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Identification of shark and ray fins using DNA barcoding

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ABSTRACT

Fisheries managers and scientists worldwide are struggling with a lack of basic information for many shark and ray species. One factor hampering the data collection is inaccurate identification of many chondrichthyan species and their body parts. Morphologically similar species, and specimens which are poorly preserved or have had key diagnostic features removed, can be difficult to identify. This study examined DNA barcoding as a method to identify shark species from dried fins, confiscated from a vessel fishing illegally in Australian waters. 211 left pectoral fins were examined. 18 either did not provide a sequenceable product or yielded a microbial sequence, while 193 fins (91.5%) provided a chondrichthyan sequence. All of these could be matched to reference specimens in a DNA barcode database, and so were able to be identified. 27 species were detected, 20 species of sharks and seven species of rays The most abundant species (22% of fins) was *Carcharhinus dussumieri*. Many of these species are listed on the World Conservation Union (IUCN) Red List and include one, *Anoyxpristis cuspidata* (3%), rated as critically endangered. Fishing authorities can use DNA barcoding to gather data on which chondrichthyan species are targeted by illegal fishers, information that will greatly assist in management and conservation.

1. Introduction

Shark fins are a highly prized commodity in many Asian cultures. Shark fins infer financial status and are traditionally used in soups served at important occasions. The increase in middle-class wealth in many shark fin consumer countries has significantly increased the demand for such products (Fong and Anderson, 2002); the largest market is Hong Kong (Clarke et al., 2005). Sharks sourced for fins come from legal fisheries, by-catch, and illegal, unreported and unregulated (IUU) fisheries. The number of sharks caught as undeclared by-catch and in IUU fisheries probably far outweighs those from legal fisheries (Bonfil, 1994), and many of these would be destined for the lucrative fin trade. The 2005 FAO estimate of world exports of dried shark fins was US$ 220 million (estimate from FISHSTAT FAO, 2000).

There is worldwide concern that the increased demand for shark fins will have a devastating impact on shark populations and stocks. Many shark species are highly vulnerable to fishing pressure as they are slow to mature and have long gestation periods and low fecundity. Historically, less effort has been put into gathering catch data records for sharks than teleosts due to the lower commercial value of shark fisheries. Morphologically similar species are often grouped together in catch records, and carcasses processed at sea can be misidentified on landing if characteristic body parts have been removed. Incomplete catch statistics make it impossible for fisheries managers and scientists to get a true picture of the status of shark species. An FAO report (Castro et al., 1999) lamented the paucity of good fisheries data and noted that severe declines were seen for nearly all shark species with ten years of catch and landing data. Capture production figures for sharks, rays and chimaerids in 2005 are listed as 771,105 tonnes (estimate from FISHSTAT (FAO, 2000)), a figure known to be an underestimate as many countries do not report by-catch statistics and it excludes illegally taken chondrichthyans. Bonfil (1994) and Vannuccini (1999) both suggest that true capture production figures are twice the FAO estimates. Clarke et al. (2006) used a fishery-independent method to estimate the worldwide shark catch for the shark fin trade. They analysed trade data from Asian markets and estimated 1.7 million tonnes of shark per year were caught for the fin trade, a value 3–4 times higher than the FAO estimate of the shark fin trade for 2000. An accurate picture of the status of shark populations worldwide requires major improvements to catch data records, and one of the limiting factors has been the problem of accurate identification of morphologically similar species and processed shark material.

Over the past few decades, molecular techniques have been promoted for shark species identification, particularly in instances when traditional taxonomic methods fail due to insufficient morphological information. Protein electrophoresis has been extensively used (e.g. Tenge et al., 1993; Yearsley et al., 1999) and has been applied to shark fins and fillets (Smith and Benson, 2001).

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Heist and Gold (1999) used restriction fragment length polymorphisms (RFLPs) of sections of the mitochondrial genes cytochrome *b* and threonine tRNA to identify 11 species of carcharhinids. Chan et al. (2003) also used RFLP analysis of the cytochrome b gene to identify 6 carcharhinid species commonly found off the coast of New South Wales, Australia. Hoelzel (2001) designed species-specific primers in the cytochrome *b* gene for *Cetorhinus maximus*. Mahmood Shivji's laboratory has developed multiplexed species-specific primers targeting the nuclear ribosomal ITS2 region for several shark species: *Carcharhinus obscurus*, *C. plumbeus* (Pank et al., 2001); *Isurus oxyrinchus*, *I. paucus*, *Lamna nasus*, *Prionace glauca*, *C. falciformis* (Shivji et al., 2002); *Alopias vulpinus*, *A. superciliosus*, *A. pelagicus* (Abercrombie, 2004); *Galeocerdo cuvier*, *C. leucas*, *C. brevipinna* (Nielsen, 2004); *Sphyrna lewini*, *S. mokarran*, *S. zygaena* (Abercrombie et al., 2005); *Carcharodon carcharias* (Shivji et al., 2005); *Cetorhinus maximus* (Magnussen et al., 2007). Other studies advocate using universal primers to amplify specific regions in the mitochondrial genome, regions which generally show low levels of intraspecific sequence variation but adequate levels of interspecific sequence variation for species identification. Hoelzel (2001) examined short segments of the mitochondrial NADH2 and cytochrome *b* genes for 15–16 Lamniform species. Greig et al. (2005) sequenced 35 shark species representing 5 families (Carcharhinidae, Sphynidae, Triakidae, Alopiidae and Lamnidae) from the western North Atlantic Ocean for a 1400 base pair (bp) mitochondrial DNA fragment spanning 12S rDNA, valine tRNA and 16S rDNA. Giles and Ovenden (personal communication) used the mitochondrial d-loop region to identify about 35 species of sharks and rays.

Despite the great potential of genetics to assist in the identification of shark species, there has been little consensus on which gene region would be the most suitable. Hebert et al. (2003) promoted DNA barcodes as a means to identify all animal species. The DNA barcode technique uses universal primers to PCR amplify an approximately 650 bp region of the mitochondrial cytochrome *c* oxidase I (COI) gene. This region is sequenced to give the DNA barcode for the specimen in question, and compared to barcodes from reference specimens to obtain a species identification. DNA barcoding surveys of 207 Australian fish species (Ward et al., 2005; 143 teleost species, 61 shark and ray species, 3 chimaerid species) and 210 Australasian shark and ray species (Ward et al., 2008) have concluded that DNA barcoding can be used for teleost and chondrichthyan species identification. The ability of DNA barcoding to identify a species relies on the degeneracy of the genetic code. This was confirmed by Ward and Holmes (2007), who analysed the DNA barcode region in 388 species of fishes, including 4 holocephali and 61 elasmobranchs. These studies show that barcoding discriminates 98–99% of fish species examined thus far.

An important advantage that COI DNA barcoding has over universal primer techniques that target other gene regions is the Barcode of Life Data System (BOLD), a large, rapidly growing, and searchable repository of COI DNA barcode sequences (Ratnasingham and Hebert, 2007). Few fish studies to date have taken advantage of this database, although Pegg et al. (2006) used DNA barcoding to identify planktonic fish larva, and Smith et al. (2008) and Wong and Hanner (2008) used it to identify seafood products. In our study, the DNA barcode technique was used to identify dried shark fins confiscated from vessels fishing illegally in Australian waters.

