS are highlighted in bold. 40 samples identified are independent of CO1 results (*)

	Correct names				
Family	Scientific name	Common Name	Labelled as	Sold in	Number
Carcharhinidae	*Carcharhinus spp.*		Bronze Whaler	WA	8*
			Flake	SA	4*
			Flake	QLD	10*
			Aus. Shark	QLD	5*
			Shark Barrel	QLD	2*
	Carcharhinus leucas	**Bull shark**	**Flake**	ACT	4
Orectolobidae	***Orectolobus spp.***	**Wobbegongs**	**Carpet Shark**	WA	4
			Flake	NSW	2
			Boneless Fillet	NSW	2*
Triakidae	*Mustelus spp.*	Gummy sharks	Flake	SA	1
			Gummy Flake	VIC	4
Pristiophoridae	***Pristiophorus spp.***	**Sawshark**	**Flake**	ACT	10*
			Gummy Shark	ACT	3
			Boneless Sweetfish	NSW	1
			Flake fillet	NSW	2*
Callorhinchidae	***Callorhinchus capensis***	**Cape elephantfish**	**Flake**	QLD	3*
Squalidae	***Squalus montalbani***	**Philippine spurdog**	**Flake**	VIC	2
	Squalus spp.	**Greeneye dogfishes/Dogfishes**	**Flake**	VIC	2
Centrophoridae	***Deania calcea***	**Roughskin dogfish**	**Flake**	VIC	4*
			Flake	NSW	3*
			Boneless Fillet	NSW	2*
Squatinidae	***Squatina spp.***	**Angel sharks**	**Flake**	VIC	4*
Sphyrnidae	***Sphyrna lewini***	**Scalloped hammerhead**	**Flake**	QLD	3

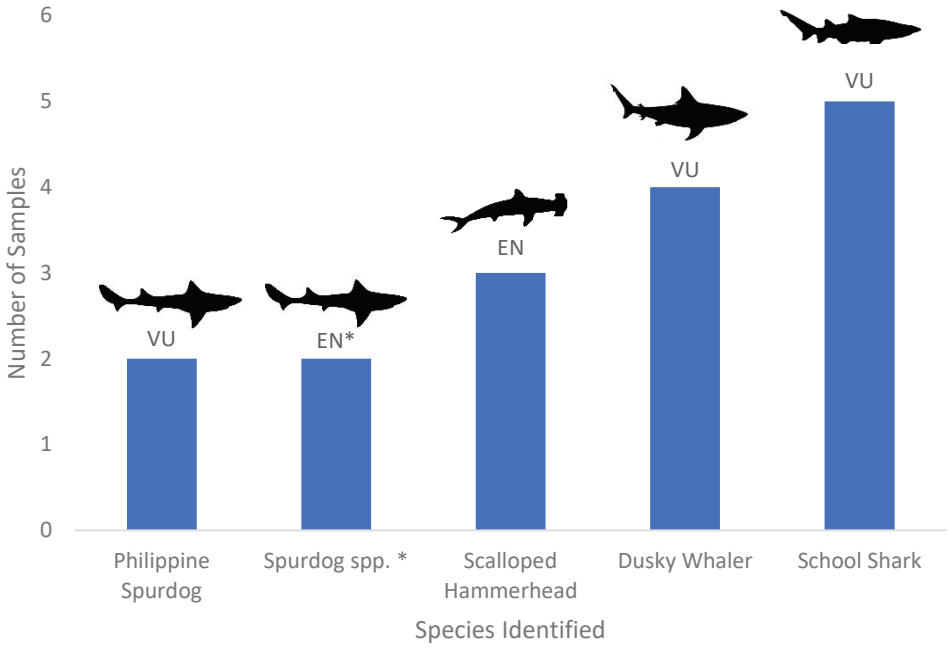


Fig 2. Bar chart of species listed under IUCN threatened categories, identified from samples collected at fish markets. The global IUCN Red List conservation status of each species is listed (VU: Vulnerable, EN: Endangered). Highest Red List category for potential species match (*).

3.3 Labelling

A total of eight different labels were used by retailers around the country including "Aus Shark", "Boneless Fillet", "Shark Barrel" and "Flake" (Fig.3). Species-specific labels included "Carpet Shark", "Bronze Whaler", "Gummy Shark" and "School Shark". "Flake" was by far the most frequently used label, found at all takeaway stores sampled and at 44% of wholesalers and fish markets.

According to the AFNS, shark meat consumed as Flake must be derived from only *M. mustelus* and *M. lenticulatus.* The standard also includes the use of "Bronze Whaler" as a label for both *C. brachyurus* and *C. obscurus.* Further, "Carpet Shark" as a label is not applicable to species of Wobbegong (Orectolobidae spp.*)* and the label "Wobbegong" should instead be applied to only Orectolobidae spp. "Boneless Fillet", "Aus Shark" and "Sweetfish" do not occur under the fish names standard and could instead be invented names by retailers. These names, including the non-specific label "Shark Barrel", comprised 14.3% of all samples.

