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DNA barcoding of fresh seafood in Australian markets reveals misleading labelling and sale of endangered species

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Abstract

Flake and shark samples were purchased from outlets in several coastal Australian regions and genetically barcoded using the cytochrome oxidase subunit 1 (CO1) gene to investigate labelling reliability and species-specific sources of ambiguously labelled fillets. Of the 41 shark fillet samples obtained, 23 yielded high-quality CO1 sequences, out of which 57% ($n = 13$) were labelled ambiguously (misleading) and 35% ($n = 8$) incorrectly. In contrast, barramundi fillets, which are widely available and sought after in Australian markets, were shown to be accurately labelled. Species identified from shark samples, including the shortfin mako ($n = 3$) and the scalloped hammerhead ($n = 1$), are assessed by the IUCN as endangered and critically endangered, respectively, with several others classified as vulnerable and near threatened.

KEYWORDS

CO1 barcoding, fisheries, flake, shark, sustainability

Seafood is one of the most traded food commodities in the world, with apparent consumption increasing at almost twice the rate of annual global population growth between 1961 and 2019 (Food and Agriculture Organisation of the United Nations, 2022). Nonetheless, limited transparency and traceability from source to sale has led to increasing concern regarding the mislabelling of seafood products (Kroetz *et al*., 2020; Luque & Donlan, 2019). Using molecular barcoding techniques, several studies have investigated widespread seafood mislabelling on both smaller scales in Singapore, Brazil, Italy, Greece and Malaysia (Chin *et al*., 2016; Marchetti *et al*., 2020; Merten Cruz *et al*., 2021; Neo *et al*., 2022; Pazartzi *et al*., 2019) and wider regions in the USA, European Union and global data sets (Cawthorn *et al*., 2018; Khaksar *et al*., 2015; Pardo *et al*., 2016; Pardo *et al*., 2018). "Sustainable seafood guides" for eco-conscious consumers are available in several regions, ranking seafood products based on assessed sustainability. These include guides by World Wildlife Fund tailored for 25 different countries (World Wildlife Fund, 2020), as well as the Australian GoodFish guide (GoodFish, 2019). These guides are frequently informed by accreditation schemes such as Marine Stewardship Council that take parameters such as life histories, IUCN status, by-catch concerns and population trends (regional and global) into consideration, offering informed choices for consumers, as well as accreditation to fisheries that wish to use their sustainability status as a marketing tool (Marine Stewardship Council, 2022). As legislation to reduce damaging fishing practices and regulate the harvest and sale of seafood products is implemented, greater incentives exist to circumvent regulations and market unsustainable products to consumers (Liu *et al*., 2013).

Consumer choice when buying seafood in Australia may be diminished through ambiguous labelling, such as labelling a diverse range of shark species as "flake" at the point of sale (Bornatowski *et al*., 2014). Although the term is misleading and holds no legal obligation to refer to any particular species (Ciconte, 2014), it has historically been used in Australia for shark fillets sold at market since the mid-1920s where it originated in relation to the Southern Australian/Victorian gummy shark fishery (Ciconte, 2014). This fishery, once considered to be well managed and sustainably fished (Ward-Paige *et al*., 2012), is now of concern due to by-catch and depleting stock structure (Petrolo *et al*., 2021).

Here, the authors applied a genetic barcoding technique to evaluate the labelling of shark products sold at Australian markets directly to consumers. DNA barcoding provides an effective tool for differentiating species present within the fresh seafood market, particularly when sold as fillets where accurate identification by other means is challenging. This study aims to establish how accurately and informatively shark fillets are labelled at the point of sale in Australian outlets by (a) identifying which shark species are being sold as flake and the prevalence of each identified species within the study, (b) assessing to what degree species are labelled accurately for consumers at point of sale and (c) determining if low-cost shark fillets are being sold in place of other fish species of higher value, such as Australian barramundi.

Shark and barramundi fillets were sampled from fresh seafood outlets including supermarkets and fishmongers around coastal Australia. A total of 41 fillets labelled as shark or flake and 18 fillets labelled as barramundi were sampled from nine locations, with fresh or frozen fillets preserved in 95% high-grade ethanol on purchase. The chance of sampling the same individual multiple times was controlled through purchase of one fillet per species per outlet.