2. Materials and methods

2.1. Sample collection for reference database

Sample collection for the reference database is part of an on-going effort for the Fish Barcode of Life initiative (FISH-BOL, www.fishbol.org). The elasmobranch tissue samples (muscle or fin clips) for our study were collected from various locations in northern Australia and Indonesia and were either preserved in a NaCl-saturated 20% DMSO solution or 95% ethanol, or were frozen at −80 °C. Whole specimens, identified by an expert taxonomist, were retained where possible as voucher specimens for each species. Specimens which were too large to be retained were photographed. Voucher specimens are stored at the Australian National Fish Collection at CSIRO Marine and Atmospheric Research, Hobart, Tasmania. Collection data are recorded on the Barcode of Life Data System (BOLD, www.boldsystems.org) and results are described in Ward et al. (2008).

2.2. Sample collection for dried sharkfins

Unidentified fin samples ($n = 211$) collected from confiscated illegal fishing catches were received from the Australian Fisheries Management Authority. Only the left pectoral fins were used to avoid analysing the same specimen twice. No information indicating possible species identification was provided.

2.3. DNA barcode protocol

Approximately 2 mm^3 cube of tissue sample was subsampled for DNA extractions.

Samples from 145 of the 211 fins gave sequences in stage 1 of the project. The remaining 66 fins were re-screened in stage 2, and 48 of these yielded sequences. 18 failed to provide good sequences.

Stage 1: DNA extractions used a chelex resin following minor modifications of the protocol outlined in Walsh et al. (1991); see also Hajibabaei et al. (2005). Essentially 10 μL of proteinase K (25 mg/mL) and 190 μL of 5% chelex solution were added to each sample. Reactions were incubated overnight at 55 °C. The next day the samples were heated to 100 °C for 20 min and then stored at −20 °C until required for PCR. The samples were centrifuged just prior to the PCR reaction. PCR reactions were in 25 μL volumes containing 1× PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.1 μM of each primer (Table 1), 1 unit Taq DNA polymerase (Fisher Biotech) and 3 μL DNA template (concentration not measured, supernatant from the chelex extraction). Problematic samples were PCR amplified using 0.2 μM of each primer and 1 unit of Platinum® Taq DNA polymerase (Invitrogen). Initial amplifications used the primer combination FishF1 and FishR1, which amplified the barcode region for the majority of fins. When this failed to produce a PCR product, primers FishF2 and FishR2 were used, and if the PCR was still unsuccessful, primer combination FishF1 and HCO2198 was used. Cycling parameters were an initial denaturation at 94 °C/2 min followed by 35 cycles of 94 °C/30 s 54 or 51 °C/30 s and 72 °C/1 min with a final extension step at 72 °C for 10 min. The annealing temperature was 54 °C unless HCO2198 was used as a reverse primer where it was lowered to 51 °C. PCR products ranged from 652–655 bp depending on primer combination and were visualised on 1.2% agarose gels. All but the very faintest bands were sequenced. Excess dNTPs and unincorporated primers were removed from the

Table 1
Primers used in Stage 1 of this study, those in Stage 2 were from Ivanova et al. (2007).

Name	Sequence (5′–3′)	Reference
FishF1 (fwd)	TCA ACC AAC CAC AAA GAC ATT GGC AC	Ward et al., 2005
FishF2 (fwd)	TCG ACT AAT CAT AAA GAT ATC GGC AC	Ward et al., 2005
FishR1 (rev)	TAG ACT TCT GGG TGG CCA AAG AAT CA	Ward et al., 2005
FishR2 (rev)	ACT TCA GGG TGA CCG AAG AAT CAG AA	Ward et al., 2005
HCO2198 (rev)	TAA ACT TCA GGG TGA CCA AAA AAT CA	Folmer et al., 1994

PCR products using Exo-SAP-IT (USB) following the manufacturer's protocol. Sequencing reactions (1/8 reaction) used a BigDye® Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Inc.) and 1 μL of purified PCR product. Cycling conditions were according to the manufacturer's protocol. All samples were sequenced in both directions. Sequencing products were cleaned using CleanSeq (Agencourt) following the manufacturer's protocol and then run on an ABI 3100 DNA sequencer using an 80 cm array and POP4 polymer.

Stage 2: DNA was extracted using an automated Glass Fiber protocol (Ivanova et al., 2006). The 650 bp barcode region of COI was subsequently amplified under the following thermal conditions: 2 min at 95 °C; 35 cycles of 0.5 min at 94 °C, 0.5 min at 52 °C, and 1 min at 72 °C; 10 min at 72 °C; held at 4 °C. The 12.5 μL PCR reaction mixes included 6.25 μL of 10% trehalose, 2.00 μL of ultrapure water, 1.25 μL 10× PCR buffer [200 mM Tris–HCl (pH 8.4), 500 mM KCl], 0.625 μL $MgCl_2$ (50 mM), 0.125 μL of each primer cocktail (0.01 mM, using primer cocktails C_FishF1t1 and C_FishR1t1 from Ivanova et al., 2007), 0.062 μL of each dNTP (10 mM), 0.060 μL of Platinum® Taq Polymerase (Invitrogen), and 2.0 μL of DNA template. PCR amplicons were visualised on a 1.2% agarose gel E-Gel® (Invitrogen) and bidirectionally sequenced using sequencing primers M13F or M13R (Ivanova et al., 2007) and the BigDye® Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Inc.) on an ABI 3730 capillary sequencer following manufacturer's instructions.

The sequence data from the unknown fins were assembled using Sequencing Analysis v.5.1 and SeqScape v.2.5 (Applied Biosystems).

Fins were identified to species after considering the results of two analyses. In the first analysis, fasta files for each fin were pasted one at a time into the BOLD search engine. This matched those sequences with every reference sequence (chondrichthyan or not) present in BOLD and provided percent similarities with each of the top 99 matching specimens. In the second analysis, the set of Australasian reference chondrichthyan samples (see Ward et al., 2008 for further information, including GenBank accession numbers) were assembled together with all the unknown fasta fin sequences, and clustered in MEGA 4 (Tamura et al., 2007) using a neighbour-joining tree with Kimura 2-parameter distance (Kimura, 1980). The pairwise deletion option was selected to account for missing sequence information between each compared specimen. The tree was bootstrapped (1000 iterations) to assign confidence levels to each branch in the tree.

3. Results

193 of the 211 confiscated fins (91.5%) yielded DNA that could be amplified and sequenced, 18 (8.5%) did not. Most likely the DNA of the failed fins had degraded, or perhaps there might be species present that did not amplify with the 'universal' primers used. The latter concern is only likely to affect a few species (Ward et al., 2005; Ward et al., 2008). Further work may well give barcodes for some if not all of these 'failed' samples.

3.1. Matches with BOLD

Appendix A provides, for each fin, the percent matches to the best matched and second best matched species in the global BOLD reference database of animal barcodes. 165 of the 193 fins that yielded DNA barcodes gave perfect, 100% matches, to chondrichthyan species. The average best match was 99.97%, and 96.89% to second best matches.

