Genetic identification of sharks in the U.S. Atlantic large coastal shark fishery*

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Abstract.–Nucleotide sequences of a 394–396 base pair fragment of mitochondrial (mt) DNA, including parts of the cytochrome *b* and threonine tRNA genes, were obtained for eleven species of carcharhiniform sharks important to the U.S. Atlantic large coastal shark fishery. Sequences were used to predict sizes of restriction fragments produced by 118 restriction enzymes with unique recognition sequences. Seven restriction enzymes were chosen that produce an array of species-specific fragments for the eleven species. Geographic variation was examined in several species by surveying specimens from geographically distant regions. Only one of the species, the spinner shark *(Carcharhinus brevipinna),* exhibited geographic variation in mtDNA restriction fragments. The sandbar shark (*C. plumbeus*) exhibited sequence polymorphism that did not produce differences in restriction patterns of any of the seven enzymes. We detected numerous differences between observed restriction patterns in ten tiger sharks (*Galeocerdo cuvier*) and patterns predicted from a published sequence. We concluded that the published sequence is incorrect. Amplification of a single PCR product from a sample of meat, digestion of aliquots of the product with restriction enzymes, and sizing of fragments on agarose gels is an efficient method for distinguishing among these eleven carcharhiniform sharks. The method can be applied when only a small amount of tissue is available.

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The U.S. Atlantic large coastal shark fishery grew rapidly during the 1980s when commercial landings increased from 135 metric tons (t) in 1979 to a high of 7122 t in 1989 (NMFS, 1993). In 1993, a quota of 2750 t was established; in 1997 this quota was halved to 1375 t in order to rebuild depleted shark stocks. This fishery targets several species of sharks that are valued for fins (exported to Asia) and meat (sold domestically). Because of the slow growth rate, high age at maturity, and low fecundity of most shark species (Pratt and Casey, 1990), commercial shark fisheries typically collapse after a brief period unless strict conservation measures are implemented (Holden, 1974, 1977; Hoenig and Gruber, 1990). The 1993 shark fishery management plan divided exploited species into three categories: large coastal sharks, small coastal sharks, and pelagic sharks (NMFS, 1993). The category that grew most rapidly was the large coastal shark fishery, which is dominated by several species of requiem sharks and two species of hammerhead sharks.

Sound management of a multispecies fishery requires information on the vulnerability of each component of the fishery. Differences in life history characters, e.g. intrinsic growth rates, locations of nursery areas, or migration within or outside of the fished area, can result in different vulnerabilities to overfishing of exploited species. For example, Musick et al. (1993) reported a relative decline in the dusky shark (*Carcharhinus obscurus*) off Virginia during expansion of the large coastal shark fishery. Thus, it is important to estimate catches on a species-by-species basis and to implement species-specific management. In the event that regulations (e.g. moratoria or minimum size limits) are applied to individual species, enforcement will rely on identification of protected species within the catch. The manner in which sharks are processed at sea, however, makes it difficult to accurately identify species at landing. Sharks typically are headed, gutted, and finned (i.e. fins are removed), thus destroying morphological characters necessary for species identification. Although Castro (1993) recommended a suite of characters for identification of shark carcasses, the limited number of available morphological characters makes it difficult to distinguish among several species. Martin (1993) suggested that use of restriction-frag-

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ment differences in polymerase chain reaction (PCR) amplified DNA might provide a rapid and inexpensive means of identifying carcasses and fins. We develop this technique as a means of identifying the eleven most frequently landed carcharhiniform sharks in the U.S. east coast longline fishery.

Materials and methods

Tissues (heart, white muscle, or fin) from nine species of carcharhinid sharks and two species of sphyrnid sharks were obtained from commercial fishermen, sport tournaments, and research longlining cruises (Table 1). Tissues were either frozen in the field and stored at –80°C or preserved immediately in 10× Longmire's lysis buffer (0.1 M tris, 0.1 M Na_2EDTA, 0.01 M NaCl, 0.5% SDS, pH 8.0) at room temperature. Genomic DNA was isolated by first powdering tissue under liquid nitrogen with a prechilled mortar and pestle. Approximately 100 mg of powdered tissue were suspended in 500 µL STE buffer (0.1 M NaCl, 50 mM tris, 1 mM EDTA; pH 7.5) and lysed with 25 µL 20% SDS. Genomic DNA was extracted twice with phenol:chloroform:isoamyl alcohol (25:24:1) and twice with chloroform:isoamyl alcohol (24:1). DNA in aqueous phase was precipitated by adding 2.5 volumes of ice-cold absolute ethanol and 0.1 volumes of 3M NaOAC, stored at –20°C for at least two hours, centrifuged for 15 min at 4°C at maximum speed in a microcentrifuge, and rinsed with 70% ethanol.