DNA was isolated from tissue *via* a modified salting-out procedure (Sunnucks & Hales, 1996). Nanodrop spectrophotometry was applied for quality assurance and control of extracted DNA, resulting in yields between 400 and 1500 ng μl^{-1} of genomic DNA. Amplification of 606 and 575 bp partial fragments of mitochondrial cytochrome oxidase subunit 1 (CO1) was carried out using universal shark and fish primers (Liu *et al*., 2013, Ward *et al*, 2008). PCR was performed using BIOTAQ Polymerase kits (Bioline), and visually confirmed using standard gel electrophoresis. Purification and standard bi-directional sequencing were carried out by Macrogen Inc. (Seoul, South Korea) using Applied Biosystems technology. Consensus sequences were generated in Geneious R8 (Kearse *et al*., 2012) and manually edited in MEGA6 (Tamura *et al*., 2016, https://www.megasoftware.net) for phylogenetic analyses following MUSCLE alignment (Tamura *et al*., 2016). Sequence hits with highest query coverage and maximum identical values (>98%) were retained following NCBI BLAST of nucleotide and protein databases. Along with the acquired sequences, 77 voucher sequences (Supporting Information Table TABLE S1) representing 28 commercially important shark species and 6 voucher sequences representing *Lates calcarifer* (Supporting Information Table S2) were added to the analysis.

An alignment of all sequences, including GenBank-sourced voucher specimens, was used in phylogenetic analysis and comparison to a known species with best substitution models for shark [HKY + G + I (G = 1.19, I = 0.53)], and barramundi sequences (K2) was used as calculated *via* a likelihood method in MEGA6. Phylogenetic analyses included estimates of maximum likelihood (ML) from MEGA6 and Bayesian inference using MrBayes (Huelsenbeck & Ronquist, 2001) in Geneious R8. ML analyses used 77 consensus sequences, with 10 independent runs, all displaying identical tree length and topology. Majority rule support values derived from consensus trees based on 1000 bootstrap replicates were accepted. Bayesian inference was estimated with 100,000 iterations and 10,000 tree burn-in, with otherwise default settings. Consensus support values of >70% are displayed on the best ML tree, edited in FigTree 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/). Blue grenadier (*Macruronus novaezelandiae*) and Nile perch (*Lates niloticus*) were chosen as out-groups for shark and barramundi phylogenetic trees, respectively. Partial COI sequences were resolved for 23 flake and 15 barramundi samples. Nominal flake samples had 280 variable sites and 264 parsimony informative sites across all species, compared to barramundi samples where 69 sites varied, 63 of which were informative. Of the 59 samples obtained, 12 were re-sequenced with a final total of 46 yielding DNA of sufficient quality for analysis. Combined with voucher specimens, resulting phylogenetic trees are displayed in Figures 1 and 2, respectively. Of 23 samples labelled "flake" or "shark," one was identified as blue grenadier, a species currently harvested sustainably where conservation concerns are limited to issues with by-catch (Hume *et al*., 2004; Waugh *et al*., 2005; West *et al*., 1999). Another eight fillets labelled "flake" or "shark" produced moderate-quality sequences and resembled Japanese anchovy (*Engraulus japonicas*) based on the NCBI BLAST results. Despite DNA re-extraction, re-amplifying and re-sequencing, BLAST results consistently identified these samples as *E. japonicas* with a mean identity value of 87.3% compared to shark and barramundi samples whose mean identity values were 99.4% and 99.9%, respectively. These results indicate a potential case for further study regarding seafood mislabelling, although the exact origin cannot be confirmed using the methods of this study. These eight samples were excluded from further analysis along with the samples that failed to produce PCR products or sequences of sufficient quality for analysis.

The remaining 23 samples sold and identified as shark represented 11 commercially fished species of shark (plus one teleost) where members of the Carcharhinidae were most common (52% of all samples, $n = 12$; Table 1). The Australian blacktip, *Carcharhinus tilstoni*, was dominant within Carcharhinidae sampled (22%, $n = 5$), followed by the spot-tail shark, *C. sorrah* (17%, $n = 4$), obtained from Queensland and Western Australian outlets. In addition to the Carcharhinidae, the shortfin mako, *Isurus oxyrinchus* (13%, $n = 3$) was identified from several southeast Queensland flake samples. The remainder of samples represented a mix of other species, often unique to one location (Table 1).

