Abstract—Molecular-based approaches for shark species identification have been driven largely by issues specific to the fishery. In an effort to establish a more comprehensive identification data set, we investigated DNA sequence variation of a 1.4-kb region from the mitochondrial genome covering partial sequences from the 12S rDNA, 16S rDNA, and the complete valine tRNA from 35 shark species from the Atlantic fishery. Generally, within-species variability was low in relation to interspecific divergence because species haloptypes formed monophyletic groups. Phylogenetic analyses resolved ordinal relationships among Carcharhiniformes and Lamniformes, and revealed support for the families Sphyrnidae and Triakidae (within Carcharhiniformes) and Lamnidae and Alopidae (within Lamniformes). The combination of limited intraspecific variability and sufficient between-species divergence indicates that this locus is suitable for species identification.

Mitochondrial gene sequences useful for species identification of western North Atlantic Ocean sharks

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Seventy-three species of sharks inhabit the United States territorial waters of the Atlantic Ocean, Gulf of Mexico, and Caribbean Sea (Compagno, 1984a, 1984b). All but one (spiny dogfish, *Squalus acanthias*, managed separately) are managed under the current Fisheries Management Plan (FMP) for highly migratory species (NMFS[1]). Thirty-three species are of lesser commercial importance and are relegated to the "deepwater and other" species management group, and 19 species cannot be landed commercially or recreationally ("prohibited species" group). The remaining 20 species are of interest to the commercial shark fishery and are categorized as large coastal species (LCS), small coastal species (SCS), and pelagic species management units in the current FMP. Although these management units are practical, it is clear that species respond uniquely to exploitation and therefore should be managed on a species-by-species basis (Castro et al., 1999; NMFS[2]). Species-level management is widely recommended (e.g., FAO Marine Resource Service, 2000) but is complicated by the paucity of species-specific fisheries data, stemming, in part, from an inability to accurately identify species.

Many commercially important species (e.g., within Carcharhiniformes) are difficult to identify whole, and this task is more daunting if individuals are processed (head, entrails, and fins are removed); unfortunately, at-sea processing is widespread in the industry (Castro[3]). Although current U.S. legislation prohibits the practice of "finning" (where fins are retained and carcasses are discarded at sea),

[1] NMFS (National Marine Fisheries Service). 2003. Final amendment 1 to the fishery management plan for Atlantic tunas, swordfish and sharks, 599 p. Office of Sustainable Fisheries, Highly Migratory Species Management Division, NMFS, NOAA, 1315 East West Highway, SSMC3, Silver Spring, MD 20910.

[2] NMFS (National Marine Fisheries Service). 2001. Final United States national plan of action for the conservation and management for sharks, 90 p. Office of Sustainable Fisheries, Highly Migratory Species Management Division, NMFS, NOAA, 1315 East West Highway, SSMC3, Silver Spring, MD 20910.

[3] Castro, J. I 1993. A field guide to the sharks commonly caught in commercial fisheries of the southeastern United States. NOAA Tech. Memo. NMFS-SEFSC-338, 47 p. Southeast Fisheries Science Center, NMFS, NOAA, 75 Virginia Beach Dr., Miami, FL 33149.

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the landing of fins is allowed where carcasses and fins are off-loaded at the same time in a no more than 1:20 (fin-to-carcass) weight ratio. However, serious problems can arise in matching off-loaded fins to processed carcasses. In and of itself, the landing of shark fins can be lucrative; fins accounted for more than 50% of the total Atlantic shark fishery value in 2002 (NMFS[4]). Because preferences exist for fins from certain species, exvessel prices for specific types of fin vary considerably (e.g., Weber and Fordham, 1997). It is perhaps not surprising that augmenting the fin-to-carcass ratio with spoiled meat or "finning" target species out of season (and subsequently attributing the fins to fish that are allowed to be caught during the season) might not be uncommon (Vannuccini, 1999). Clearly, these possibilities lead to the challenge of matching collected fins to processed carcasses. Therefore, accurate and reliable species identification methods are paramount for law enforcement and sound species management.