PCR amplification for cycle sequencing or restriction-enzyme digestion was accomplished by using a suite of PCR primers. The "diagnostic" fragment used in digestions consisted of a single segment, 394–396 bp in length, that was amplified by using light-strand primer Cb3RL (CATATTAAACCCGAATGATAYTT) located within the 3' domain of the mitochondrially encoded cytochrome *b* (cyt *b*) gene and heavy-strand primer Cb6H (CTCCAGTCTTCGRCTTACAAG) located within the mitochondrially encoded threonine tRNA ($tRNA^{THR}$) gene (Martin and Palumbi, 1993). This fragment was sequenced through the primer sites by using additional primer sets within and outside the diagnostic fragment. PCR fragments were prepared for sequencing by using the Bio-Rad Prep-a-Gene DNA purification system that removes extraneous salts, primers, and small fragments of DNA prior to cycle sequencing. Dideoxy DNA sequencing was performed with the Promega *fmol* DNA sequencing system by using ^{32}P end-labeled primers. Cycle sequencing reactions consisted of a single two-minute denaturing process at 95°C, followed by thirty cycles of 1 min at 95°C, 30 sec at 64°C, and 30 sec at 72°C. Sequencing reactions were scored on 6% denaturing

Table 1

Sources of tissues or DNA sequences. EMBL/Genbank accession numbers identify sequences from Martin and Palumbi (1993).

Species	Acronym	Source of sequence	Source of specimen
Bignose shark (*Carcharhinus altimus*)	Cal-A	This study	Virginia, Atlantic Ocean
Blacktip shark (*C. limbatus*)	Cli-A	"	Virginia, Atlantic Ocean
Bull shark (*C. leucas*)	Cle-A	"	Florida, Gulf of Mexico
Dusky shark (*C. obscurus*)	Cob-A	"	Virginia, Atlantic Ocean
Sandbar shark (A) (*C. plumbeus*)	Cpl-A	GenBank L08032	Hawaii, Pacific Ocean
Sandbar shark (B)	Cpl-B	This study	Florida, Gulf of Mexico
Sandbar shark (C)	Cpl-C	"	Hawaii, Pacific Ocean
Silky shark (*C. falciformis*)	Cfa-A	"	Hawaii, Pacific Ocean
Spinner shark (A) (*C. brevipinna*)	Cbr-A	"	Pacific Ocean, Australia
Spinner shark (B)	Cbr-B	"	Virginia, Atlantic Ocean
Lemon shark (*Negaprion brevirostris*)	Nbr-B	GenBank L08039	Florida, Atlantic Ocean
"Tiger shark" (A)[1] (*Galeocerdo cuvier*)	Gcu-A	GenBank L08034	Hawaii, Pacific Ocean
Tiger shark (B)	Gcu-A	This study GenBank AF004288	Florida, Gulf of Mexico
Great hammerhead (*Sphyrna mokarran*)	Smo-A	"	Florida, Gulf of Mexico
Scalloped hammerhead (*S. lewini*)	Sle-A	GenBank L08041	Hawaii, Pacific Ocean

[1] It was determined that this sequence was not tiger shark (see text).

polyacrylamide gels and examined by autoradiography. DNA sequences were read on an IBI gel reader and entered directly into computer text files. Sequences were confirmed by recording a minimum of two separate sequencing runs through each base. Sequences were aligned by using the ESEE software package (Cabot and Beckenback, 1989). Four cyt *b* sequences—those of sandbar (*C. plumbeus*), lemon (*Negaprion brevirostris*), tiger (*Galeocerdo cuvier*), and great hammerhead sharks (*Sphyrna mokarran*)—were downloaded from the EMBL/NCBI Genbank database (Martin and Palumbi, 1993). All other cyt *b* sequences and all $tRNA^{THR}$ sequences were acquired from our laboratory.

Sequences from each species between primers Cb3RL and Cb6H were concatenated with sequences of the primers to produce a single sequence for each of the eleven species (Table 2). Sequences were examined for predicted restriction sites with IBI MacVector software (IBI Mac Vector, 1991). One hundred eighteen restriction enzymes with unique recognition sequences were used in the search for restriction sites.

Amplified "diagnostic" fragments were prepared by amplifying genomic DNA at thirty cycles of 95°C for 1 min, 48 to 52°C for 30 sec, and 72°C for 30 sec. Amplified fragments were ethanol precipitated as above and reconstituted in water. Generally, one 100 µL PCR reaction produced enough diagnostic fragment for all seven restriction digestions. Fragments were digested by using manufacturer's buffers and specifications. Most restriction patterns were scored on 2% agarose gels run on 1X TAE. Fragments between 40 and 100 bp were scored on either vertical nondenaturing polyacrylamide gels or 2% nusieve 3:1 agarose gels. Fragments less that 40 bp were not scored, although loss of fragments as small as 24 bp could be inferred from mobility shifts in larger fragments. All gels were stained with ethidium bromide and examined under UV light (Sambrook et al., 1989).