Only one specimen (shark fin #154) yielded a match of <99.5%, and that was 99.2% to *Chiloscyllium punctatum*. However, this is likely to be a correct species match as the next best match was only 91.7% to *C. hasselti*, followed by 91.6% to *C. griseum*, 91.5% to *C. plagiosum* and 90.2% *C. indicum*. Three other congeners, currently un-barcoded, are not known from the region of this catch.

The five shark fins that matched to the great hammerhead *Sphyrna mokarran* (all at 100%) had second best matches not to other members of the *Sphyrna* genus but to non-congeners, *Eusphyra blochii* and *Carcharhinus acronotus*. *E. blochii* is the only hammerhead shark not in the genus *Sphyrna*, and its generic status has been debated (Cavalcanti, 2007). It falls within the cluster of *Sphyrna* species following COI barcoding (see the NJ tree provided as Supplementary Data), and identification of this species as a second match is not unexpected. More surprising is the identification by BOLD of the western Atlantic *C. acronotus* as an equal second match. However, matches to both these species are only at 92%–93%; values too low to attach any reliability to.

A few specimens showed an equivocal BOLD match, matching at 100% to the species pair *C. obscurus*/*C. galapagensis* and 99.8–100% to the species pair *C. tilstoni*/*C. limbatus*. The former two species are very similar to one another morphologically and genetically, the latter two form part of a trio of species (the third one being *C. amblyrhynchoides*) which are also very closely related to one another (Ward et al., 2008).

3.2. Tree matches

The NJ tree matches fin sequences to reference specimens from Australasian waters (Ward et al., 2008), and the bootstrapped tree itself is provided herein as Supplementary Data. Appendix A provides, for each fin, the percent bootstrap value to the species clade that includes that fin.

118 of the 193 sequenced fins gave 99% or 100% bootstrap values to a single species clade. The bootstrap value of 42 fins to the *Carcharhinus dussumieri* clade was only 76%, but no fins had sequences which matched those of its paired species *C. sealei*. The three closely related species *C. tilstoni*, *C. limbatus*, and *C. amblyrhynchoides* together with 33 fins form a close-knit and well-defined group, with a bootstrap value of 98%. 26 of these fins group with *C. tilstoni* (49% bootstrap value) with six fins grouping with *C. limbatus* (60% bootstrap value). One of the fins, #204, appeared as basal to the three species.

3.3. Final identifications

Considering the results of the global BOLD matches and the bootstrapped Australasian tree, all fins that produced sequences were able to be assigned to a particular species (Appendix A). Fin #204, with an uncertain provenance in the tree (*Carcharhnus tilstoni*, *C. limbatus*, or *C. amblyrhynchoides*) was assigned to *C. tilstoni* based on its 100% match to a *C. tilstoni* specimen in the BOLD database. On the other hand, those 18 specimens that from BOLD matches could have been either *C. tilstoni* or *C. limbatus* were all assigned to *C. tilstoni* based on their clustering in the tree. The three specimens with 100% BOLD matches to both *C. obscurus* and *C. galapagensis* are assigned to *C. obscurus* based on their sequence identity or near-identity to the tree specimens of *C. obscurus*; however, no specimens of *C. galapagensis* were present in the Australian reference collection (Ward et al., 2008) so these identifications must be regarded as provisional.

The 193 dried fins were found to comprise 27 species of sharks and rays. The most common species was *C. dussumieri* (whitecheek shark), making up 22% of the sample (see Table 2). The next most common species were *C. tilstoni* (14%), *C. sorrah* (9%), *Sphyrna*

Table 2
Catch composition of confiscated fins from northern Australian waters.

Species	Common name[a]	IUCN Red List status[b,c]	*n*	%
Carcharhinus dussumieri	Whitecheek Shark	NT, LC Australia	42	21.8
Carcharhinus tilstoni	Australian Blacktip Shark	LC	27	14.0
Carcharhinus sorrah	Spot-tail Shark	DD, LC Australia, NT South East Asia	18	9.3
Sphyrna lewini	Scalloped Hammerhead	NT, LC Australia	13	6.7
Carcharhinus amboinensis	Pigeye Shark	DD, NT Southwest Indian Ocean	12	6.2
Carcharhinus macloti	Hardnose Shark	NT, LC Australia	11	5.7
Carcharhinus brevipinna	Spinner Shark	NT, VU Northwest Atlantic	8	4.2
Rhynchobatus cf. *laevis*	cf. Smoothnose Wedgefish	VU, NT Australia	7	3.6
Anoxypristis cuspidata	Narrow Sawfish	CR	6	3.1
Carcharhinus limbatus	Blacktip Shark	NT, VU Northwest Atlantic	6	3.1
Eusphyra blochii	Winghead Shark	NT, LC Australia	6	3.1
Sphyrna mokarran	Great Hammerhead	DD, LC Australia	6	3.1
Triaenodon obesus	Whitetip Reef Shark	NT	5	2.6
Carcharhinus leucas	Bull Shark	NT	3	1.6
Carcharhinus obscurus	Dusky Shark	NT, VU Northwest Atlantic and Gulf of Mexico	3	1.6
Rhina ancylostoma	Shark Ray	VU, NT Australia	3	1.6
Rhynchobatus australiae	White-spotted Guitarfish	VU, NT Australia	3	1.6
Carcharhinus amblyrhynchos	Grey Reef Shark	NT	2	1.0
Rhinobatos typus	Giant Shovelnose Ray	VU, NT Australia	2	1.0
Rhizoprionodon acutus	Milk Shark	LC	2	1.0
Rhizoprionodon taylori	Australian Sharpnose Shark	LC	2	1.0
Carcharhinus falciformis	Silky Shark	LC, DD North Indian, Tropical Pacific and Western North Atlantic	1	0.5
Chiloscyllium punctatum	Brownbanded bamboo shark	NT	1	0.5
Galeocerdo cuvier	Tiger Shark	NT	1	0.5
Himantura hortlei	Hortle's Whipray	Not on list	1	0.5
Negaprion acutidens	Sharptooth Lemon Shark	VU, EN South East Asia, LC Australia	1	0.5
Pastinachus sephen	Cowtail Stingray	Not on list	1	0.5

[a] Common names taken from IUCN Red List.
[b] IUCN = The world conservation union.
[c] IUCN categories: Critically Endangered (CR), Endangered (EN), Vulnerable (VU), Near Threatened (NT), Least Concern (LC); Data Deficient (DD), with regions when given, see www.iucnredlist.org for more details.

lewini (7%), *C. amboinensis* (6%) and *C. macloti* (6%). The remaining 21 species each individually represented 0.54% of the fin collection. 18 of these species are listed in the World Conservation Union (IUCN) Red List (Kyne et al., 2006), with their current status ranging from near threatened through to critically endangered.

While most fins were sequenced for all or virtually all bases of the 655 bp barcode region, a few generated shorter sequence reads. The shortest sequence was 398 bp. Nevertheless, from both the BOLD matches and the tree placement, this fin (#94) was readily allocated to a species, *Rhynchobatus* cf. *laevis*. To ensure that this species match was not in error due to insufficient sequence data, the sequence data from all of the unknown fin samples were shortened to this same stretch of sequence and compared to specimens in the reference database. The K2P neighbouring-joining tree (not shown) produced from this data was very nearly identical to that produced from sequences with full length data, showing that a shortened sequence will not necessarily compromise the identification process.