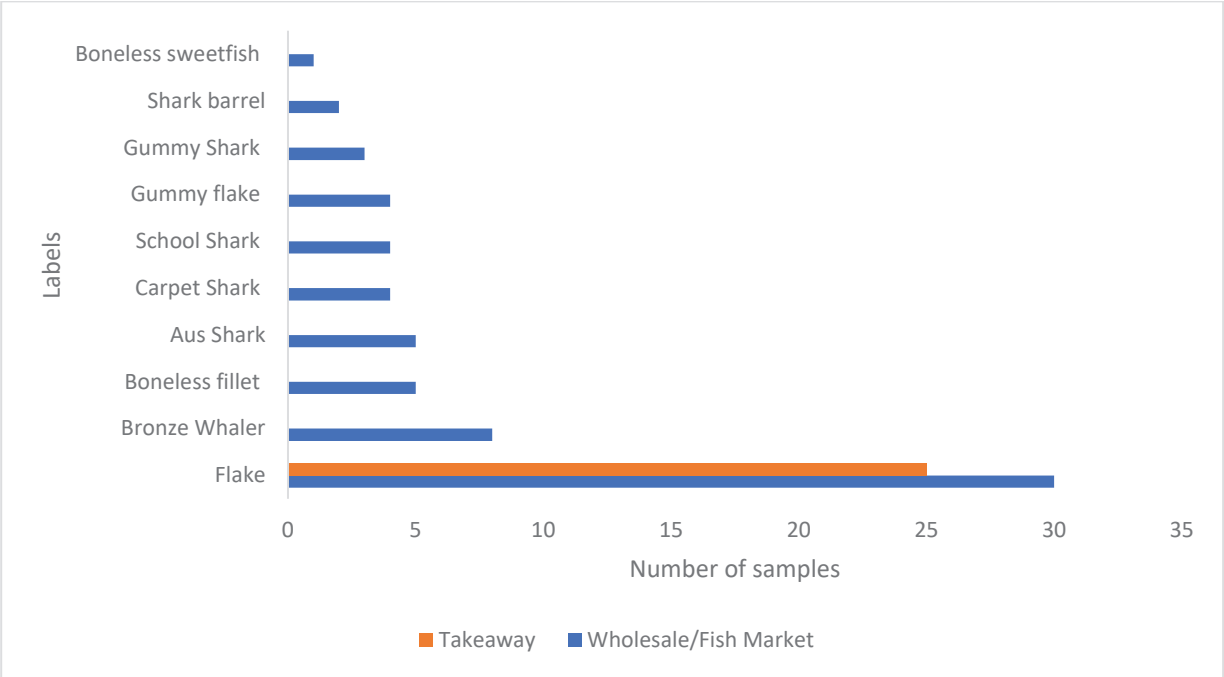


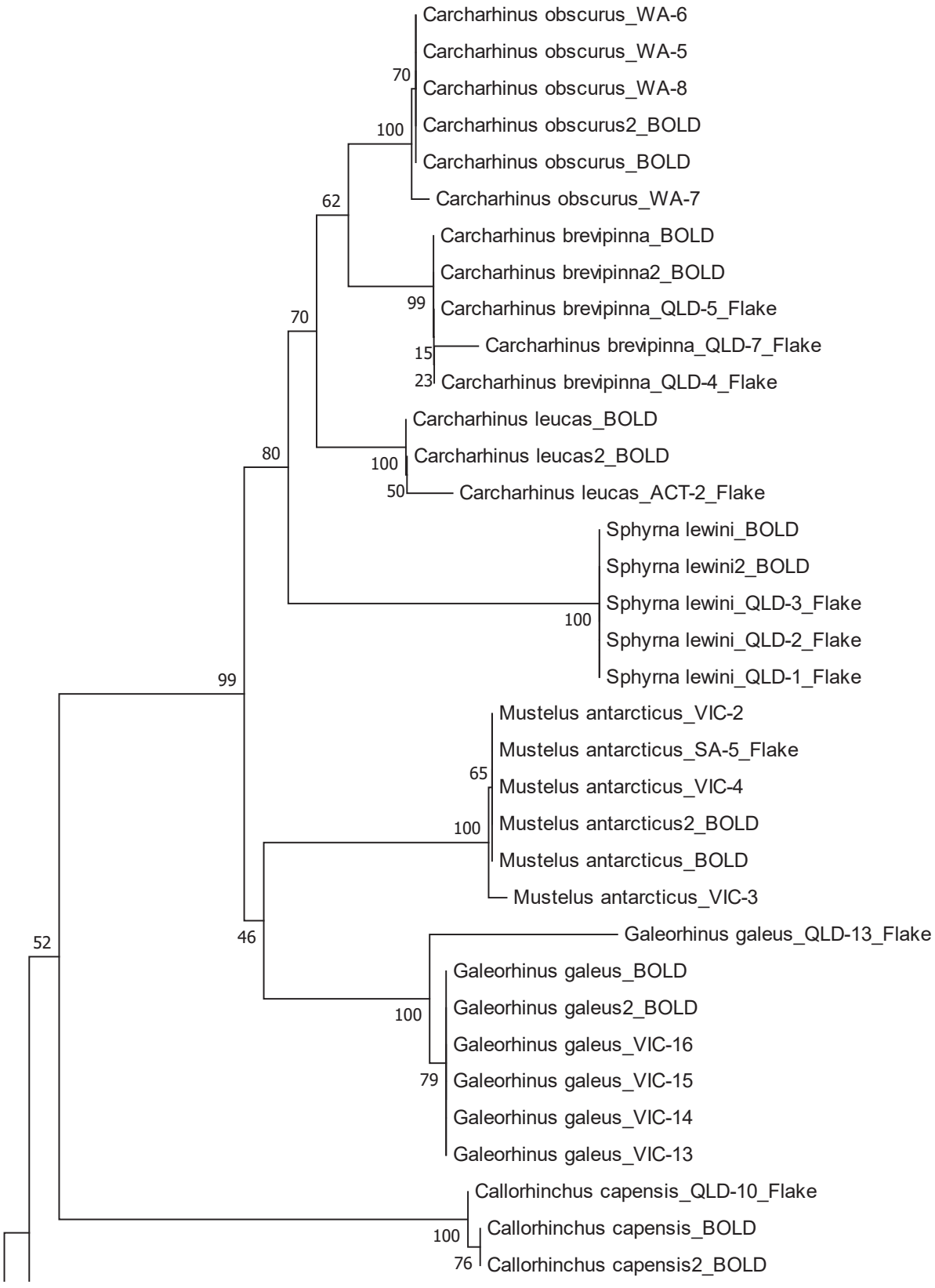
Fig 3. Labels identified from retailers (n = 91; Takeaway = 25, Wholesale/Fish Market = 66).

3.4 Phylogenetic analysis

The neighbour-joining tree constructed from sequences generated using the CO1 Fish1 and Fish2 primers supported 13 species-specific clades with >95% bootstrap support (Fig 4a). These 13 species-specific clades spanned four orders: Carcharhiniformes, Pristiophoriformes, Orectolobiformes and Squaliformes. Within the Carcharhiniformes, three monophyletic species-specific clades of *Carcharhinus* were recovered: *C. obscurus, C. brevipinna* and *C.leucus.* Clades of *Mustelus antarcticus, Galeorhinus galeus* and *Sphryna lewini* were also identified. *Orectolobus halei, Orectolobus maculatus* and *Orectolobus hutchinsi* belong to the Orectolobiformes. *Pristiophorus nudipinnis* belong to Pristiophoriformes. Within Squaliformes, two monophyletic clades of *Squalus montalbani* and *Squalus spp.* were recovered. One clade of *Callorhinchus capensis* (Order Chimaeriformes) was also supported with >80% bootstrap support.