Results

A single mtDNA fragment of 394–396 bp that contained the 3' end of cyt *b* and part of the $tRNA^{THR}$ gene was amplified in all species. Except for two hammerhead sharks, the fragment was 395 bp in length (Table 2). Cyt *b* sequences from all eleven species were identical in length and unambiguously aligned. In comparison with the other nine species, the scalloped hammerhead (*S. lewini*) possessed a single-base deletion in the $tRNA^{THR}$ sequence, and the great hammerhead possessed a single-base insertion in the $tRNA^{THR}$ sequence.

In bignose shark (*C. altimus*), three fragments of sizes 395 bp, 720 bp, and 1040 bp were produced repeatedly. The additional bands were more pronounced at lower (48°C) than higher (52°C) annealing temperatures. Further investigation with additional primers revealed that bignose shark possessed a much larger mitochondrial D-loop region than any other shark in the study. Amplification with a light-strand primer located within cyt *b* and a heavy-strand primer located within the 12S ribosomal RNA gene produced an approximately 1400-bp fragment in all species except bignose shark, where a single fragment of approximately 2000 bp was produced. We hypothesize that the Cb6H recognition site within the $tRNA^{THR}$ gene is duplicated twice within the D-loop of the bignose shark, resulting in a three-banded amplification product. Duplication of segments of flanking regions within the mitochondrial D-loop have been reported in other vertebrates (Broughton and Dowling, 1994, and citations within). To our knowledge, this is the first evidence of this phenomenon in elasmobranchs. To obtain the single product for sequencing and restricting, the 395-bp fragment was excised from an agarose gel and reamplified to produce sufficient amounts of the diagnostic fragment.

Of 118 restriction enzymes surveyed by MacVector software, 34 were predicted to have restriction sites within one or more of the eleven species. Seven restriction enzymes (*Alu*I, *Dde*I, *Fok*I, *Hae*III, *Hinc*II, *Hinf*I, and *Rsa*I) were chosen for screening because use of these seven enzymes allowed all eleven species to be distinguished (Table 3). The number of sharks whose mtDNA was subjected to restriction-enzyme digestion is given in Table 4. The initial screen of restriction sites was undertaken with only a single sequence from each species. Differences in predicted and observed restriction patterns in three species (sandbar, spinner, and tiger sharks) made it necessary to sequence additional animals in order to investigate whether differences were due to sequencing errors or intraspecific variation.

Observed restriction patterns in sandbar sharks from the Gulf of Mexico differed by restriction site from the pattern predicted from the published sequence of Martin and Palumbi (1993) for a sandbar shark (Cpl-A) from Hawaii (Martin[1]). Although we predicted that *Fok*I would not cut the sandbar shark fragment, *Fok*I digestion produced two fragments of 310 and 85 bp. We sequenced additional sandbar sharks from the Gulf of Mexico (Cpl-B) and from Hawaii (Cpl-C) and found that both sequences possessed the *Fok*I restriction site. Sequence of the speci-

[1] Martin, A. P. 1997. University of Nevada-Las Vegas, Las Vegas, NV 89154-4004. Personal commun.

Table 2

DNA Sequences used to predict restriction digests. Boxes surround primer sequences used in PCR amplification (A=adenine, C=cytosine, G=guanine, T=thymidine, Y=C or T). A vertical line is drawn between the end of the cytochrome *b* sequence and the beginning of the threonine tRNA sequence. See Table 1 for description of samples.

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          5'  Cb3RL  ->

Cal-A   CATATTAAACCCGAATGATAYTT CTTATTTGCTTATGCAATCCTGCGCTCAATCCCTAATAAACTAGGAGGAGTCCTAGC
Cbr-A   ....................... ............C......T.A.............C.....................
Cbr-B   ....................... ............C......T.A.............C.....................
Cfa-A   ....................... ......C.....C......T.A........T.........................
Cle-A   ....................... ........................A...........C.....................
Cli-A   ....................... ......C..C............A........T.........................
Cob-A   ....................... ...................TT.A...........C.....................
Cpl-A   ....................... ...........................................................
Cpl-B   ....................... ...........................................................
Cpl-C   ....................... ...........................................................
Gcu-A   ....................... .C.......C.........T.C..G...........C.....................
Gcu-B   ....................... ...................T.A..T..T.....C..................T.....
Nbr-A   ....................... .........C............A.....T..T.....C.....................
Sle-A   ....................... .C....C..C.........T.A.....T..T.....C..............T....
Smo-A   ....................... ......C..C.........T.A.....C..T.....C.....................