Molecular species identification research on sharks has been driven largely by resolution of specific problems associated with the fishery. For example, Heist and Gold (1999) used mtDNA sequence data to develop restriction fragment assays that differentiate 11 species of carcharhiniform sharks commonly encountered in the LCS fishery. Similarly, Pank et al. (2001) used multiplex PCR to differentiate two morphologically similar shark species (*Carcharhinus obscurus* and *C. plumbeus*)—an approach that was expanded by Shivji et al. (2001) to include five additional species (with some overlap of species included by Heist and Gold 1999). Both approaches are relatively rapid, inexpensive, and easily implemented; however, they appear most applicable when the number of species investigated is limited. In sum, of the thirty-nine species of sharks that are not in the "deepwater and other" management group, molecular species identification assays have been developed for fifteen species (9 LCS, 3 pelagic, and 3 in the prohibited species management groups) (Heist and Gold, 1999; Pank et al., 2001; Shivji et al., 2001), leaving 24 species without molecular methods for identification.

Some investigators have instead turned to DNA sequence analysis to resolve issues of species identification (Takeyama et al., 2001; Akimoto et al., 2002; Jerome et al., 2003). This approach is exemplified best by the recent development of computer interfaces that allow access to and analysis of large DNA databases (DNA Surveillance, Ross et al., 2003; ARB, Ludwig et al., 2004). Simply put, these databases circumvent the tedious process of scanning large taxonomically diverse DNA repositories (e.g., GenBank) by allowing the user to access (DNA Surveillance) or maintain (ARB) taxonomically restricted sets of reference sequences. Users can submit "unknown" sequences to compare against specified sequence subsets; subsequent analyses are returned as genetic distances (between unknown and reference sequences) and include a phylogenetic hypothesis.

The power of this approach lies in the ease with which reference sequences can be added to the database, in the "quality-control" that can be exerted over subsequent additions to the reference sequences, and in the ease with which geographic variation within species can be included. The success of this approach, however, hinges on the information contained in the gene in the reference database. The inception of this approach, as applied to commercially important sharks, requires a sufficiently informative set of reference sequences against which searches can be made. The aforementioned molecular approaches (RFLP, multiplex PCR) include a diversity of gene regions (mitochondrial DNA, nuclear ITS); thus no comprehensive data set exists for commercially landed Atlantic shark species. Fortunately, recent work with a 2.4-kb fragment of the mitochondrial genome (spanning 12S rDNA to 16s rDNA) to examine the phylogenetic relationships among shark orders has shown that this region may be useful in resolving relationships at this taxonomic level (Douady et al., 2003). Unfortunately, sampling within orders was limited, and it is thus unknown whether this region contains sufficient phylogenetic signal at lower taxonomic levels.

We present here mtDNA sequence data of a smaller fragment of the same region containing partial sequence information for the mitochondrial 12S rDNA, 16S rDNA, and the complete valine tRNA from 35 shark species (including all 20 commercially exploited species, 12 of 19 prohibited species, the spiny dogfish, and two species of *Mustelus*). We suggest that a suitable locus for species-identification purposes will permit identification of unequivocally distinct species (i.e., large genetic differentiation between species compared to within species) and offer the potential for meaningful phylogenetic comparisons (important when "query" animals are absent or not adequately represented in a molecular database). Keeping in mind issues of species identification and fisheries management, we examine this mtDNA region for patterns of genetic variability and assess its utility in phylogenetic reconstruction. We then discuss the use of this region for the underpinnings of a validated reference DNA database suitable for forensic and fisheries management applications.

Methods

Sample collection

Voucher Atlantic Ocean shark samples (muscle, fin, or blood) were obtained from the CCEHBR Marine Forensics archive in Charleston, SC (Table 1). Samples were accompanied by species certification and chain-of-custody forms. Muscle and fin samples were either frozen at

[4] NMFS (National Marine Fisheries Service). 2003. Stock assessment and fishery evaluation report for Atlantic highly migratory species (SAFE), 274 p. Office of Sustainable Fisheries, Highly Migratory Species Management Division, NMFS, NOAA, 1315 East West Highway, SSMC3, Silver Spring, MD 20910.

Table 1

Scientific and common names of samples, number of individuals sampled (*n*), species codes, and Genbank accession numbers. Taxonomy follows Campagno (1984, 2001). Species codes correspond to a representative individual in the National Ocean Service Marine Forensics Program (CCEHBR, Charleston, SC) tissue archive with that particular haplotype (except for *Heterodontus francisci* Hfra1).