The NJ tree generated using 12S primers was less discriminatory, identifying species from six orders: Carcharhiniformes, Pristiophoriformes, Orectolobiformes, Squaliformes, Squatiniformes and Chimaeriformes with limited individual species specificity and many nodes with bootstrap values <90% (Fig 4b). Within Carcharhiniformes, 37 instances of *Carcharhinus spp.* were recovered and three instances of *S. lewini.* Two separate clades of *Mustelus manazo* and *Mustelus canis* were also recovered. Within Squaliformes, two clades of *Deania calcea* and *Squalus spp.*

were recovered and one clade of *Squatina spp.* in the order Squatiniformes. Clades with bootstrap values of >90% included *C. capensis* in the order Chimaeriformes, *Orectolobus japonicus* in the order Orectolobiformes, *Pristiophorus nudipinnis* in the order Pristiophoriformes and the *Mustelus* spp.



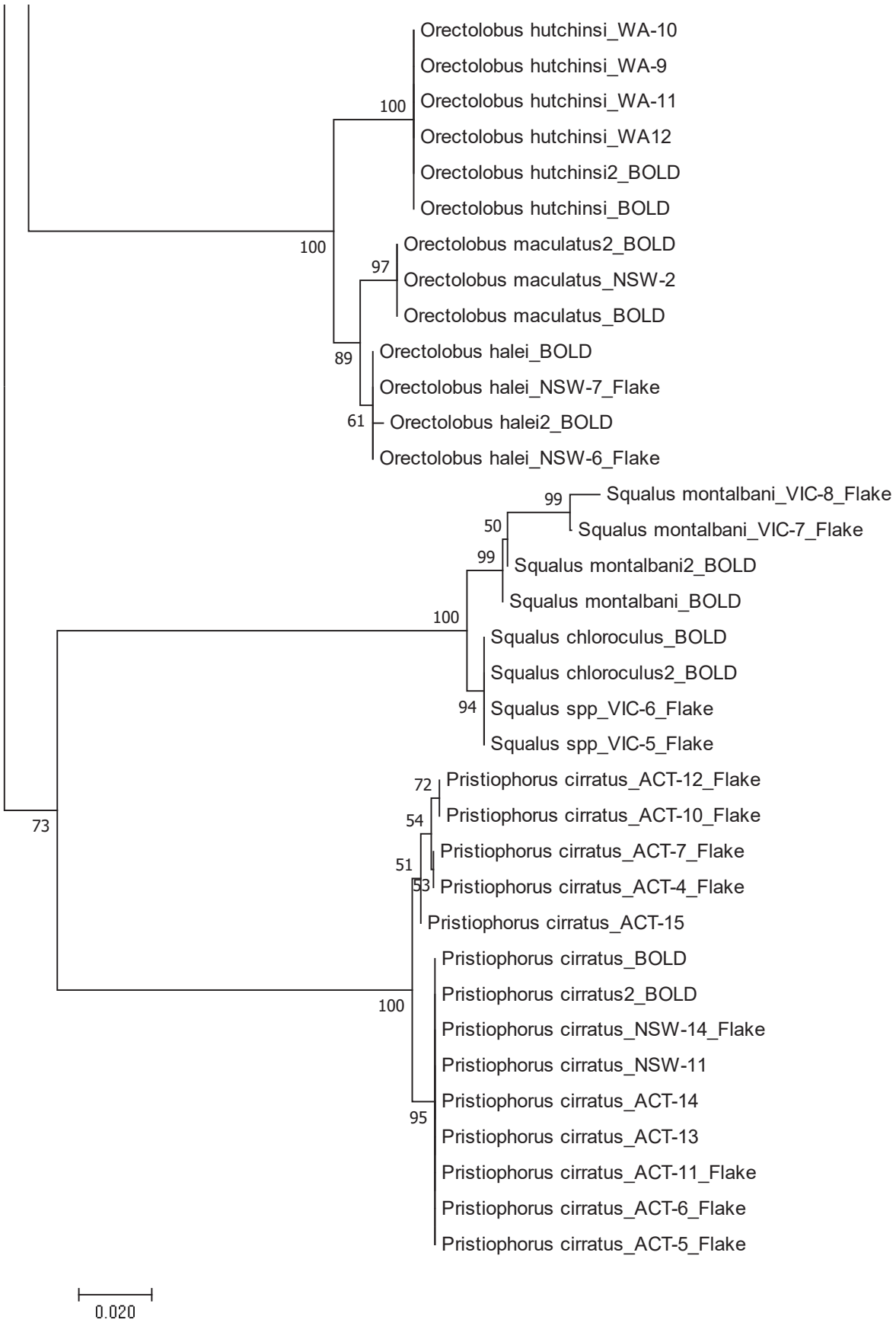
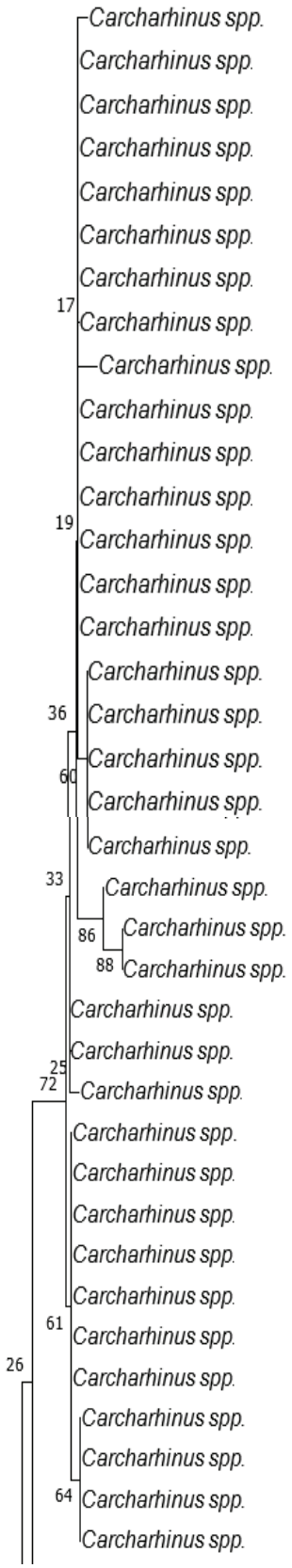


Fig 4(a). Neighbour-joining tree of CO1 gene sequences, corresponding to 13 different shark species from four orders. Sequences obtained from samples sold under the label Flake are identified. Sequences downloaded as reference data from BOLD are also identified.