Cal-A   TCTCCTATTCTCTATCTTCATCCTTATATTGGTGCCCCTCCTCCACACCTCCAAACAACGAAGTACCATCTTCCGACCCA
Cbr-A   C.............T..T.....C.....A..C..T........T...................T.............
Cbr-B   C.............T..T.....C.....A..C..T........T.....................................
Cfa-A   ......................C........T..T............................C........T.......
Cle-A   C........T..C..T..T...........A..T..T.....................................T.
Cli-A   ............C.....T...........AA.T..T.............T.............................T
Cob-A   C...........G.....T.....C.....A..T..T.............................C...G............
Cpl-A   .................T.....................................................................
Cpl-B   .....................................................................................
Cpl-C   .....................................................................................
Gcu-A   C........T.....TC.T...........A..T..T.....T..A.....T.........................A.
Gcu-B   C..AG.........T........C...C.AA.T..T..T...........T...........C.G..C........AAC
Nbr-A   C...........C..T........C...C.A..C.....T...........A.............T............TC
Sle-A   C..TT.......A.....T.....C...C.A..T..A..A..T.....................A............AC
Smo-A   ............C.....T.....C...C.A..C.TTTA...T........T............A............AC
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continued

men from the Gulf of Mexico (Cpl-B) differed from that of the specimen from Hawaii (Cpl-C) by three base-pair substitutions. The sequence from the specimen from Hawaii (Cpl-C) differed from that of Martin and Palumbi (1993) by one base-pair (and which affected the *Fok*I restriction site). Whether the single base pair difference between our specimen from Hawaii and the one reported in Martin and Palumbi (1993) represents an uncommon polymorphism in sandbar sharks from the Pacific or an error in reading the original sequence cannot be determined. We examined restriction digestions from three sandbar sharks collected near Hawaii and all possessed the *Fok*I restriction site.

We also found a populational difference between spinner sharks (*C. brevipinna*) collected from the North Atlantic (including the Gulf of Mexico) and from the Pacific coast of Australia. Initially, we sequenced a spinner shark from Australia and predicted that *Rsa*I would not cut the diagnostic fragment. We restricted fragments from seven spinner sharks, two from Australia, and five from the U.S. Atlantic and Gulf of Mexico coasts. Although fragments from the two spinner sharks from Australia were not cut with *Rsa* I, each of the five specimens from the Atlantic produced fragments of 251 and 144 bp. We sequenced one specimen from the Atlantic (spinner B) and found two nucleotide substitutions, including one that resulted in the restriction site difference between spinner sharks from the Atlantic and Pacific.

We detected numerous differences between restriction patterns predicted from the sequence of Martin and Palumbi (1993) for tiger shark collected near Hawaii and our tiger sharks collected from the Gulf of Mexico (Atlantic) and Hawaii and Australia (Pacific).

Table 2 (continued)

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Cal-A   TAACACAAATCTTCTTCTGACTTCTTGTGGCCAACTCAATTATTTTAACTTGAATTGGAGGTCAACCAGTAGAACAACCA
Cbr-A   ..........T.................A..T..T.............................C..................
Cbr-B   ..........T.................A..T..T.............................C..................
Cfa-A   ........................C..A..............C..................C..................
Cle-A   ..........TC............C..A..T..T.......................T.....................
Cli-A   ............................A..T..T.....C.........................C............
Cob-A   ...G........................A.....T.............................C..............
Cpl-A   ...........................................................................
Cpl-B   ...........................................................................
Cpl-C   ...........................................................................
Gcu-A   ....C......C.........T.C.....A..T.........C..CC.................C..................
Gcu-B   .T.......................C..A..T.................C............A...................
Nbr-A   .C...................T.......A..T.................................C..................
Sle-A   ........................C..A..............CC.................C..................
Smo-A   ...................T.....C.A..T...........C.....C..............................

Cal-A   TTCATTATAGTAGGACAAATCGCCTCAATCTCCTACTTTTCCTTATTCCTTATTATTATACCATTCACTAGCTGATGAGA
Cbr-A   ....................T..T.............................C..C.....T..T..C............
Cbr-B   ....................T..T.............................C..C.........T..C............
Cfa-A   ..T.....................................G.....................C........C..
Cle-A   .......C............T.....................................C.........T..............
Cli-A   ...........................................T......G.C.....................................
Cob-A   ...............T.....................................C..C......C.T..............
Cpl-A   ....................T.....................................C..............
Cpl-B   ...........................................................................
Cpl-C   ....................T.....................................C..............
Gcu-A   ....................T.....................A....T...........T......G.C........C..
Gcu-B   ..T....C............T..T.....................T.....C..C........T.TC........C..
Nbr-A   ..T....................T.....................................T...........T..
Sle-A   ..T....C..............................C..TC....T.....C............G.C........C..
Smo-A   ..T.....................................A..........C............G..........C..