4. Discussion

Varying molecular approaches have been recommended for shark species identification. One approach is to design species-specific primers which can be multiplexed in a PCR reaction. For example, Abercrombie et al. (2005) designed species-specific primers to identify three species of hammerhead shark. An advantage of species-specific primers is that multiple species in a processed product can be identified, provided that the specific primers to identify all the species present in the product are available (Hoelzel, 2001). Disadvantages include a limitation to the current suite of species-specific primers available, and the possibility that the primers may not be 100% species-specific.

The species identification technique we chose, DNA barcoding, uses universal primers to amplify part of the COI gene. We used this approach to identify shark and ray species from dried fins, confiscated from a vessel fishing illegally in northern Australian waters. Barcoding success is dependent on low levels of sequence variation within species and much higher levels between species. Barcodes can be submitted to the publicly available database, the Barcode of Life Data System (BOLD, www.boldsystems.org) (Ratnasingham and Hebert, 2007), where the query DNA barcode is aligned and compared to the barcodes stored in BOLD. The unknown specimen will only be identified to species level if there is less than 1% divergence between the query sequence and the reference sequence. While the mean sequence divergence between congeneric species of sharks and rays is 7.48% (Ward et al., 2008), some congeners are known to have very low sequence divergence. For example, the interspecies sequence divergence between the sharks *Carcharhinus limbatus*, *C. amblyrhynchoides* and *C. tilstoni* averages only 0.45% (although intraspecies sequence divergence is substantially lower at 0.04%, Ward et al., 2008). In situations where taxa share sequences with less than 1% divergence, BOLD shows all possible species assignments. In a few instances, fins matched equally well to two species in BOLD. These species were always very closely related congeners. In our case, we had already assembled a collection of shark and ray reference sequences specifically from Australasian waters, and were able to use these sequences in conjunction with the fin sequences to produce a combined NJ tree. Consideration of the tree results together with the BOLD similarity matches enabled us to resolve the identities of all fins. In fact, shark fin species identification turned out to be quite a demanding task for barcoding, as nearly 70% of fins in our sample came from the single genus *Carcharhinus*, which consists of many closely related species (Ward et al., 2008).

27 species of sharks and rays were found to be present among the 193 left pectoral fins that were sequenced (Table 2). 85% of fin sequences matched perfectly with sequences from known reference samples (sequence similarity = 100%, K2P distance = 0). All remaining sequences had similarity levels to the best matched reference sequence no lower than 99.2% and K2P distances no greater than 0.77%, and clustered tightly with known species sequences in the neighbour-joining tree of Australasian reference specimens (Ward et al., 2008). 20 species were sharks, accounting for 88.1% of the fins, while seven species of ray made up the remaining 11.9%.

At the time of writing, some 359 of the world's approximately 1000 shark, ray and chimaerid species have been DNA barcoded (see www.fishbol.org). Thus globally, it is likely that not all confiscated fins or fins passing through the large Hong Kong market can be presently identified by barcoding. However, the barcodes that are collected from fins are available for subsequent matching as the reference database expands; two of our specimens were only identifiable at the very end of our study when our reference database (available in BOLD) expanded from 205 to 210 shark and ray species.

18 fins (<1% of the total) did not yield chondrichthyan COI sequences. Some of these did not produce a PCR product and others amplified fragments of microbial origin. Poor preservation conditions onboard fishing vessels will lead to DNA degradation and to high bacterial levels. Targeting a shorter region within the COI barcode region would increase the likelihood of good amplification from degraded DNA. *In silico* studies of fish DNA barcode sequences show that a shorter DNA barcode fragment will still usually identify a specimen to its origin species. Hajibabaei et al. (2006) split the 650 bp barcode region into short segments (109 and 218 bp) and compared each segment's ability to successfully identify a specimen based on its percent intraspecific and intergeneric variation and the percent of parsimony informative sites in each segment. They found that these mini-barcodes generally had sufficient sequence variation and divergence to give the same information as a full length barcode sequence. In our study, one of the fins could only be sequenced for 398 bp, and was still readily matched to a species. We have not attempted to design primers targeting shorter segments of the barcode region as this was beyond the scope of this study. Avoiding bacterial contamination is a more difficult problem. In this situation a species-specific primer should be effective, but this approach is restricted by the available suite of species primers. A cloning and sequencing approach might also work but would be time-consuming. Less than 20 elasmobranch species currently have species-specific primers (see Section 1), and only seven of these correspond to species we detected in our fin collection using the barcode approach. There are currently no species-specific primers for the most abundant species, *C. dussumieri*. In fact the abundance of *C. dussumieri* in the fin collection is a little surprising; while it is a common species in tropical coastal waters of the Indo-West Pacific, it is quite small – only about one metre long when mature – and would only yield relatively small fins.

There are some disadvantages to the barcoding approach for species identification. It requires access to sequencing technology, but this is becoming faster, more commonplace and increasingly inexpensive. Identifying the component species of a blended product comprising multiple species would be difficult unless a cloning step is included; this would be time-consuming. Further, mitochondrial DNA is maternally inherited so any identification procedure that relies on mtDNA only gives information on the maternal parent; hybrids would be wrongly characterised as the maternal species. However, hybridisation appears to be rare or absent in elasmobranchs (Gardner, 1997) and we could not find any documented instances in a literature search. Possibly its rarity or absence in chondrichthyans might be attributable to their ubiquitous internal fertilisation.

The number of vessels caught fishing illegally in Australia's northern waters has been increasing (AFMA, 2007). These vessels are mainly targeting sharks for their fins. Intercepted boats have catches confiscated but fishers often only retain fins, discarding the lower value carcasses at sea. This makes it extremely difficult for authorities to determine species composition and consequently fisheries agencies are unable to get a good understanding of what species are most affected by this wasteful practice. Many of the elasmobranch species identified in our study are listed on the IUCN (World Conservation Union) Red List of threatened species (Table 2). This includes a critically endangered species, narrow sawfish (*Anoxypristis cuspidata*), of which six specimens were identified, and 11 species listed as near threatened in Australian waters (*Carcharhinus amblyrhynchos*, *Carcharhinus brevipinna*, *Carcharhinus leucas*, *Carcharhinus limbatus*, *Carcharhinus obscurus*, *Galeocerdo cuvier*, *Rhina ancylostoma*, *Rhinobatos typus*, *Rhynchobatus australiae*, *Rhynchobatus laevis*, *Triaenodon obesus*).

5. Conclusion

We conclude that DNA barcoding is an accurate and rapid technique for shark fin identification. It can be used by fishery and law enforcement authorities to gather data from legal and illegal shark fisheries, in Australian waters and elsewhere, giving improved information and more scope to manage chondrichthyan species. It could also be used to assess exactly what shark fins are passing through the shark fin markets of the world, including Hong Kong.

Supplementary data available on-line

Neighbour-joining tree of Kimura 2 parameter distances of shark and ray COI sequences. Samples from shark fins are labelled (in bold face) as SF_xx and samples from identified chondrichthyans (from Australasian waters, as in Ward et al., 2008) are labelled by species and with Barcode of Life Database specimen designations. Bootstrap values ≥70% indicated, from 500 replicates.

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Appendix A

Shark fins that were sequenced and identified. Given are the consensus identification (considering the tree and BOLD results), the tree-based identification (with percent bootstrap value after 500 bootstraps), and the best and second best matched species from BOLD (with percent similarity).