Among five Carcharhinid taxa identified in this study, the most common, *C. tilstoni*, is listed as least concern with a stable population trend (Pillans & Stevens, 2003). Together with *C. sorrah*, these species were most frequently sold as fresh flake fillets and were known to contribute up to 50% of the catch within the Great Barrier Reef World Heritage Area from 2006 to 2009 (Harry *et al*., 2011). Nonetheless, the status of *C. sorrah* differs in that it is classified as near threatened with a decreasing population trend. The labelling of these species was among the most inaccurate, with two samples of *C. sorrah* labelled for sale as "blacktip reef shark," suggesting that the samples were *C. melanopterus*, a species not found among the samples collected. Another sample of *C. sorrah* was labelled as "carpet shark," suggesting

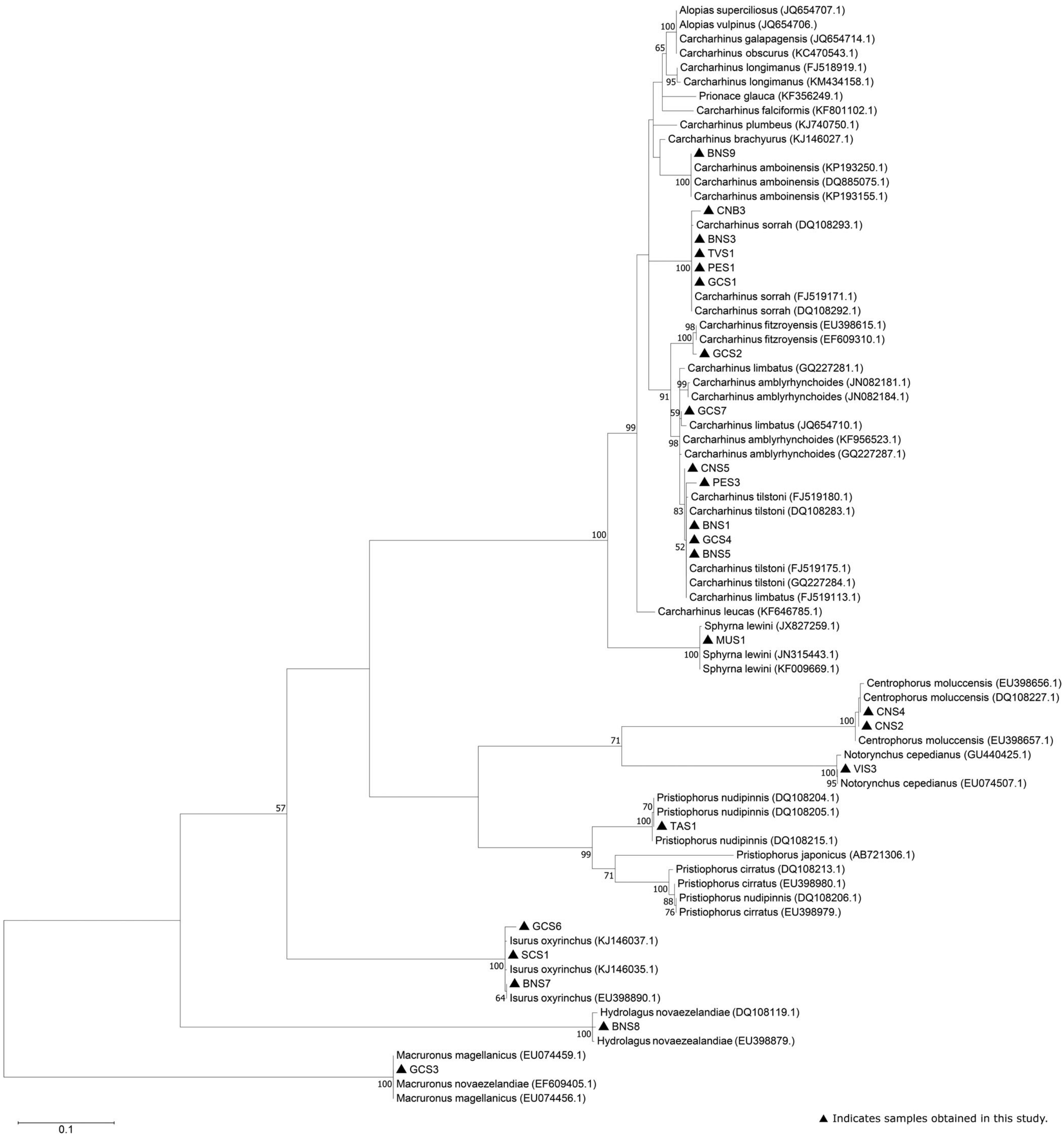

FIGURE 1 Out-group rooted phylogenetic tree of partial cytochrome oxidase subunit 1 (CO1) for shark species sold as flake by Australian seafood retailers. Maximum likelihood (ML) bootstrap support values are indicated. The best ML tree (lnL = −4425.90) includes 23 samples from seafood outlets obtained in this study (▲) from Brisbane (BN, $n = 6$), Cairns (CN, $n = 3$), Gold Coast (GC, $n = 6$), Launceston (TA, $n = 1$), Melbourne (VI, $n = 1$), Mullumbimby (MU, $n = 1$), Perth (PE, $n = 2$), Sunshine Coast (SC, $n = 1$) and Townsville (TV, $n = 1$), Australia.