Cal-A   AAACAAAATCCTCAGCCTAAATTAG | TTTTGGTAACTTAACT-AAAAAGCGTCGAC | CTTGTAAGYCGAAGACTGGAG
Cbr-A   ......................... | ........G..................... | .....................
Cbr-B   ......................... | ........G..................... | .....................
Cfa-A   ...T..................... | ........G..................... | .....................
Cle-A   ......................... | ........G..................... | .....................
Cli-A   ......................... | ........G..................... | .....................
Cob-A   ......................... | ........G..................... | .....................
Cpl-A   ......................... | ........G..................... | .....................
Cpl-B   ......................... | ........G..................... | .....................
Cpl-C   ......................... | ........G..................... | .....................
Gcu-A   .........T............C... | ........G..................... | .....................
Gcu-B   ......................C... | ........G..................... | .....................
Nbr-A   ...T..................... | ........G..................... | .....................
Sle-A   .........T............C... | .......GG........-............ | .....................
Smo-A   .........T............C... | .......GG.....T.CGT........... | .....................

                                                                    <-  Cb6H  3'
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We sequenced a tiger shark from the Gulf of Mexico and found 46 nucleotide differences between our sequence and that of Martin and Palumbi (1993). This difference is greater than that seen between all pairs of species in this study and is clearly too large of a difference to be explained by intraspecific polymorphism. Because our observed restriction patterns for tiger sharks from the Atlantic and Pacific match predictions made from our tiger shark sequence, our sequence for the tiger shark is likely correct, and part of the sequence listed in Martin and Palumbi (1993) is not that of tiger shark.

The remaining eight species—bignose, blacktip (*C. limbatus*), bull (*C. leucas*), dusky, silky (*C. falciformis*), scalloped hammerhead, great hammerhead, and lemon sharks—exhibited restriction patterns

identical to those predicted based on sequence data (Table 4). The list of species that showed no variation in restriction pattern included silky and dusky sharks from widely disparate geographic locales.

Discussion

Restriction digests of the diagnostic 394–396 bp fragment proved a reliable way to distinguish among the

Table 3

Predicted restriction fragments for seven enzymes based on nucleotide sequences.

Species	*Alu*I	*Dde*I	*Fok*I	*Hae*III	*Hinc*II	*Hinf*I	*Rsa*I
Bignose	232, 84, 79	331, 64	310, 85	205, 190	223, 148, 24	324, 71	251, 144
Blacktip	121, 111, 79, 43, 41	333, 64	395	395	223, 148, 24	324, 71	251, 144
Bull	190, 121, 43, 41	331, 64	395	395	223, 148, 24	324, 71	251, 144
Dusky	311, 43, 42	331, 64	395	221, 174	371, 24	324, 71	395
Sandbar A	223, 79, 43, 41	331, 64	395	205, 190	223, 148, 24	324, 71	251, 144
Sandbar B	223, 79, 43, 41	331, 64	310, 85	205, 190	223, 148, 24	324, 71	251, 144
Sandbar C	223, 79, 43, 41	331, 64	310, 85	205, 190	223, 148, 24	324, 71	251, 144
Silky	232, 79, 43, 41	331, 64	310, 85	221, 174	371, 24	324, 71	395
Spinner A	190, 121, 43, 41	331, 64	395	221, 174	371, 24	324, 71	395
Spinner B	190, 121, 43, 41	331, 64	395	221, 174	371, 24	324, 71	251, 144
Lemon	190, 121, 43, 41	331, 64	310, 85	221, 174	371, 24	324, 71	395
"Tiger" A[1]	190, 121, 43, 41	331, 64	208, 187	221, 174	371, 24	216, 108, 71	251, 144
Tiger B	190, 121, 43, 28, 13	331, 64	310, 85	395	371, 24	395	395
Great Hammerhead	121, 111, 85, 89	331, 65	396	396	223, 149, 24	325, 71	396
Scalloped Hammerhead	311, 83	256, 75, 63	394	221, 173	370, 24	323, 71	395

[1] It was determined that this sequence was not that of a tiger shark. See text.

Table 4

Number and location of sharks whose mtDNA was subjected to restriction enzyme digestion. Numbers in parentheses represent number of sharks from each source location in the sample.