Order	Family and species	Common name	Code (*n*)	Accession
Carcharhiniformes	Carcharhinidae			
	Carcharhinus acronotus	Blacknose	Cacr003(3)	AY830721
	C. altimus	Bignose	Calt001(2)	AY830722
	C. brevipinna	Spinner	Cbre001(3)	AY830723
	C. falciformis	Silky	Cfal003(1)	AY830725
			Cfal006(1)	AY830726
	C. isodon	Finetooth	Ciso004(1)	AY830727
			Ciso010(1)	AY830728
			Ciso015(1)	AY830729
	C. leucas	Bull	Cleu003(3)	AY830730
	C. limbatus	Blacktip	Clim004(1)	AY830731
			Clim006(2)	AY830732
	C. longimanus	Oceanic whitetip	Clon000(1)	AY830736
			Clon002(1)	AY830733
			Clon005(1)	AY830734
			Clon006(1)	AY830735
	C. obscurus	Dusky	Cobs000(1)	AY830737
			Cobs001(3)	AY830738
	C. perezi	Caribbean reef	Cper001(2)	AY830739
			Cper002(2)	AY830740
	C. porosus	Smalltail	Cpor001(1)	AY830743
	C. plumbeus	Sandbar	Cplu004(2)	AY830741
			Cplu023(1)	AY830742
	C. signatus	Night	Csig002(1)	AY830744
	Galeocerdo cuvier	Tiger	Gcuv003(3)	AY830746
	Negaprion brevirostris	Lemon	Nbre005(1)	AY830756
	Prionace glauca	Blue	Pgla004(1)	AY830760
			Pgla0020(1)	AY830761
			Pgla0022(1)	AY830762
	Rhizoprionodon terraenovae	Sharpnose	Rter001(2)	AY830763
			Rter026(1)	AY830764
	Sphyrnidae			
	Sphyrna lewini	Scalloped hammerhead	Slew003(2)	AY830768
	S. mokarran	Great hammerhead	Smok003(3)	AY830769
	S. tiburo	Bonnethead	Stib016(2)	AY830770
			Stib018(1)	AY830771
	S. zygaena	Smooth hammerhead	Szyg681(6)	AY830772
	Triakidae			
	Mustelus canis	Smooth dogfish	Mcan003(3)	AY830754
	M. norrisi	Florida smoothhound	Mnor001(2)	AY830755
Lamniformes	Alopiidae			
	Alopias superciliosus	Bigeye thresher	Asup001(1)	AY830718
			Asup006(1)	AY830719
	A. vulpinus	Thresher	Avul002(1)	AY830720
	Lamnidae			
	Carcharodon carcharias	White	Ccar002(3)	AY830724
	Isurus oxyrinchus	Shortfin mako	Ioxy005(1)	AY830747
			Ioxy032(1)	AY830748
			Ioxy051(1)	AY830749
	I. paucus	Longfin mako	Ipau002(2)	AY830750
			Ipau005(1)	AY830751
	Lamna nasus	Porbeagle	Lnas001(2)	AY830752
			Lnas003(1)	AY830753

continued

Table 1 (continued)

Order	Family and species	Common name	Code (*n*)	Accession
	Odontaspididae			
	Carcharius taurus	Sand tiger	Otau004(1)	AY830757
			Otau005(1)	AY830758
			Otau007(1)	AY830759
Orectolobiformes	Ginglymostomatidae			
	Ginglymostoma cirratum	Nurse	Gcir001(2)	AY830745
Hexanchiformes	Hexanchidae			
	Hexanchus vitulus	Bigeye sixgill	Hvit1(1)	AY830716
	Heptranchias perlo	Sevengill	Hper1(1)	AY830715
Squaliformes	Squalidae			
	Squalus acanthias	Spiny dogfish	Saca002(1)	AY830765
			Saca003(2)	AY830766
Squatiniformes	Squatinidae			
	Squatina dumeril	Atlantic angel	Sdum001(3)	AY830767
Heterodontiformes	Heterodontidae			
	Heterodontus francisci	Horn shark	Hfra(1)	NC003137

–80°C, dried, or stored in 70% EtOH. Blood was stored at room temperature in sodium dodecyl sulfate-urea (SDS-urea: 1% SDS, 8M urea, 240 mM Na_2HPO_4, 1mM EDTA pH 6.8). Total nucleic acids were extracted from frozen, dried, and EtOH-preserved samples by using DNeasy Tissue Kits and following manufacturer's recommendations (Qiagen, Valencia, CA). DNA was isolated from blood in SDS-urea according to White and Densmore (1992; protocol 11). Extracted DNA was visualized by electrophoresis in a 1% agarose gel stained with 0.4 ng/mL of ethidium bromide in 1× Tris-borate-EDTA (TBE: 89 mM Tris-borate, 2 mM Na_2EDTA, pH 8). A 1-kb DNA ladder (Promega, Madison, WI) was used as a size standard.