Carcharhinus spp.
Carcharhinus spp.
Carcharhinus spp.
Carcharhinus spp.
Carcharhinus spp.
Carcharhinus spp.
Carcharhinus spp.
17
Carcharhinus spp.
Carcharhinus spp.
Carcharhinus spp.
Carcharhinus spp.
Carcharhinus spp.
19
Carcharhinus spp.
Carcharhinus spp.
Carcharhinus spp.
Carcharhinus spp.
36
Carcharhinus spp.
Carcharhinus spp.
60
Carcharhinus spp.
Carcharhinus spp.
33
Carcharhinus spp.
86
Carcharhinus spp.
88
Carcharhinus spp.
Carcharhinus spp.
Carcharhinus spp.
25
72
Carcharhinus spp.
Carcharhinus spp.
Carcharhinus spp.
Carcharhinus spp.
Carcharhinus spp.
61
Carcharhinus spp.
26
Carcharhinus spp.
Carcharhinus spp.
Carcharhinus spp.
64
Carcharhinus spp.
Carcharhinus spp.

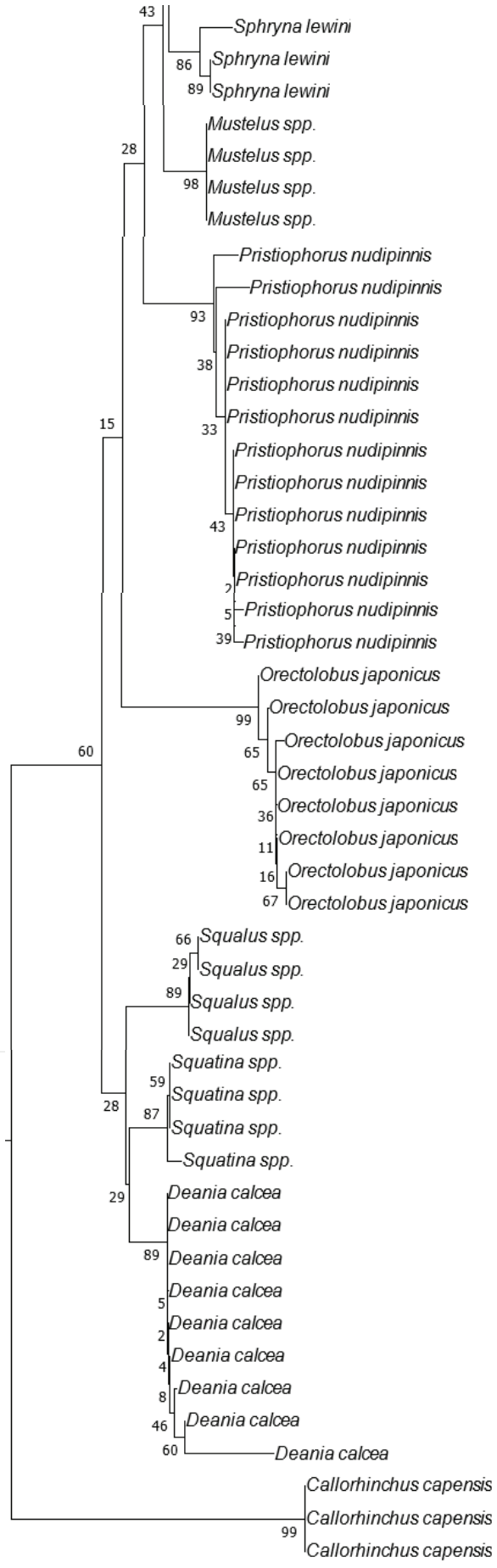


Fig 4(b). Neighbour-joining tree of 90 12S gene sequences generating with the Elas02 primers, corresponding to shark species from six orders.

4. Discussion

We successfully sequenced and identified 85 samples of shark flesh from five Australian states and found a mislabelling rate of 78.8%. We further identified four threatened species (35.3%). In total, 91 samples were collected with eight different labels, including 13 representing invented or non-specific names that were not listed on the AFNS. This use of DNA barcoding to uncover instances of mislabelling or substitution in the seafood market is becoming increasingly common, and several recent studies have used this technique to demonstrate seafood mislabelling around the world including India (Nagalakshmi et al. 2016), Europe (Barbuto et al. 2010; Di Pinto et al. 2015; Gunther et al. 2017; Tinacci et al. 2018; Hobbs et al. 2019), Greece (Pazartzi et al. 2019), Argentina (Delpiani et al. 2019), USA (Hellberg et al. 2019), and Brazil (Carvalho et al. 2015; Almeron-Souza et al. 2018; Bunholi et al. 2018; Feitosa et al. 2018; Calegari et al. 2019). Clearly this is a global issue that is likely contributing to global overexploitation of shark fisheries.

Our analysis shows labelling frequently does not adhere to Australian guidelines. Flake was the most frequently observed label (n= 55) of the eight different labels used for all (91) samples. Species-specific labels complied with the standard except for *Orectolobus* spp. sold as “Carpet Shark”. However, invented and non-specific names (excluding Flake) made up 14.3% of all shark meat products sold including the label “Sweetfish”. Sweetfish is not a label designated under the AFNS and could potentially refer to the River Ayu or Sweetfish (*Plecoglossus altivelis*) a river fish from East Asia that is extremely popular in Japanese cuisine. As the species identified was *P. cirratus* this sample could represent a potential species misidentification, however as the retailer personally identified the product as shark meat we treated it as a case of invented labelling. Invented names and labels are often applied in place of widely known common names, thereby providing non-specific or deliberately misleading identification (Delpiani et al. 2019; Pazartzi et al. 2019). For instance, in Argentina invented names have been found in place of widely used common names where *Squatina* spp. and *Mustelus* spp. were found marketed under names that did not correspond to official common names (Delpiani et al. 2019). These instances of misleading labelling demonstrates the difficulties facing consumers should they want to know what species they are eating.