Species	*n*	Source of specimen
Bignose shark	4	Virginia, Atlantic Ocean (4)
Blacktip shark	7	Virginia, Atlantic Ocean (1); Florida, Gulf of Mexico (6)
Bull shark	7	Florida, Gulf of Mexico (7)
Dusky shark	7	Virginia, Atlantic Ocean (2); Florida, Atlantic Ocean (1); Australia, Pacific Ocean (3); Australia, Indian Ocean (1)
Lemon shark	7	Florida, Gulf of Mexico (6); Mexico, Gulf of Mexico (1)
Sandbar shark	10	Florida, Gulf of Mexico (7); Hawaii, Pacific Ocean (3)
Silky shark	7	Texas, Gulf of Mexico (3); Hawaii, Pacific Ocean (4)
Spinner shark	7	Virginia, Atlantic Ocean (1); Florida, Atlantic Ocean (3); Florida, Gulf of Mexico (1); Australia, Pacific Ocean (2)
Tiger shark	10	Florida, Gulf of Mexico (5); Australia, Pacific Ocean (2) Hawaii, Pacific Ocean (3)
Great hammerhead	7	Florida, Gulf of Mexico, Florida (6); Florida, Atlantic Ocean (1)
Scalloped hammerhead	7	Virginia, Atlantic Ocean (1); Florida, Gulf of Mexico (2); Alabama, Gulf of Mexico (1); Texas, Gulf of Mexico (1); Campeche, Gulf of Mexico (2)

eleven species of carcharhiniform sharks. Each species possessed a unique set of restriction fragments, and even in the species where polymorphism was detected, misidentification was not a problem. This technique can be performed on small pieces of tissue collected at dockside and stored indefinitely at room temperature and moreover can distinguish between species whose carcasses may be difficult to discriminate (Fig. 1). Similar DNA technology has been used for identifying carcasses in teleosts (Chow et al., 1993; Bartlett and Davidson, 1991), and for identifying planktonic fish eggs (Daniel and Graves, 1993)—cases where morphological characters proved insufficient for species level identification .

Use of genetic characters to identify species can be complicated by population structure. Although most marine fishes that are distributed across vast geographic stretches are open-ocean pelagics (Briggs, 1960), with presumed minor genetic differences across the range of a species (but see Crosetti et al., 1994; Graves et al., 1992), many large coastal sharks are distributed as multiple discrete populations (Compagno, 1984). It is thus important to know whether genetic differences among individuals are diagnostic of species or populations. Some questions, among others, are the following: to what degree are published genetic data useful in regions other than those from which the original specimens were collected, and can this method be used to identify populations as well as species? Baker et al. (1996), for example, were able to determine geographic origin of marine mammals they identified from flesh samples on the basis of genetic data. In our study, regional differences in restriction patterns were observed in only one species, spinner shark, whereas sequence differences that did not affect restriction patterns were observed in sandbar shark. In four species (dusky, silky, scalloped hammerhead, and tiger), sequences from one individual accurately predicted restriction sites in specimens collected thousands of kilometers from where the original specimen was collected.