Amplification and sequencing

Primers 12SA-5′ and 16SA-3′ (Palumbi, 1996) were used to amplify an approximately 1400-bp region spanning the 3′ end of the 12s rDNA, the valine tRNA, and the 5′ end of the 16s rDNA region of mitochondrial DNA (mtDNA). Samples were amplified in 50 µL reactions containing ~50 ng of template DNA, 20 mM Tris-HCl pH 8.4, 50 mM KCl, 0.2 mM each dNTP, 2 mM $MgCl_2$, 20 mM each primer, and 2.5 units *Taq* DNA polymerase (Gibco BRL, Rockville, MD). Thermal cycling consisted of an initial denaturation at 94°C for 1.5 minutes, followed by 30 cycles of 40 seconds at 94°C, 40 seconds at 52°C, and 50 seconds at 72°C, and a final extension step of 15 minutes at 72°C. Negative controls (no template) were included in each set of reactions. PCR products were gel-purified as described in Rosel and Block (1996) and 20–50 ng were used as template for ABI Big Dye Terminator (v. 1.0, Applied Biosystems, Foster City, CA) cycle sequencing reactions. Sequence was obtained with amplification primers 12SA-5′, 16SA-3′ and two additional internal sequencing primers. Sequencing reaction products were precipitated with ethanol, washed according to sequencing kit instructions, dried in a Savant Speedvac Plus, and resuspended in 4 μL of loading dye (5:1 Hi-Di formamide:dextran blue). Fragments were analyzed on an Applied Biosystems 377 automated DNA sequencer.

Sequence analysis and alignment

Sequences were edited with SEQUENCHER (vers. 3.0; Gene Codes Corp., Detroit, MI). We included three additional sequences from GenBank: horn shark (*Heterodontus francisci*, NC003137) to represent the family Heterodontidae, thorny skate (*Raja radiata*, AF106038), rabbit fish (*Chimaera monstrosa*, AJ310140), and the Atlantic guitarfish (*Rhinobatis lentiginosus*, AY830717—this study) to serve as outgroups for phylogenetic analyses. Sequences were aligned by using a linear hidden Markov model (HMM) as implemented in SAM (Sequence Alignment and Modeling System; Hughey and Krogh, 1996; Karplus et al., 1998) with default settings. The alignment file is available from the senior author.

Phylogenetic hypotheses were constructed by using the maximum parsimony (MP) and neighbor-joining (NJ) algorithms implemented in PAUP 4.0b10 (Sinauer Associates, Sunderland, MA). NJ analyses employed a variety of pairwise distance measures, but the distance measure used had little or no effect on the recovered topologies. Phylogenies recovered with MP with equally weighted characters were generally concordant with those recovered by NJ, particularly when bootstrap consensus trees were compared. For ease of interpretation, we report NJ analyses using p-distances as a metric. Bootstrapping (Felsenstein, 1985) was used to estimate the reliability of NJ reconstructions (1000 pseudoreplicates).

Results

Sequence variation and divergence

An approximately 1.4-kb gene region was amplified and sequenced from 93 samples representing 35 shark species. Fifty-seven of the 93 individuals had unique haplotypes (Table 1, Fig. 1). An alignment of these haplotypes with several outgroups with the SAM algorithm resulted in a 1510 position consensus alignment after the introduction of gaps. Of these 1510 aligned positions, 717 positions were variable and 543 were parsimony informative. Transition outweighed transversion substitutions by a factor of 4.27. Considering only phylogenetically informative sites within the ingroup, we found that nucleotide composition did not differ significantly among haplotypes (A: 35.9%, C: 21.9%, G: 16.9%, T: 25.3%; χ^2=175.6, P=0.39).