Overall, 67 out of the 85 identified samples were mislabeled under the AFNS, including 53 (62.35%) that were mislabeled as Flake. The term Flake was designated to *M. antarcticus* and *M. lenticulatus* in 2014 and therefore potentially is still not widespread knowledge, particularly in the states furthest from South Australia where *M. antarcticus* does not occur and where the appearance of this species on local markets is of low frequency and potentially less recognisable (FRDC 2016). Eight species labelled as Flake did not comply with AFNS regulation, and samples collected from

Victoria showed the lowest level of Flake being mislabelled (n= 4,). This is likely a direct result of close proximity to the SESSF fishery. Queensland had a significantly higher level of mislabelling, where all Flake samples collected were mislabelled (n= 8, 100%). In Queensland sharks are caught primarily in the East Coast Inshore Fin Fish, Gulf of Carpentaria Inshore Fin Fish and Gulf of Carpentaria Line fisheries. Another instance of species mislabelling was three samples labeled as Gummy Shark that were identified as *P. cirratus* (Common sawshark). This could be a result of deliberate or mis-informed labelling whereby the inconsistency arises from either the retailer itself or the information they were given at time of sourcing.

Labelling was also found to be inaccurate for *Orectolobus hutchinsi* being sold under the label "Carpet Shark". The AFNS name for *Orectolobus* spp. is "Wobbegong", and some confusion can be explained due to the species taxonomic identification under the order Orectolobiformes (Carpet Sharks) (Compagno 2001). Carpet Shark as a label remains designated to *Parascyllium, Hemiscyllium* and *Chiloscyllium* spp.. The specific designation of Wobbegong, to ensure differentiation of all *Orectolobus spp.* from other carpet sharks is likely due to their history in Australian fisheries, where they have been targeted commercially in NSW since 1990/91 (FRDC 2016). Total catch declined by 50% in 1997-98 and 2007-08, leading to the introduction of management regulations by NSW for commercial and recreational fishers in 2008 (FRDC 2016). The current catch of Wobbegong in Australia is considered sustainable (SAFS), with NSW the only state to continue targeting the species' commercially (Simpfendorfer et al. 2019).

We identified 16 out of 45 samples (35.6%) as species listed under threatened categories on IUCN. This consisted of four species (*S. lewini, S. montalbani, C. obscurus, G. galeus)* listed as Vulnerable or Endangered on the IUCN Red List of Threatened Species (IUCN 2019). *Sphyrna lewini* (Scalloped Hammerhead)*, S.montalbani* (Philippine Spurdog, AFNS: Roughskin Dogshark) and *G. galeus* (School shark) were identified under the label Flake, suggesting that the commercial label is contributing to unidentified trade of threatened species. A similar study in Greece found 55.81% of samples were threatened species sold under the commercial name 'galeos' despite regulations stating Galeos was a label designated for three *Mustelus* species (Pazartzi et al. 2019). The use of a widespread commercial name applied across many species makes monitoring the trade of threatened species increasingly complicated, particularly when the commercial name has a species standard but remains unregulated (Barbuto et al. 2010; Almeron-Souza et al. 2018; Pazartzi et al. 2019).

Trade of threatened species is allowable in Australia under EPBC regulations where species listed on CITES Appendix II like *S. lewini* can be landed under certain conditions. The Scalloped Hammerhead (*S. lewini)* is found in waters globally and is listed as Endangered on the IUCN Red

List. The species has been identified as at risk of detrimental overfishing in fishing ports globally (Abercrombie et al. 2015; Dulvy et al. 2017), including in countries like Bangladesh (Haque et al. 2019), Taiwan (Liu et al. 2013), Brazil (Feitosa et al. 2018) and Hong Kong (Shea and To 2017). In Australia, *S. lewini* has been listed as 'Conservation Dependent' under the EPBC Act as of 2018. The School Shark (*G. galeus)* has been listed as 'Conservation Dependent' since 2009*,* with a history in targeted fishing operations since the 1930's (FRDC 2016). In 1990 *G. galeus* was listed as bycatch only in all fisheries around Australia, with a total allowable catch in 2018 of 215tonnes (Patterson et al. 2019). However, there remains limited statistics on current stock size and biomass mortality and *G. galeus* remains classified as overfished and globally Vulnerable under IUCN Red List listing (IUCN 2019; Patterson et al. 2019). Despite this the shark remains the second most important species in the Eastern and Southern Scalefish and Shark Fishery (Patterson et al. 2019).

In Australia, Spurdogs (*Squalus* spp.*)* are currently caught in trawl and long-line fisheries around Australia and are extremely susceptible to overfishing due to low reproductive rates and time to reproduction characteristic of many deep-sea fishes (Graham et al. 2001; Last et al. 2007; Simpfendorfer and Kyne 2009; Simpfendorfer et al. 2019). From 1970-1990 *Squalus* spp. saw population declines of up to 97% in Australia, mainly due to bottom trawling, and are now regulated under a management plan that includes catch limits and depth and spatial closures of fishing ranges (Graham et al. 2001; AFMA 2012). In 2019, *S. chloroculus* was listed as Endangered on the IUCN Red List, and *S. montalbani* (Philippine spurdog) is currently classified as IUCN: Vulnerable (IUCN 2019). Two sequences obtained showed a 95% similarity to both *S. chloroculus* and *S. mitsukurii* (Shortnose spurdog)*,* with a >94% match on BOLD to *S. chloroculus.* Prior to 2007, both species were considered conspecific and further often confused with *S. montalbani,* potentially accounting for the very strong similarity (Last et al. 2007). *S. chloroculus* has been found only along Australia's southern coast from New South Wales to The Great Australian Bite and is considered endemic to Australia, while *S. mitsukurii* is restricted to Japanese waters suggesting that a match to *S. chloroculus* is likely (Last et al. 2007; Last and Stevens 2009). Further, the NJ tree for Fish2 supports two separate clades of *Squalus* spp. suggesting a species designation other than *S. montalbani* for these two sequences. Both species are listed as 'Transitional Recovering' under the EPBC Act (Simpfendorfer et al. 2019).