Fin[a]	Consensus identification	Tree-based species	% bootstrap	Best matched species	% Similarity	2nd Best matched species[b]	% Similarity
SF 1	*Carcharhinus tilstoni*	*C. tilstoni*	49	*C. tilstoni*	100	*C. limbatus*	99.84
SF 5	*Carcharhinus dussumieri*	*C. dussumieri*	76	*C. dussumieri*	100	*C. sealei*	99.07
SF 6	*Carcharhinus dussumieri*	*C. dussumieri*	76	*C. dussumieri*	100	*C. sealei*	99.07
SF 7	*Carcharhinus dussumieri*	*C. dussumieri*	76	*C. dussumieri*	100	*C. sealei*	99.07
SF 8	*Carcharhinus dussumieri*	*C. dussumieri*	76	*C. dussumieri*	100	*C. sealei*	99.07
SF 9	*Carcharhinus dussumieri*	*C. dussumieri*	76	*C. dussumieri*	100	*C. sealei*	99.07
SF 10	*Carcharhinus dussumieri*	*C. dussumieri*	76	*C. dussumieri*	100	*C. sealei*	99.07
SF 11	*Carcharhinus dussumieri*	*C. dussumieri*	76	*C. dussumieri*	100	*C. sealei*	99.07
SF 12	*Carcharhinus tilstoni*	*C. tilstoni*	49	*C. tilstoni*/*C. limbatus*	100	*C. amblyrhynchoides*	99.54
SF 13	*Carcharhinus tilstoni*	*C. tilstoni*	49	*C. tilstoni*/*C. limbatus*	100	*C. amblyrhynchoides*	99.54
SF 14	*Carcharhinus dussumieri*	*C. dussumieri*	76	*C. dussumieri*	100	*C. sealei*	99.07
SF 15	*Carcharhinus dussumieri*	*C. dussumieri*	76	*C. dussumieri*	100	*C. sealei*	99.07
SF 16	*Carcharhinus dussumieri*	*C. dussumieri*	76	*C. dussumieri*	99.84	*C. sealei*	98.91
SF 18	*Carcharhinus dussumieri*	*C. dussumieri*	76	*C. dussumieri*	100	*C. sealei*	99.07
SF 19	*Carcharhinus dussumieri*	*C. dussumieri*	76	*C. dussumieri*	100	*C. sealei*	99.07
SF 20	*Carcharhinus macloti*	*C. macloti*	100	*C. macloti*	100	*C. obscurus*/*C. galapagensis*	97.38
SF 21	*Carcharhinus macloti*	*C. macloti*	100	*C. macloti*	99.85	*C. obscurus*/*C. galapagensis*	97.22
SF 22	*Carcharhinus sorrah*	*C. sorrah*	100	*C. sorrah*	100	*C. obscurus*	95.81
SF 24	*Carcharhinus sorrah*	*C. sorrah*	100	*C. sorrah*	100	*C. obscurus*	95.79
SF 25	*Carcharhinus sorrah*	*C. sorrah*	100	*C. sorrah*	99.84	*C. obscurus*	95.64
SF 26	*Sphyrna lewini*	*S. lewini*	100	*S. lewini*	100	Not generated	na
SF 27	*Carcharhinus limbatus*	*C. limbatus*	60	*C. limbatus*	100	*C. amblyrhynchoides*	99.68
SF 28	*Carcharhinus tilstoni*	*C. tilstoni*	49	*C. tilstoni*	100	*C. limbatus*	99.84
SF 29	*Carcharhinus tilstoni*	*C. tilstoni*	49	*C. tilstoni*/*C. limbatus*	100	*C. amblyrhynchoides*	99.54
SF 30	*Carcharhinus tilstoni*	*C. tilstoni*	49	*C. tilstoni*/*C. limbatus*	100	*C. amblyrhynchoides*	99.54
SF 31	*Carcharhinus tilstoni*	*C. tilstoni*	49	*C. tilstoni*	100	*C. limbatus*	99.84
SF 32	*Carcharhinus tilstoni*	*C. tilstoni*	49	*C. tilstoni*/*C. limbatus*	100	*C. amblyrhynchoides*	99.54
SF 33	*Sphyrna lewini*	*S. lewini*	100	*S. lewini*	100	Not generated	na
SF 34	*Carcharhinus tilstoni*	*C. tilstoni*	49	*C. tilstoni*	100	*C. limbatus*	99.84
SF 35	*Carcharhinus falciformis*	*C. falciformis*	100	*C. falciformis*	100	*C. amblyrhynchos*	97.22
SF 36	*Carcharhinus brevipinna*	*C. brevipinna*	100	*C. brevipinna*	100	*C. brachyurus*	98.61
SF 38	*Carcharhinus dussumieri*	*C. dussumieri*	76	*C. dussumieri*	100	*C. sealei*	99.07
SF 39	*Carcharhinus dussumieri*	*C. dussumieri*	76	*C. dussumieri*	99.85	*C. sealei*	98.92
SF 40	*Carcharhinus brevipinna*	*C. brevipinna*	100	*C. brevipinna*	99.85	*C. brachyurus*	98.46
SF 41	*Carcharhinus dussumieri*	*C. dussumieri*	76	*C. dussumieri*	99.84	*C. sealei*	98.91