it was *Orectolobus* sp. (wobbegong), an unrelated genus found in the same region, but not identified here. Ultimately, *C. sorrah* and *C. tilstoni* fillets were labelled as a mixture of "shark" or "flake," with two *C. tilstoni* fillets marketed as "blacktip shark," a term used interchangeably for several carcharhinids sold, including another species (*C. limbatus*) identified in this study. The term "blacktip" continues to cause confusion due to the multiple common names it contains, none of which generally correspond to their labels. The term "blacktip shark" alone generally refers to *C. limbatus*, which was found in this study, but labelled as "flake." *C. tilstoni*, the Australian blacktip shark,

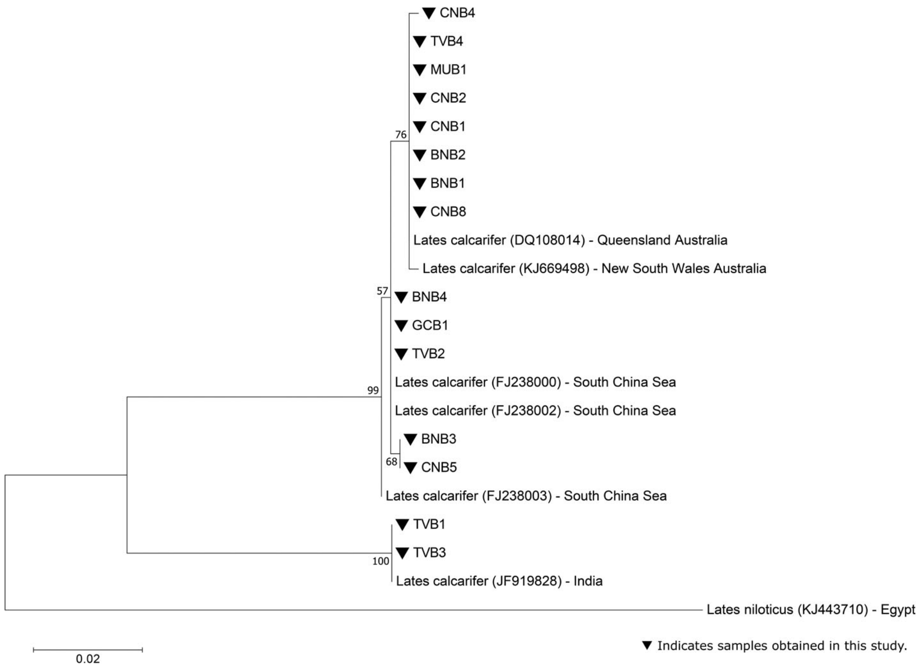

FIGURE 2 Out-group rooted phylogenetic tree of partial cytochrome oxidase subunit 1 (CO1) for barramundi (*Lates calcarifer*) samples sold by Australian seafood distributors. Bootstrap support values are indicated. The best maximum likelihood tree (lnL = −2687.11) includes 15 samples from seafood outlets obtained in this study (▼) from Brisbane (BN, $n = 4$), Cairns (CN, $n = 5$), Gold Coast (GC, $n = 1$), Mullumbimby (MU, $n = 1$) and Townsville (TV, $n = 4$), Australia.

on the contrary, was labelled as "blacktip shark" in two instances, which is unsatisfactory given that the two "blacktip shark" species differ in conservation status (Table 1). Finally, a purchased fillet labelled "blacktip reef shark" matched another carcharhinid, *Carcharhinus sorrah*, the spot-tail shark, rather than *C. melanopterus*, as the common name implies. Indeed, until 2018, stocks of these species were not independently monitored and grouped together making individual species assessments from fisheries data around Northern Australia challenging (Johnson *et al*., 2018). Labelling at the point of sale and differences in IUCN conservation status, (Rigby, Carlson *et al*., 2021; Pillans & Stevens, 2003) and life-history characteristics of species are inconsistent, highlighting a notable gap between informed fisheries management and consumers, a significant seafood stakeholder group.