Also listed under the EPBC Act as 'Transitional Recovering' are Dusky Whaler sharks (*C. obscurus)*, now considered recovering after historic declines from overfishing, and listed as globally Vulnerable under IUCN (Graham et al. 2001; Simpfendorfer et al. 2019). The AFNS designation for both *C. obscurus* and *C. brachyurus* is Bronze Whaler, with no required differentiation between the two species. This is likely encouraged by the very strong morphological similarities between the

two species. However *C. obscurus* has a history of overfishing in Australia, and *C. brachyurus* is currently listed as Near Threatened (IUCN 2019). An important parameter for stock assessments is recording species-specific landing data, and therefore greater transparency by separately labelling the two species would benefit conservation and fishery sustainability of *C. brachyurus* and *C. obscurus* in Australia (Liu et al. 2013).

In 2017, a review was conducted on the sustainability of a number of shark fisheries around the world based on stock assessments and availability of science-based management plans (Simpfendorfer and Dulvy 2017). In southern Australia, the annual catch of *M.antarcticus* was considered both sustainable and sustainably managed, with adequate management plans in place to ensure populations are biologically sustainable, including annually reviewed Recommended Biological Catch (RBC) quotas (Simpfendorfer and Dulvy et al. 2017; Woodhams et al. 2018; Woodhams and Hart et al. 2018; Patterson et al. 2019). In the past 5 years, commercial take of *M. antarcticus* in the SESSF has not exceeded the annual RBC quota limit (Woodhams and Hart 2018). Annual catch of blacktip sharks in eastern Queensland was similarly considered sustainable, however at the time of report, not sustainably managed (Simpfendorfer and Dulvy 2017). This is of concern when sharks such as *C. brevipinna* are IUCN listed as Near Threatened (IUCN 2019). Only 86% of the annual commercial catch of all sharks in Queensland was recorded in logbooks prior to 2018, with the introduction of the Shark and Ray logbook now aimed at reliably listing species records (Leigh et al. 2015; AFMA 2018). This variation in sustainable management of fisheries in Australia is a pressing conservation concern. Such inconsistency in management, coupled with poor labelling standards, does not encourage sustainability in the trade of seafood.

Providing consumers with the option of making sustainable decisions when purchasing seafood not only encourages an understanding of where seafood has been sourced but also encourages the continued growth of sustainably managed fisheries (Dudgeon et al. 2012; Lamendin et al. 2015). Exploitation of fisheries often arises from increasing market demand, with potential for economic gain often overriding sustainable recommendations (Clarke et al. 2006; Baker 2008; McClenachan et al. 2016). The catch quotas and management of *M. antarcticus* in Australia is contributing to a growth in sustainable practices, ensuring consumers are aware of how their seafood is sourced and maintained. However, with the discrepancies between management practices of fisheries around the country it is increasingly important that labelling standards are maintained to ensure consumers are aware of the species they are purchasing.

Nearly all threatened species identified were being sold under the label Flake (except for *C. obscurus)* confirming that the label is being used as an umbrella term for a number of shark species, and providing an avenue for unidentified trade. Encouragement for retailers to follow the AFNS

standard, through the introduction of an ID system, allowing consumers to reliably identify retailers supplying correctly labelled products would be beneficial while the standard remains voluntary. Increased enforcement of the guidelines and stronger regulations on labelling of threatened species would also benefit both consumers and species conservation. While the fishery in southern Australia is currently considered sustainable and fisheries in northern Australia have introduced updated logbook systems, the ability to regulate and manage effectively is unlikely given there is an unknown level of trade that is not included in management plans due to inaccurate labelling. This is of particular concern for catch of threatened species that remains legal under obviously unregulated quota limits, with room for further improvement in sustainable management.

With current data suggesting many fisheries have the potential for sustainable management, and with chondrichthyan species under increasing threat, the use of a DNA barcoding approach for genetic identification has shown significant utility (Dudgeon et al. 2012; Simpfendorfer and Dulvy et al. 2017). However, advancements in barcoding including the use of the 12S RNA gene are limited. While CO1 barcoding has the advantage of a large background database, the use of 12S barcoding in this study was impacted by limited available sequences. In comparison, while CO1 could provide greater species-specific identification the use of 12S primers ensured identification of >90% of our samples whereas CO1 accounted for <50%. Having both genes to cross-reference for identification purposes allowed increased reliability of identification, despite the limitations of both genes. The use of a metabarcoding approach for species identification remains probable, however future studies on the use of the 12S gene for metabarcoding should focus on a stronger experimental design. Where invented labels were found further research could be conducted into reviewing any labels potentially in use for both shark and fish (eg. Sweetfish). This could also potentially apply to the label Boneless Fillet, as many species of fish are considered ‘boneless’ after filleting, further representing ambiguity on the market.

Conclusions

This study demonstrates that substitution and mislabelling of shark products is prevalent in Australia and that naming standards, which are currently voluntary, require greater encouragement in order to be effective. Potentially a lack of understanding among retailers of the specific guidelines (as in the case of “Flake” and “Carpet Shark”) could contribute to inadvertent mislabelling and greater awareness is needed with stronger enforcement and increased encouragement. This includes greater public awareness of Australia’s fisheries and sustainable options available on the market. There is potential for further study in the use of the 12S gene for a

metabarcoding approach for species identification in the seafood trade. Our results suggest that enforcement of labelling standards remains inadequate across most of Australia, and further emphasises the importance of accurate and reliable labelling to not only educate and protect consumers, but to encourage sustainable management. Addressing the issue of shark meat labelling may contribute to improving the conservation status of sharks traded in Australia.

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Appendix A – Sequence Results

Table 1A. Results of BLAST searches for 600bp of all CO1 sequences (n= 25), obtained using Ward et al. 2005 Fish1 primer. E-value identified when available (*)