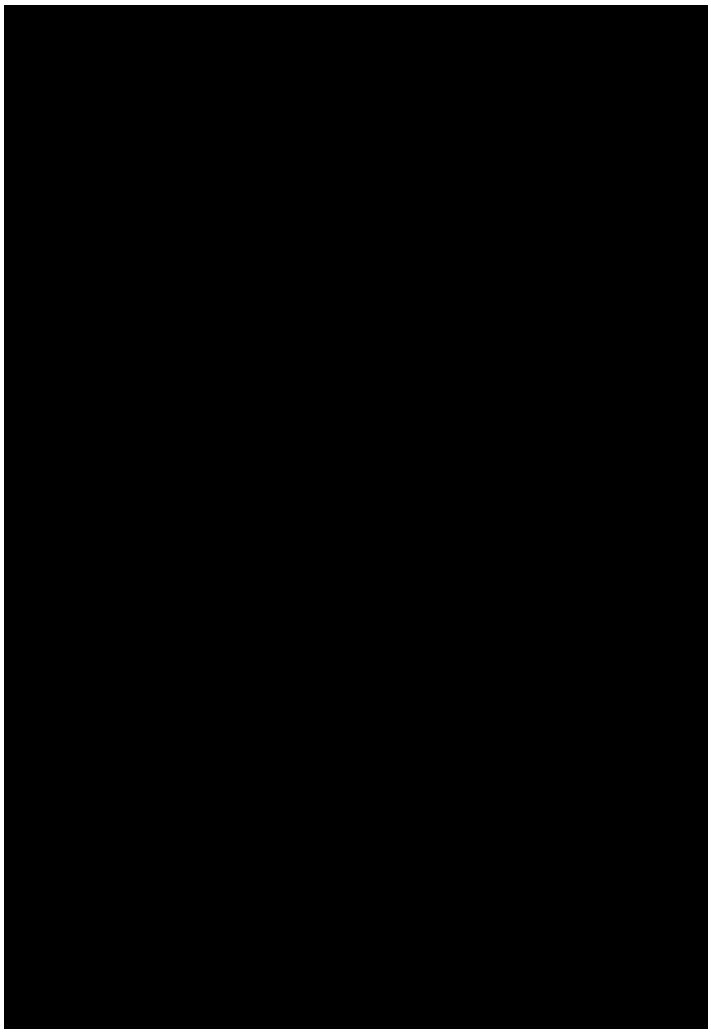

Figure 1

Ten-percent polyacrylamide gel of *Alu*I digests in three species that produce very similar carcasses. Lane zero is a size standard, lanes one to three are sandbar sharks, lanes four to six are bignose sharks, and lanes seven to nine are dusky sharks. Numbers at left refer to sizes (base pairs) of size standards. Predicted sizes of fragments in each species are given in Table 3.

The low level of intra- and interregional polymorphism within species is not surprising given the low genetic diversity typically reported for sharks (Smith, 1986). In restriction fragment length polymorphism (RFLP) studies of whole mtDNA molecules, Heist et al. (1995, 1996) found a very low nucleotide sequence diversity of 0.036% in sandbar shark and 0.13% in Atlantic sharpnose shark (*Rhizoprionodon terraenovae*). The small numbers of substitutions between geographically distant populations of sandbar and spinner shark observed in this study indicate that intraspecific diversity should not hinder species identification of these eleven species.

The erroneous tiger shark sequence in the study of Martin and Palumbi (1993) indicates a further strength of the PCR-RFLP technique beyond use for forensic identification; it can be used to evaluate

validity of sequence data. Many molecular genetic or phylogenetic studies (or both) are based on sequences sampled from only one individual of a species. Discovery of erroneous sequence data in such studies is becoming increasingly common (Derr et al., 1992; Helbig and Seibold 1996; Ledje and Arnason, 1996). PCR reactions are easily contaminated by carryover DNA from other organisms or by other DNAs in the laboratory (Thomas, 1994). Mislabeling of tubes also can lead to incorrect assignment of sequence data to species. We sequenced the entire cyt *b* gene in tiger shark from Florida and, in comparison with the "tiger shark" sequence reported by Martin and Palumbi (1993), found one nucleotide difference in the first (5'-most) 540 bp of the gene and 64 nucleotide differences in the remaining 606 bp of the gene. We hypothesize that the "tiger shark" sequence of Martin and Palumbi (1993) is a mixture that includes sequence data from another species. The discrepancy between published and newly determined sequences is similar to observations on published bird sequence by Helbig and Seibold (1996) and on mammalian sequences reported by Ledje and Arnason (1996). In each case, the published sequences differed so greatly from newly obtained sequences that conclusions of the prior works were called into question. The free exchange of sequence data through Genbank also facilitates replication of errors. Validity of sequence data can, and perhaps should, be tested by amplifying DNA from several individuals of the same species, and then by determining whether predicted restriction sites are present in all amplifications.

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Literature cited

Baker, C. S., F. Cipriano, and S. R. Palumbi.
1996. Molecular genetic identification of whale and dolphin products from commercial markets in Korea and Japan. Mol. Ecol. 5:671–685.

Bartlett, S. E., and W. S. Davidson.
1991. Identification of *Thunnus* tuna species by the polymerase chain reaction and direct sequence analysis of their mitochondrial cytochrome *b* genes. Can. J. Fish. Aquat. Sci. 48:309–317.

Briggs, J. C.
1960. Fishes of worldwide (circumtropical) distribution. Copeia 1960:171–180.

Broughton, R. E., and T. E. Dowling.
1994. Length variation in mitochondrial DNA of the minnow *Cyprinella spiloptera*. Genetics 138:179–190.

Cabot, E. L., and A. T. Beckenback.
1989. Simultaneous editing of multiple nucleic acid and protein sequences with ESEE. Comp. Appl. Biol. 5:233–234.

Castro, J. I.
1993. A field guide to the sharks commonly caught in commercial fisheries of the southeastern United States. U.S. Dep. Commer., NOAA Tech. Memo. NMFS-SEFSC-338.

Chow, S., M. E. Clarke, and P. J. Walsh.
1993. PCR-RFLP analysis on thirteen western Atlantic snappers (subfamily Lutjanidae): a simple method for species and stock identification. Fish. Bull. 91:619–627.

Compagno, L. J. V.
1984. FAO species catalogue, vol 4, part 1: sharks of the world. An annotated and illustrated catalogue of shark species known to date. FAO Fish. Synop. 125, 655 p.