SF 42	*Carcharhinus dussumieri*	*C. dussumieri*	76	*C. dussumieri*	100	*C. sealei*	99.07
SF 43	*Carcharhinus limbatus*	*C. limbatus*	60	*C. limbatus*	100	*C. amblyrhynchoides*	99.69
SF 44	*Carcharhinus dussumieri*	*C. dussumieri*	76	*C. dussumieri*	100	*C. sealei*	99.07
SF 46	*Rhizoprionodon acutus*	*R. acutus*	100	*R. acutus*	100	*(R. porosus)*	94.6
SF 47	*Carcharhinus dussumieri*	*C. dussumieri*	76	*C. dussumieri*	100	*C. sealei*	99.07
SF 48	*Carcharhinus dussumieri*	*C. dussumieri*	76	*C. dussumieri*	100	*C. sealei*	99.07
SF 49	*Carcharhinus dussumieri*	*C. dussumieri*	76	*C. dussumieri*	100	*C. sealei*	99.07
SF 50	*Carcharhinus brevipinna*	*C. brevipinna*	100	*C. brevipinna*	100	*C. brachyurus*	98.61
SF 51	*Carcharhinus tilstoni*	*C. tilstoni*	49	*C. tilstoni*/*C. limbatus*	100	*C. amblyrhynchoides*	99.54
SF 53	*Carcharhinus tilstoni*	*C. tilstoni*	49	*C. tilstoni*/*C. limbatus*	100	*C. amblyrhynchoides*	99.54
SF 54	*Carcharhinus sorrah*	*C. sorrah*	100	*C. sorrah*	100	*C. obscurus*	95.81
SF 55	*Eusphyra blochii*	*E. blochii*	100	*E. blochii*	100	*Sphyrna mokarran*	92.75
SF 56	*Carcharhinus tilstoni*	*C. tilstoni*	49	*C. tilstoni*/*C. limbatus*	100	*C. amblyrhynchoides*	99.54
SF 57	*Sphyrna lewini*	*S. lewini*	100	*S. lewini*	100	Not generated	na
SF 58	*Carcharhinus brevipinna*	*C. brevipinna*	100	*C. brevipinna*	99.85	*C. brachyurus*	98.46
SF 59	*Carcharhinus dussumieri*	*C. dussumieri*	76	*C. dussumieri*	100	*C. sealei*	99.07
SF 60	*Carcharhinus dussumieri*	*C. dussumieri*	76	*C. dussumieri*	100	*C. sealei*	99.07
SF 61	*Carcharhinus tilstoni*	*C. tilstoni*	49	*C. tilstoni*/*C. limbatus*	99.83	*C. amblyrhynchoides*	99.32
SF 62	*Carcharhinus tilstoni*	*C. tilstoni*	49	*C. tilstoni*/*C. limbatus*	100	*C. amblyrhynchoides*	99.54
SF 63	*Carcharhinus sorrah*	*C. sorrah*	100	*C. sorrah*	100	*C. longimanus*	95.66
SF 64	*Carcharhinus limbatus*	*C. limbatus*	60	*C. limbatus*	100	*C. amblyrhynchoides*	99.69
SF 65	*Carcharhinus dussumieri*	*C. dussumieri*	76	*C. dussumieri*	100	*C. sealei*	99.07
SF 66	*Carcharhinus dussumieri*	*C. dussumieri*	76	*C. dussumieri*	100	*C. sealei*	99.07
SF 67	*Carcharhinus tilstoni*	*C. tilstoni*	49	*C. tilstoni*	100	*C. limbatus*	99.84
SF 68	*Carcharhinus dussumieri*	*C. dussumieri*	76	*C. dussumieri*	100	*C. sealei*	99.07
SF 69	*Carcharhinus brevipinna*	*C. brevipinna*	100	*C. brevipinna*	100	*C. brachyurus*	98.61
SF 72	*Carcharhinus sorrah*	*C. sorrah*	100	*C. sorrah*	100	*C. obscurus*	95.81
SF 73	*Carcharhinus tilstoni*	*C. tilstoni*	49	*C. tilstoni*	100	*C. limbatus*	99.84
SF 74	*Carcharhinus dussumieri*	*C. dussumieri*	76	*C. dussumieri*	100	*C. sealei*	99.07
SF 75	*Carcharhinus dussumieri*	*C. dussumieri*	76	*C. dussumieri*	100	*C. sealei*	99.07
SF 76	*Carcharhinus tilstoni*	*C. tilstoni*	49	*C. tilstoni*/*C. limbatus*	99.85	*C. amblyrhynchoides*	99.38
SF 77	*Carcharhinus dussumieri*	*C. dussumieri*	76	*C. dussumieri*	100	*C. sealei*	99.07
SF 78	*Carcharhinus limbatus*	*C. limbatus*	60	*C. limbatus*	100	*C. amblyrhynchoides*	99.69
SF 79	*Eusphyra blochii*	*E. blochii*	100	*E. blochii*	100	*Sphyrna mokarran*	92.75
SF 80	*Eusphyra blochii*	*E. blochii*	100	*E. blochii*	100	*Sphyrna mokarran*	92.75
SF 81	*Carcharhinus brevipinna*	*C. brevipinna*	100	*C. brevipinna*	99.85	*C. brachyurus*	98.46
SF 82	*Carcharhinus tilstoni*	*C. tilstoni*	49	*C. tilstoni*/*C. limbatus*	100	*C. amblyrhynchoides*	99.54