Overall, 14% of samples were accurately labelled, matching the species identity characterised phylogenetically. These included three *I. oxyrinchus* samples, labelled as "mako" and "shortfin mako," and one *Pristiophorus nudipinnis* fillet, labelled as "saw shark." Over half of the samples (55%) were labelled exclusively as "flake" or "shark" (or a minor variation) with no attempt to inform the consumer of species. The remainder of samples (14%) was identified as unrelated species to those named on retailer labels.

The shortfin mako, *I. oxyrinchus*, was the third most common shark species identified in this study and is both commercially important and heavily exploited internationally (Dulvy *et al*., 2008) despite being classified as endangered by the IUCN (Rigby, Barreto, *et al*., 2019). It was the only species sampled that is officially protected from commercial fishing under the Environmental Protection and Biodiversity Conservation Act 1999 (EPBC Act), Australia's legal framework protecting threatened species, listed as a migratory species in 2010 (Commonwealth of Australia, 2014a). An exemption of sale exists if captured within recreational fisheries as by-catch only (Commonwealth of Australia, 2014a), whereby sale is allowed despite the commercial targeting of shortfin mako being outlawed (Government, 2010 #756). Stock assessments vary throughout much of its global range and, at the other extreme, is classified as critically endangered in the Mediterranean (Dulvy *et al*., 2008). Although population declines globally have been due to a combination of targeted exploitation and by-catch (Rigby, Carlson *et al*., 2021; Dulvy *et al*., 2008; Stevens, 1983), the lack of catch regulation in high-seas, except where regional management is enforced (Cullis-Suzuki & Pauly, 2010), has contributed to its exploitation. Although there is evidence that South Pacific populations around New Zealand and Australia have begun to recover with a 35.2% increase recorded over 72 years (Francis *et al*., 2014), evidence of this population being isolated from the global population [classified as endangered with a decreasing trend (Rigby, Barreto, *et al*., 2019)] is weak (Schrey & Heist, 2003). Therefore, until further studies confirm the multiple stocks of *I. oxyrinchus*, a precautionary approach should continue to be taken and the stock treated as circum-global.

The sample identified as *Centrophorus moluccensis* from a Cairns outlet was originally labelled for sale as "northern gummy shark," a non-existent species name used in place of the common names "smallfin gulper shark" or "endeavour dogfish" at the point of sale, which may be less appealing and/or familiar to the consumer. Mislabelling in this case may incorrectly market products as purportedly sustainably fished, even though the product sold (*C. moluccensis*) is near threatened due to low fecundity (Graham & Daley, 2011). The true gummy shark, *Mustelus antarcticus*, is a southern species restricted in range southwards of Newcastle, New South Wales, located approximately 2600 km south of the Cairns region (Kailola *et al*., 1993). Gummy shark fisheries in southern Australia have historically been reported as sustainable (Walker, 1998) based solely on biomass of *M. antarcticus* (Petrolo *et al*., 2021). Nonetheless, over the past two decades, concerns around the population structure (Petrolo

TABLE 1 Summary of shark species identity matches using phylogenetic analyses of partial cytochrome oxidase subunit 1 (COI) sequences obtained from fillets purchased from four broad coastal regions of Australia

Species name	Common name	Product label	Point-of-sale label	IUCN status	Population trend	EPBC status	*n*	Sample origin (sequence)
Carcharhinus amboinensis	Pigeye shark	Reef shark	Incorrect	V	Decreasing	Not listed	1	Brisbane (BNS9)
Carcharhinus fitzroyensis	Creek whaler	Flake	Ambiguous	LC	Unknown	Not listed	1	Gold Coast (GCS2)
Carcharhinus limbatus	Blacktip shark	Flake	Ambiguous	V	Decreasing	Not listed	1	Gold Coast (GCS7)
Carcharhinus sorrah	Spot-tail shark	Blacktip reef shark, blacktip shark, carpet shark	Incorrect	NT	Decreasing	Not listed	5	Brisbane (BNS3); Gold Coast (GCS1); Townsville (TSV1); Cairns (CNB3); Perth (PES1)
Carcharhinus tilstoni	Australian blacktip shark	Blacktip shark, flake, shark	Ambiguous	LC	Stable	Not listed	5	Brisbane (BNS1 & BNS5); Gold Coast (GCS4); Cairns (CNS5); Perth (PES3)
Centrophorus moluccensis	Smallfin gulper shark	Flake, northern gummy shark	Ambiguous and incorrect	NT	Stable	Not listed	2	Cairns (CNS4 & CNS2)
Hydrolagus novaezealandiae	Dark ghost shark	Flake	Ambiguous	LC	Stable	Not listed	1	Brisbane (BNS8)
Isurus oxyrinchus	Shortfin mako	Shortfin mako, Queensland flake	Correct and ambiguous	E	Decreasing	Not listed	3	Brisbane (BNS7); Gold Coast (GCS6); Sunshine Coast (SCS1)
Macruronus novaezelandiae	Blue grenadier	Flake	Incorrect	-	-		1	Gold Coast (GCS3)
Notorynchus cepedianus	Broadnose sevengill shark	Tiger shark	Incorrect	V	Decreasing	Not listed	1	Melbourne (VIS3)
Pristiophorus nudipinnis	Shortnose sawshark	Saw shark	Ambiguous	LC	Stable	Not listed	1	Launceston (TAS1)
Sphyrna lewini	Scalloped hammerhead	Flake	Ambiguous	CE	Decreasing	Conservation Dependant	1	Mullumbimby (MUS1)