Crosetti, D., W. S. Nelson, and J. C. Avise.
1994. Pronounced genetic structure of mitochondrial DNA among populations of the circumglobally distributed grey mullet (*Mugil cephalus*). J. Fish. Biol. 44:47–58.

Derr, J. N., S. K. Davis, J. B. Wolley, and R. A. Wharton.
1992. Reassessment of the 16S rRNA nucleotide sequence from members of the parasitic Hymenoptera. Mol. Phylogenet. Evol. 2:136–147.

Daniel, L. B. III, and J. E. Graves.
1993. Morphometric and genetic identification of eggs of spring-spawning sciaenids in lower Chesapeake Bay. Fish. Bull. 92:254–261

Graves, J. E., J. R. McDowell, A. M. Beardsley, and D. R. Scoles.
1992. Stock structure of the bluefish *Pomatomus saltatrix* along the mid-Atlantic coast. Fish Bull. 90:703–710.

Heist, E. J., J. E. Graves, and J. A. Musick.
1995. Population genetics of the sandbar shark (*Carcharhinus plumbeus*) in the Gulf of Mexico and Mid-Atlantic Bight. Copeia 1995:555–562.

Heist, E. J., J. A. Musick, and J. E. Graves.
1996. Mitochondrial DNA diversity and divergence among sharpnose sharks, *Rhizoprionodon terraenovae*, from the Gulf of Mexico and Mid-Atlantic Bight. Fish. Bull. 94:664–668.

Helbig, A. J., and I. Seibold.
1996. Are storks and new world vultures paraphyletic? Mol. Phylogenet. Evol. 6:315–319

Hoenig, J. M., and S. H. Gruber.
1990. Life-history patterns in the elasmobranchs: implications for fisheries management. *In* H. L. Pratt Jr, S. H. Gruber, and T. Taniuchi (eds.), Elasmobranchs as living resources: advances in the biology, ecology, systematics, and the status of the fisheries, p. 1–16. U.S. Dep. Commerce, NOAA Tech. Rep. NMFS 90.

Holden, M. J.
1974. Problems in the rational exploitation of elasmobranch populations and some suggested solutions. *In* F. R. Harden-Jones (ed.), Sea fisheries research, p. 117–137. John Wiley and Sons, New York, NY.

1977. Elasmobranchs. *In* J. A. Gulland (ed.), Fish population dynamics, p. 187–214. John Wiley and Sons, New York, NY.

IBI Mac Vector.

1991. IBI Mac Vector, 3.5 International Biotechnologies Inc. New haven, CT.

Ledje, C., and U. Arnason.

1996. Phylogenetic analyses of complete cytochrome *b* genes of the order Carnivora with particular emphasis on the Carniformia. J. Mol. Evol. 42:135–144.

Martin, A. P.

1993. Application of mitochondrial DNA sequence analysis to the problem of species identification of sharks. U.S. Dep. Commer., NOAA Tech. Rep. NMFS 115:53–59.

Martin, A. P., and S. R. Palumbi.

1993. Protein evolution in different cellular environments: cytochrome *b* in sharks and mammals. Mol. Biol. Evol. 10:873–891.

Musick, J. A., S. Branstetter, and J. A. Colvocoresses.

1993. Trends in shark abundance from 1974 to 1991 for the Chesapeake Bight region of the U.S. Mid-Atlantic coast. U.S. Dep. Commer., NOAA Tech. Rep. NMFS 115: 1–18.

NMFS (National Marine Fisheries Service).

1993. Fishery management plan for sharks of the Atlantic Ocean. U.S. Dep. Commer., Natl. Mar. Fish. Serv. Southeast Regional Office, St Petersburg, FL 33702, 167 p.

Pratt, H. L., Jr., and J. G. Casey.

1990. Shark reproductive strategies as a limiting factor in directed fisheries, with a revision of Holden's method of estimating growth parameters. *In* H. L. Pratt Jr, S. H. Gruber, and T. Taniuchi (eds.), Elasmobranchs as living resources: advances in the biology, ecology, systematics, and the status of the fisheries, p. 97–109. U.S. Dep. Commer., NOAA Tech. Rep. NMFS 90.

Sambrook, L., E. F. Fritsch, and T. Maniatis.

1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, NY.

Smith, P. J.

1986. Low genetic variation in sharks (Chondrichthyes). Copeia 1986:202–207.

Thomas, R. H.

1994. Analysis of DNA from natural history museum collections. *In* B. Schierwater, B. Streit, G. P. Wagner, R. Desalle (eds.), Molecular ecology and evolution: approaches and applications, p. 311–321. Birkhauser Verlag, Basel.