					*E-value**	
Sample	*BLAST/species*	*Max Score*	*Query Cover*	*Per. Identity*	*CO1 complete barcode*	*CO1 partial sequence*
VIC-1	Mustelus antarcticus	928	95%	95.46%		0.0
VIC-3	Mustelus antarcticus	928	96%	95.46%		0.0
VIC-13	Galeorhinus galeus	950	95%	96.13%		0.0
VIC-14	Galeorhinus galeus	946	96%	96.13%		0.0
VIC-15	Galeorhinus galeus	946	96%	95.68%		0.0
VIC-16	Galeorhinus galeus	946	96%	95.68%		0.0
						0.0
NSW-2	Orectolobus maculatus	950	95%	96.13%		0.0
NSW-6	Orectolobus halei	947	94%	96.09%		0.0
NSW-7	Orectolobus halei	937	94%	96.09%		0.0
WA-5	Carcharhinus obscurus	953	96%	96.00%	0.0	
WA-6	Carcharhinus obscurus	959	96%	96.17%	0.0	
WA-7	Carcharhinus obscurus	942	96%	95.67%	0.0	
WA-8	Carcharhinus obscurus	959	96%	96.17%	0.0	
WA-9	Orectolobus hutchinsi	950	95%	96.13%		0.0
WA-10	Orectolobus hutchinsi	950	95%	96.13%		0.0
WA-11	Orectolobus hutchinsi	950	95%	96.13%		0.0
WA-12	Orectolobus hutchinsi	950	95%	96.13%		0.0
SA-5	Mustelus antarcticus	950	95%	96.13%		0.0
QLD-1	Sphyrna lewini	963	97%	96.18%	0.0	0.0
QLD-2	Sphyrna lewini	963	97%	96.18%	0.0	0.0
QLD-3	Sphyrna lewini	963	97%	96.18%	0.0	0.0
QLD-4	Carcharhinus brevipinna	965	96%	96.19%	0.0	0.0
QLD-5	Carcharhinus brevipinna	965	96%	96.19%	0.0	0.0
QLD-7	Carcharhinus brevipinna	959	96%	96.02%	0.0	0.0
QLD-13	Galeorhinus galeus	933	95%	95.63%		0.0

Table 2A. Results of BLAST searches for 651bp of all CO1 sequences (n= 20), obtained using Ward et al. 2005 Fish2 primer. E-value identified when available (*)

					*E-value**	
Sample	*BLAST/species*	*Max Score*	*Query Cover*	*Per. Identity*	*CO1 complete barcode*	*CO1 partial sequence*
VIC-8	Squalus montalbani	1074	97%	97.18%	0.0	0.0
VIC-7	Squalus montalbani	1074	97%	97.17%	0.0	0.0
VIC-6	Squalus spp.	1050	95%	100.00%	0.0	0.0
VIC-5	Squalus spp.	1144	95%	100.00%	0.0	0.0
VIC-4	Mustelus antarcticus	1149	94%	100.00%	0.0	0.0
VIC-2	Mustelus antarcticus	1151	95%	100.00%	0.0	0.0
QLD-10	Callorhinchus capensis	1146	96%	99.52%	0.0	0.0
NSW-14	Pristiophorus cirratus	1144	93%	100.00%	0.0	0.0
NSW-11	Pristiophorus cirratus	1147	94%	100.00%	0.0	0.0
ACT-15	Pristiophorus cirratus	1146	94%	100.00%	0.0	0.0
ACT-14	Pristiophorus cirratus	1144	93%	100.00%	0.0	0.0
ACT-13	Pristiophorus cirratus	1146	94%	100.00%		
ACT-12	Pristiophorus cirratus	1138	93%	99.84%	0.0	0.0
ACT-11	Pristiophorus cirratus	1146	94%	100.00%	0.0	0.0
ACT-10	Pristiophorus cirratus	1140	94%	99.84%	0.0	0.0
ACT-7	Pristiophorus cirratus	1140	94%	99.84%	0.0	0.0
ACT-6	Pristiophorus cirratus	1147	94%	100.00%	0.0	0.0
ACT-5	Pristiophorus cirratus	1149	94%	100.00%	0.0	0.0
ACT-4	Pristiophorus cirratus	1144	93%	100.00%	0.0	0.0
ACT-2	Carcharhinus leucas	1167	97%	98.13%	0.0	0.0

Table 3A. Results of BLAST searches for approx. 148bp from all 12S sequences (n= 91), obtained using Taberlet et al. 2018 primer. E-value identified when available (*)

					*E-value**	
Sample	*BLAST/species*	*Max Score*	*Query Cover*	*Per. Identity*	*12S complete barcode*	*12S partial sequence*
WA-12	Orectolobus japonicus	231	100%	95.83%	4.00E-57	2.00E-56
WA-11	Orectolobus japonicus	250	100%	97.92%	1.00E-62	4.00E-62
WA-10	Orectolobus japonicus	226	100%	94.63%	2.00E-55	8.00E-53
WA-9	Orectolobus japonicus	224	100%	95.10%	7.00E-55	3.00E-54
WA-8	Carcharhinus obscurus	270	100%	100.00%	9.00E-69	3.00E-68
WA-6	Carcharhinus obscurus	239	100%	96.00%	3.00E-59	9.00E-59
WA-5	Carcharhinus obscurus	272	100%	100.00%	2.00E-69	9.00E-69
WA-4	Carcharhinus obscurus	270	100%	100.00%	9.00E-69	3.00E-68
WA-3	Carcharhinus obscurus	241	100%	96.62%	7.00E-60	2.00E-59
WA-2	Carcharhinus obscurus	270	100%	100.00%	9.00E-69	3.00E-68
WA-1	Carcharhinus obscurus	270	100%	100.00%	9.00E-65	3.00E-68
ACT-15	Pristiophorus nudipinnis	219	98%	95.00%		3.00E-53
ACT-14	Pristiophorus nudipinnis	233	98%	97.10%		1.00E-57
ACT-13	Pristiophorus nudipinnis	241	100%	97.18%		6.00E-60
ACT-12	Pristiophorus nudipinnis	239	100%	97.16%		2.00E-59
ACT-11	Pristiophorus nudipinnis	233	99%	95.71%		1.00E-57
ACT-10	Pristiophorus nudipinnis	233	99%	97.10%		1.00E-57
ACT-9	Carcharhinus leucus	237	100%	95.95%	9.00E-59	3.00E-58
ACT-8	Carcharhinus leucus	254	100%	97.97%	9.00E-64	3.00E-63
ACT-7	Pristiophorus nudipinnis	233	99%	97.10%		1.00E-57
ACT-6	Pristiophorus nudipinnis	233	99%	97.10%		1.00E-57
ACT-5	Pristiophorus nudipinnis	198	99%	92.70%		5.00E-46
ACT-4	Pristiophorus nudipinnis	228	100%	95.77%		5.00E-56
ACT-3	Carcharhinus leucus	329	94%	100.00%	2.00E-86	
ACT-2	Carcharhinus leucus	329	94%	100.00%	2.00E-86	
ACT-1	Carcharhinus leucus	344	97%	100.00%	8.00E-91	