Note: IUCN status and population trends are indicated for critically endangered (CE), endangered (E), vulnerable (V), near threatened (NT), least concern (LC) and data-deficient species (DD). Six of the 11 shark species identified are species of concern based on IUCN status (highlighted grey). These are (grey shades) species of concern due to a decreasing population trend.

et al., 2021), as well as by-catch of other species, have increased, such as in the case of *Galeorhinus galeus*, a critically endangered species that occupies a similar ecological niche and is often caught alongside *M. antarcticus* (Walker *et al*., 2020). Similarly, the broadnose sevengill shark, *Notorynchus cepedianus*, identified in this study is classified as vulnerable with a decreasing population trend. As apex predators in their respective ecosystems, it is important to acknowledge the functional role of these taxa within their habitats, which bring them into close proximity with populated areas where few other sharks regularly occur (Barnett *et al*., 2010). In contrast, every sample labelled as "barramundi," *Lates calcarifer*, was positively identified following BLAST searches providing no evidence of shark fillet mislabelled as highly prized (and highly priced) Australian barramundi ($n = 15$), despite concerns. Data also suggest an indication of a second species of barramundi possibly existing across a geographic division (Figure 2); nonetheless, this is outside the scope of this study and lends itself to future investigation.

Australian legislation, including the EPBC Act, generally corresponds to the IUCN framework. Despite the scalloped hammerhead, *Sphyrna lewini*, being currently listed as critically endangered by the IUCN (Rigby, Dulvy, *et al*., 2019), the EPBC Act lists *S. lewini* as "conservation dependant," allowing commercial fisheries to remain active in Australia with a limit of 200 t, where 90% of the catch originates in four commercial fisheries located in Queensland, the Northern Territory and Western Australia (Commonwealth of Australia, 2014b). Along with life-history characteristics (Chen *et al*., 1990), unique factors such as a high value for fins (Abercrombie *et al*., 2005) and the tendency of adults to aggregate (Gallagher *et al*., 2014) increase the vulnerability of this species to fishing pressure. Despite setting 200 t *per annum* sustainable harvest levels, *S. lewini* has continued to decline in Australian waters with figures in 2018 and 2019 showing further declines of all global populations (Gallagher & Klimley, 2018, Rigby, Dulvy, *et al*., 2019). Future investigation quantifying the frequency at which *S. lewini* and other similarly vulnerable taxa are sold by Australian seafood outlets should be undertaken to better understand the exploitation and the management of target species.

Ambiguous and inaccurate labelling of shark fillets dominated the point-of-sale sampling in this study, with misleading names frequently used in Australia. Here, the harvest and sale of a diverse range of species with varying conservation status were revealed: from "least concern" to "critically endangered," according to the IUCN assessments. As such, a case exists for the development of stronger laws and enforcement regarding seafood traceability. This includes accurate labelling practices to be adopted and regulated to reliably inform consumer choice, particularly regarding the conservation status of harvested species at the point of sale. Based on the gaps in labelling accuracy and transparency revealed in this study, a precautionary approach is recommended for fisheries where species of conservation concern are clearly identified as either target or by-catch. The scale of mislabelling in the seafood industry must be understood to improve the transparency in the source to sale of seafood products, providing consumers with accurate and reliable information for making sustainable choices. This will enhance consumer certainty and serve to advance the regulation and management of the harvest and sale of species of conservation concern, including sharks.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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