Appendix A (*Continued*)

Fin[a]	Consensus identification	Tree-based species	% bootstrap	Best matched species	% Similarity	2nd Best matched species[b]	% Similarity
SF 83	*Carcharhinus sorrah*	*C. sorrah*	100	*C. sorrah*	100	*C. obscurus*	95.81
SF 84	*Carcharhinus dussumieri*	*C. dussumieri*	76	*C. dussumieri*	100	*C. sealei*	99.07
SF 85	*Carcharhinus tilstoni*	*C. tilstoni*	49	*C. tilstoni*/*C. limbatus*	100	*C. amblyrhynchoides*	99.54
SF 86	*Carcharhinus brevipinna*	*C. brevipinna*	100	*C. brevipinna*	99.83	*C. brachyurus*	98.26
SF 87	*Carcharhinus tilstoni*	*C. tilstoni*	49	*C. tilstoni*/*C. limbatus*	100	*C. amblyrhynchoides*	99.54
SF 88	*Carcharhinus sorrah*	*C. sorrah*	100	*C. sorrah*	100	*C. obscurus*	95.81
SF 89	*Carcharhinus dussumieri*	*C. dussumieri*	76	*C. dussumieri*	100	*C. sealei*	99.07
SF 90	*Carcharhinus dussumieri*	*C. dussumieri*	76	*C. dussumieri*	100	*C. sealei*	99.07
SF 91	*Eusphyra blochii*	*E. blochii*	100	*E. blochii*	100	*Sphyrna mokarran*	92.75
SF 92	*Eusphyra blochii*	*E. blochii*	100	*E. blochii*	100	*Sphyrna mokarran*	92.75
SF 93	*Rhynchobatus cf. laevis*	*R. cf. laevis*	99	*R. cf. laevis*	100	*R. australiae*	97.67
SF 94	*Rhynchobatus cf. laevis*	*R. cf. laevis*	99	*R. cf. laevis*	99.5	*R. australiae*	97.23
SF 95	*Rhynchobatus cf. laevis*	*R. cf. laevis*	99	*R. cf. laevis*	100	*R. australiae*	97.67
SF 96	*Rhynchobatus australiae*	*R. australiae*	99	*R. australiae*	99.84	*R. cf. laevis*	97.81
SF 98	*Anoxypristis cuspidata*	*A. cuspidata*	100	*A. cuspidata*	100	*Pristis zijsron*	88.57
SF 99	*Sphyrna mokarran*	*S. mokarran*	100	*S. mokarran*	100	*Eusphyra blochii*/(*Carcharhinus acronotus*)	92.75
SF 100	*Sphyrna lewini*	*S. lewini*	100	*S. lewini*	100	Not generated	na
SF 104	*Rhinobatos typus*	*R. typus*	100	*R. typus*	100	*Rhynchobatus australiae*	84.58
SF 105	*Rhinobatos typus*	*R. typus*	100	*R. typus*	100	*Rhynchobatus cf. laevis*	84.73
SF 107	*Carcharhinus dussumieri*	*C. dussumieri*	76	*C. dussumieri*	100	*C. sealei*	99.07
SF 108	*Carcharhinus dussumieri*	*C. dussumieri*	76	*C. dussumieri*	100	*C. sealei*	99.07
SF 109	*Carcharhinus dussumieri*	*C. dussumieri*	76	*C. dussumieri*	100	*C. sealei*	99.07
SF 110	*Carcharhinus dussumieri*	*C. dussumieri*	76	*C. dussumieri*	100	*C. sealei*	99.07
SF 111	*Carcharhinus dussumieri*	*C. dussumieri*	76	*C. dussumieri*	100	*C. sealei*	99.07
SF 113	*Carcharhinus sorrah*	*C. sorrah*	100	*C. sorrah*	100	*C. obscurus*	95.81
SF 114	*Carcharhinus sorrah*	*C. sorrah*	100	*C. sorrah*	100	*C. obscurus*	95.81
SF 115	*Carcharhinus sorrah*	*C. sorrah*	100	*C. sorrah*	100	*C. obscurus*	95.81
SF 116	*Carcharhinus sorrah*	*C. sorrah*	100	*C. sorrah*	100	*C. longimanus*	95.66
SF 117	*Carcharhinus macloti*	*C. macloti*	100	*C. macloti*	99.85	*C. obscurus*/*C. galapagensis*	97.22
SF 118	*Rhizoprionodon acutus*	*R. acutus*	100	*R. acutus*	99.85	*(R. porosus)*	94.6
SF 119	*Carcharhinus macloti*	*C. macloti*	100	*C. macloti*	100	*C. obscurus*/*C. galapagensis*	97.38
SF 120	*Carcharhinus macloti*	*C. macloti*	100	*C. macloti*	100	*C. obscurus*/*C. galapagensis*	97.38
SF 121	*Carcharhinus macloti*	*C. macloti*	100	*C. macloti*	100	*C. obscurus*/*C. galapagensis*	97.36
SF 122	*Carcharhinus sorrah*	*C. sorrah*	100	*C. sorrah*	100	*C. obscurus*	95.81
SF 123	*Carcharhinus sorrah*	*C. sorrah*	100	*C. sorrah*	100	*C. obscurus*	95.81
SF 124	*Carcharhinus sorrah*	*C. sorrah*	100	*C. sorrah*	100	*C. obscurus*	95.81
SF 125	*Carcharhinus sorrah*	*C. sorrah*	100	*C. sorrah*	100	*C. obscurus*	95.81
SF 126	*Carcharhinus dussumieri*	*C. dussumieri*	76	*C. dussumieri*	100	*C. sealei*	99.07
SF 127	*Carcharhinus amboinensis*	*C. amboinensis*	100	*C. amboinensis*	100	*C. brachyurus*	96.45
SF 128	*Carcharhinus amboinensis*	*C. amboinensis*	100	*C. amboinensis*	100	*C. brachyurus*	96.45
SF 129	*Sphyrna mokarran*	*S. mokarran*	100	*S. mokarran*	100	*Eusphyra blochii*/*(Carcharhinus acronotus)*	92.75
SF 130	*Carcharhinus amboinensis*	*C. amboinensis*	100	*C. amboinensis*	100	*C. brachyurus*	96.45
SF 131	*Carcharhinus obscurus*	*C. obscurus*	99	*C. obscurus*/*C. galapagensis*	100	*C. longimanus*	98.15
SF 132	*Carcharhinus amboinensis*	*C. amboinensis*	100	*C. amboinensis*	99.84	*C. brachyurus*	96.28
SF 133	*Carcharhinus amboinensis*	*C. amboinensis*	100	*C. amboinensis*	100	*C. brachyurus*	96.6
SF 134	*Carcharhinus dussumieri*	*C. dussumieri*	76	*C. dussumieri*	100	*C. sealei*	99.07
SF 135	*Carcharhinus obscurus*	*C. obscurus*	99	*C. obscurus*/*C. galapagensis*	100	*C. longimanus*	98.15
SF 137	*Carcharhinus amboinensis*	*C. amboinensis*	100	*C. amboinensis*	99.85	*C. brachyurus*	96.29
SF 139	*Sphyrna mokarran*	*S. mokarran*	100	*S. mokarran*	99.85	*Eusphyra blochii*/*(Carcharhinus acronotus)*	92.59
SF 140	*Carcharhinus amboinensis*	*C. amboinensis*	100	*C. amboinensis*	100	*C. brachyurus*	96.14
SF 141	*Carcharhinus amboinensis*	*C. amboinensis*	100	*C. amboinensis*	100	*C. brachyurus*	96.45
SF 142	*Negaprion acutidens*	*N. acutidens*	100	*N. acutidens*	100	*(N. brevirostris)*	95.06
SF 143	*Carcharhinus tilstoni*	*C. tilstoni*	49	*C. tilstoni*/*C. limbatus*	99.61	*C. amblyrhynchoides*	99.22
SF 144	*Triaenodon obesus*	*Triaenodon obesus*	100	*Triaenodon obesus*	99.85	*Carcharhinus brachyurus*	96.14
SF 145	*Triaenodon obesus*	*Triaenodon obesus*	100	*Triaenodon obesus*	99.85	*Carcharhinus brachyurus*	96.14
SF 146	*Triaenodon obesus*	*Triaenodon obesus*	100	*Triaenodon obesus*	100	*Carcharhinus brachyurus*	96.29
SF 147	*Triaenodon obesus*	*Triaenodon obesus*	100	*Triaenodon obesus*	100	*Carcharhinus brachyurus*/*C. isodon*	96.29

Appendix A (*Continued*)