					E-value*	
Sample	*BLAST/species*	*Max Score*	*Query Cover*	*Per. Identity*	*12S complete barcode*	*12S partial sequence*
NSW-14	Pristiophorus nudipinnis	239	100%	97.16%		2.00E-59
NSW-13	**No similarity**					
NSW-12	Pristiophorus nudipinnis	243	100%	97.20%		2.00E-60
NSW-11	Pristiophorus nudipinnis	196	100%	91.61%		1.00E-46
NSW-10	Deania calcea	267	100%	99.32%		1.00E-46
NSW-9	Deania calcea	219	98%	90.00%		4.00E-53
NSW-8	Carcharhinus obscurus	261	100%	99.31%	5.00E-66	2.00E-65
NSW-7	Orectolobus japonicus	246	100%	97.89%	1.00E-61	5.00E-61
NSW-6	Orectolobus japonicus	248	100%	97.90%	4.00E-62	2.00E-61
NSW-5	Deania calcea	272	100%	100.00%		3.00E-69
NSW-4	Deania calcea	267	100%	100.00%		1.00E-67
NSW-3	Deania calcea	270	100%	100.00%		9.00E-69
NSW-2	Orectolobus japonicus	252	100%	98.59%	3.00E-63	1.00E-62
NSW-1	Orectolobus japonicus	219	100%	93.96%	3.00E-53	1.00E-52
VIC-20	Deania calcea	268	99%	99.33%		3.00E-68
VIC-19	Deania calcea	267	98%	99.32%		1.00E-67
VIC-18	Deania calcea	267	98%	98.68%		1.00E-67
VIC-17	Deania calcea	267	98%	100.00%		1.00E-67
VIC-16	Mustelus spp.	202	100%	92.25%		3.00E-48
VIC-15	Mustelus spp.	204	100%	92.31%		9.00E-69
VIC-14	Mustelus spp.	206	100%	91.72%		1.00E-47
VIC-13	Mustelus spp.	206	100%	92.36%		2.00E-49
VIC-12	Squatina dumeril	239	100%	95.95%		2.00E-59
VIC-11	Squatina dumeril	222	100%	94.48%		3.00E-54
VIC-10	Squatina dumeril	226	100%	94.59%		2.00E-55
VIC-9	Squatina dumeril	233	100%	95.86%		1.00E-57
VIC-8	Squalus montalbani	265	100%	100.00%	4.00E-67	
VIC-7	Squalus montalbani	261	100%	100.00%	5.00E-66	
VIC-6	Squalus spp.	257	100%	99.30%	7.00E-65	
VIC-5	Squalus spp.	255	100%	99.29%	2.00E-64	
VIC-4	Mustelus manazo	255	100%	98.61%	2.00E-64	8.00E-64
VIC-3	Mustelus manazo	259	100%	98.63%	2.00E-65	7.00E-65
VIC-2	Mustelus manazo	261	100%	98.64%	6.00E-66	2.00E-65
VIC-1	Mustelus manazo	261	100%	98.61%	6.00E-66	2.00E-65

					E-value*	
Sample	*BLAST/species*	*Max Score*	*Query Cover*	*Per. Identity*	*12S complete barcode*	*12S partial sequence*
QLD-25	Carcharhinus brevipinna	270	100%	100.00%	9.00E-69	1.00E-66
QLD-24	Carcharhinus spp.	261	100%	99.31%	5.00E-66	5.00E-66
QLD-23	Carcharhinus sorrah	265	100%	100.00%	4.00E-67	1.00E-66
QLD-22	Carcharhinus sorrah	265	100%	100.00%	4.00E-67	1.00E-66
QLD-21	Carcharhinus spp.	248	100%	97.90%	4.00E-62	4.00E-62
QLD-20	Carcharhinus sorrah	265	100%	100.00%	4.00E-67	1.00E-66
QLD-19	Carcharhinus sorrah	265	100%	100.00%	4.00E-67	1.00E-66
QLD-18	Carcharhinus sorrah	265	100%	100.00%	4.00E-67	1.00E-66
QLD-17	Carcharhinus sorrah	265	100%	99.31%	4.00E-67	1.00E-66
QLD-16	Carcharhinus spp.	257	100%	99.30%	7.00E-65	7.00E-65
QLD-15	Carcharhinus spp.	255	100%	99.29%	2.00E-64	2.00E-64
QLD-14	Carcharhinus spp.	263	100%	100.00%	1.00E-66	1.00E-66
QLD-13	Carcharhinus spp.	265	100%	100.00%	4.00E-67	4.00E-67
QLD-12	Carcharhinus spp.	235	100%	95.36%	4.00E-58	4.00E-58
QLD-11	Carcharhinus spp.	235	100%	96.00%	4.00E-58	4.00-58
QLD-10	Callorhinchus capensis	268	100%	100.00%	3.00E-68	3.00E-68
QLD-9	Callorhinchus capensis	254	100%	98.61%	9.00E-64	9.00E-64
QLD-8	Callorhinchus capensis	259	100%	98.68%	2.00E-65	2.00E-65
QLD-7	Carcharhinus brevipinna	263	100%	100.00%	1.00E-66	2.00E-64
QLD-6	Carcharhinus brevipinna	268	100%	100.00%	3.00E-68	5.00E-66
QLD-5	Carcharhinus brevipinna	268	100%	100.00%	3.00E-68	5.00E-66
QLD-4	Carcharhinus brevipinna	268	100%	100.00%	3.00E-68	5.00E-66
QLD-3	Sphyrna lewini	268	100%	100.00%	3.00E-68	1.00E-67
QLD-2	Sphyrna lewini	239	98%	97.20%	3.00E-59	9.00E-59
QLD-1	Sphyrna lewini	268	100%	100.00%	3.00E-68	1.00E-67
SA-5	Mustelus manazo	255	100%	98.61%	3.00E-64	9.00E-64
SA-4	Carcharhinus obscurus	272	100%	100%	3.00E-64	9.00E-64
SA-3	Carcharhinus obscurus	241	100%	96.60%	7.00E-60	3.00E-59
SA-2	Carcharhinus obscurus	265	100%	100%	4.00E-67	2.00E-63
SA-1	Carcharhinus obscurus	265	100%	100%	4.00E-67	2.00E-66