Fin[a]	Consensus identification	Tree-based species	% bootstrap	Best matched species	% Similarity	2nd Best matched species[b]	% Similarity
SF 148	*Triaenodon obesus*	*Triaenodon obesus*	100	*Triaenodon obesus*	99.85	*Carcharhinus brachyurus*	96.14
SF 149	*Carcharhinus macloti*	*C. macloti*	100	*C. macloti*	99.85	*C. obscurus/C. galapagensis*	97.22
SF 150	*Carcharhinus macloti*	*C. macloti*	100	*C. macloti*	100	*C. obscurus/C. galapagensis*	97.38
SF 151	*Carcharhinus tilstoni*	*C. tilstoni*	49	*C. tilstoni*	100	*C. limbatus*	99.84
SF 152	*Carcharhinus dussumieri*	*C. dussumieri*	76	*C. dussumieri*	100	*C. sealei*	99.07
SF 153	*Carcharhinus macloti*	*C. macloti*	100	*C. macloti*	99.85	*C. obscurus/C. galapagensis*	97.22
SF 154	*Chiloscyllium punctatum*	*C. punctatum*	100	*C. punctatum*	99.23	*C. hasseltii*	91.74
SF 155	*Rhizoprionodon taylori*	*R. taylori*	100	*R. taylori*	100	*(R. porosus)*	94.42
SF 156	*Carcharhinus macloti*	*C. macloti*	100	*C. macloti*	100	*C. obscurus/C. galapagensis*	97.38
SF 157	*Carcharhinus macloti*	*C. macloti*	100	*C. macloti*	100	*C. obscurus/C. galapagensis*	97.38
SF 158	*Carcharhinus tilstoni*	*C. tilstoni*	49	*C. tilstoni/C. limbatus*	100	*C. amblyrhynchoides*	99.54
SF 159	*Rhizoprionodon taylori*	*R. taylori*	100	*R. taylori*	100	*(R. porosus)*	94.42
SF 160	*Carcharhinus amblyrhynchos*	*C. amblyrhynchos*	100	*C. amblyrhynchos*	100	*C. falciformis*	97.22
SF 161	*Sphyrna lewini*	*S. lewini*	100	*S. lewini*	100	Not generated	na
SF 162	*Carcharhinus amblyrhynchos*	*C. amblyrhynchos*	100	*C. amblyrhynchos*	100	*C. falciformis*	97.22
SF 163	*Carcharhinus tilstoni*	*C. tilstoni*	49	*C. tilstoni/C. limbatus*	100	*C. amblyrhynchoides*	99.54
SF 166	*Rhynchobatus cf. laevis*	*R. cf. laevis*	99	*R. cf. laevis*	100	*R. australiae*	97.68
SF 167	*Rhynchobatus australiae*	*R. australiae*	99	*R. australiae*	100	*R. cf. laevis*	97.66
SF 168	*Rhynchobatus cf. laevis*	*R. cf. laevis*	99	*R. cf. laevis*	100	*R. australiae*	97.52
SF 169	*Rhynchobatus cf. laevis*	*R. cf. laevis*	99	*R. cf. laevis*	100	*R. australiae*	97.67
SF 170	*Rhynchobatus cf. laevis*	*R. cf. laevis*	99	*R. cf. laevis*	100	*R. australiae*	97.67
SF 171	*Rhina ancylostoma*	*R. ancylostoma*	100	*R. ancylostoma*	100	*(Rhinobatos productus)*	86.33
SF 172	*Anoxypristis cuspidata*	*A. cuspidata*	100	*A. cuspidata*	100	*Pristis zijsron*	88.57
SF 173	*Anoxypristis cuspidata*	*A. cuspidata*	100	*A. cuspidata*	99.83	*Pristis zijsron*	88.54
SF 174	*Anoxypristis cuspidata*	*A. cuspidata*	100	*A. cuspidata*	100	*Pristis zijsron*	88.57
SF 175	*Anoxypristis cuspidata*	*A. cuspidata*	100	*A. cuspidata*	100	*Pristis zijsron*	88.57
SF 176	*Anoxypristis cuspidata*	*A. cuspidata*	100	*A. cuspidata*	100	*Pristis zijsron*	88.44
SF 177	*Carcharhinus brevipinna*	*C. brevipinna*	100	*C. brevipinna*	100	*C. brachyurus*	98.61
SF 178	*Sphyrna lewini*	*S. lewini*	100	*S. lewini*	100	Not generated	na
SF 179	*Sphyrna mokarran*	*S. mokarran*	100	*S. mokarran*	100	*Eusphyra blochii/(Carcharhinus acronotus)*	92.75
SF 180	*Sphyrna lewini*	*S. lewini*	100	*S. lewini*	100	Not generated	na
SF 181	*Sphyrna lewini*	*S. lewini*	100	*S. lewini*	100	Not generated	na
SF 182	*Galeocerdo cuvier*	*Galeocerdo cuvier*	100	*Galeocerdo cuvier*	100	Not generated	na
SF 183	*Carcharhinus sorrah*	*C. sorrah*	100	*C. sorrah*	100	*C. obscurus*	95.81
SF 184	*Carcharhinus tilstoni*	*C. tilstoni*	49	*C. tilstoni*	100	*C. limbatus*	99.84
SF 185	*Carcharhinus dussumieri*	*C. dussumieri*	76	*C. dussumieri*	100	*C. sealei*	99.07
SF 186	*Rhynchobatus australiae*	*R. australiae*	99	*R. australiae*	99.85	*R. cf. laevis*	97.82
SF 187	*Carcharhinus limbatus*	*C. limbatus*	60	*C. limbatus*	100	*C. amblyrhynchoides*	99.69
SF 188	*Carcharhinus tilstoni*	*C. tilstoni*	49	*C. tilstoni/C. limbatus*	100	*C. amblyrhynchoides*	99.54
SF 189	*Carcharhinus sorrah*	*C. sorrah*	100	*C. sorrah*	100	*C. obscurus*	95.81
SF 190	*Carcharhinus leucas*	*C. leucas*	100	*C. leucas*	100	*C. obscurus*	96.3
SF 191	*Sphyrna lewini*	*S. lewini*	100	*S. lewini*	100	Not generated	na
SF 192	*Sphyrna lewini*	*S. lewini*	100	*S. lewini*	100	Not generated	na
SF 193	*Carcharhinus amboinensis*	*C. amboinensis*	100	*C. amboinensis*	100	*C. brachyurus*	96.6
SF 195	*Carcharhinus amboinensis*	*C. amboinensis*	100	*C. amboinensis*	100	*C. brachyurus*	96.74
SF 196	*Sphyrna lewini*	*S. lewini*	100	*S. lewini*	100	Not generated	na
SF 197	*Eusphyra blochii*	*E. blochii*	100	*E. blochii*	100	*Sphyrna mokarran*	92.75
SF 198	*Carcharhinus limbatus*	*C. limbatus*	60	*C. limbatus*	100	*C. amblyrhynchoides*	99.68
SF 199	*Sphyrna lewini*	*S. lewini*	100	*S. lewini*	100	Not generated	na
SF 200	*Carcharhinus dussumieri*	*C. dussumieri*	76	*C. dussumieri*	100	*C. sealei*	99.07
SF 201	*Carcharhinus leucas*	*C. leucas*	100	*C. leucas*	100	*C. obscurus*	96.3
SF 202	*Carcharhinus dussumieri*	*C. dussumieri*	76	*C. dussumieri*	100	*C. sealei*	99.07
SF 203	*Carcharhinus leucas*	*C. leucas*	100	*C. leucas*	100	*C. obscurus*	96.3
SF 204	*Carcharhinus tilstoni*	*C. tilstoni/C. limbatus/C. amblyrhynchoides*	98	*C. tilstoni*	100	*C. limbatus*	99.69
SF 205	*Rhina ancylostoma*	*R. ancylostoma*	100	*R. ancylostoma*	100	*(Rhinobatos productus)*	86.33
SF 206	*Rhina ancylostoma*	*R. ancylostoma*	100	*R. ancylostoma*	100	*(Rhinobatos productus)*	86.33
SF 207	*Sphyrna mokarran*	*S. mokarran*	100	*S. mokarran*	100	*(Carcharhinus acronotus)*	92.96
SF 208	*Carcharhinus amboinensis*	*C. amboinensis*	100	*C. amboinensis*	100	*C. brachyurus*	96.14
SF 209	*Sphyrna mokarran*	*S. mokarran*	100	*S. mokarran*	100	*Eusphyra blochii/(Carcharhinus acronotus)*	92.75

Appendix A (*Continued*)

Fin[a]	Consensus identification	Tree-based species	% bootstrap	Best matched species	% Similarity	2nd Best matched species[b]	% Similarity
SF 210	*Carcharhinus obscurus*	*C. obscurus*	99	*C. obscurus*/*C. galapagensis*	100	*C. longimanus*	98.15
SF 211	*Sphyrna lewini*	*S. lewini*	100	*S. lewini*	99.83	Not generated	na
SF 212	*Carcharhinus amboinensis*	*C. amboinensis*	100	*C. amboinensis*	100	*C. brachyurus*	96.6
SF 213	*Himantura hortlei*	*H. hortlei*	100	*H. hortlei*	100	*H. fai*	91.45
SF 214	*Pastinachus sephen*	*P. sephen*	100	*P. sephen*	100	*P. cf. sephen*	93.83

Species in parentheses are not known from the area of collection.

[a] 18 samples did not produce usable sequences.

[b] Not generated = BOLD provides a maximum of 100 sequence matches, all 100 were to the designated species.

Appendix B. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.fishres.2008.09.036.

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