Phylogenetic analysis

Unweighted parsimony analysis produced 24 equally parsimonious trees of length 2733 (CI=0.39, RI=0.74) that differed primarily in the relationships among haplotypes within species (not shown). Neighbor-joining analyses produced nearly identical topologies regardless of the distance metric used. When differences were noted, they often involved trivial placements of individual variants within species or the placement of branches that were poorly supported by bootstrap analyses regardless of the reconstruction method employed. For this reason, we present phylogenetic hypotheses generated by neighbor-joining, using p-distances as a surrogate for all analyses.

Most clades containing multiple haplotypes within species were highly supported by bootstrap analyses. Of 16 species represented by more than a single sequence, 15 were recovered as monophyletic groups in 100% of 1000 bootstrap replicates (Fig. 1). Sequence divergence within species was generally trivial compared to among-species divergences. For example, sequence divergence among haplotypes within species of *Carcharhinus* differed by approximately two orders of magnitude from that among species within the genus (average p-distance of 0.05% and 4.16%, respectively). The exception involved haplotypes observed within *C. plumbeus* that were supported as monophyletic by fewer than 70% of 1000 bootstrap replicates in MP and NJ analyses. Interestingly, a sister group relationship between *C. plumbeus* and *C. altimus* was highly supported by bootstrapping, and average sequence divergence within species (0.14%) was only about one-third of that observed between these two (0.43%).

Some higher order relationships were recovered with high bootstrap support. Notably the Carcharhiniformes were strongly supported as monophyletic, as were the families Sphyrnidae and Triakidae. The family Carcharhinidae was poorly supported as monophyletic, although a group that included *Negaprion*, *Prionace*, and all *Carcharhinus* was observed in a large number of bootstrap replicates. *Carcharhinus* was paraphyletic in the NJ topology, and *Negaprion* was nested within the genus, but this relationship received little support from bootstrapping. The Lamniformes were monophyletic and strongly supported by bootstrapping. Within this order, only the family Lamnidae received strong support, whereas support for a monophyletic Alopidae was moderate. The order Hexanchiformes was recovered as a monophyletic group; however bootstrap support for this grouping was low.

Discussion

Our goal was to assess whether the 12s–16s region of the shark mitochondrial genome contained sufficient genetic variation and phylogenetic signal to be useful in species identification. Of the 35 species examined, 6 species were each represented by a single individual, and 16 of the remaining 29 species contained variants at the mtDNA locus examined. Importantly, all within-species variants formed strongly supported monophyletic groups concordant with morphologically based species descriptions. Intraspecific variability was low in relation to interspecific divergence at this locus and in no instance was a paraphyletic relationship between species observed. The combination of limited intraspecific variability combined with sufficient between-species divergence indicates that this locus is suitable for species identification.

Two exceptions to this generalization of low within versus large between-species differentiation exist in our phylogenetic hypothesis—one involving the sister species pair *C. plumbeus* and *C. altimus*. In an alignment of mitochondrial sequences from these species, only 5 or 7 transition substitutions were observed across approximately 1.4 kb of sequence data. Interestingly, Heist and Gold (1999) included these two taxa in their cytochrome-*b* RFLP analysis, and again, Atlantic samples of *C. plumbeus* and *C. altimus* differed by only a single transition in 395 basepairs (0.25%), and there were more substitutions observed between Atlantic and Pacific *C. plumbeus* than between Atlantic samples of *C. plumbeus* and *C. altimus* (Table 2 in Heist and Gold 1999). The next most closely related pair of taxa in our phylogenetic hypothesis comprised two other Carcharhiniforms, *C. longimanus* and *C. obscurus*, a taxon pair differing by approximately 1.44% sequence divergence, compared with an average of 0.06% within taxon diversity. These two taxa were considered by Shivji et al. (2001) while developing a multiplex PCR assay for six commercially important pelagic species. Specifically, assays developed to diagnose *C. obscurus* could not discriminate between *C. obscurus* and *C. longimanus*, two closely related species in our phylogenies. The *C. plumbeus* and *C. altimus* species pair was not considered by Shivji et al. (2001); thus no comparison to the Heist and Gold (1999) cytochrome-*b* sequence/RFLP or the 12s–16s data set presented in our study was possible. We are currently analyzing additional samples,

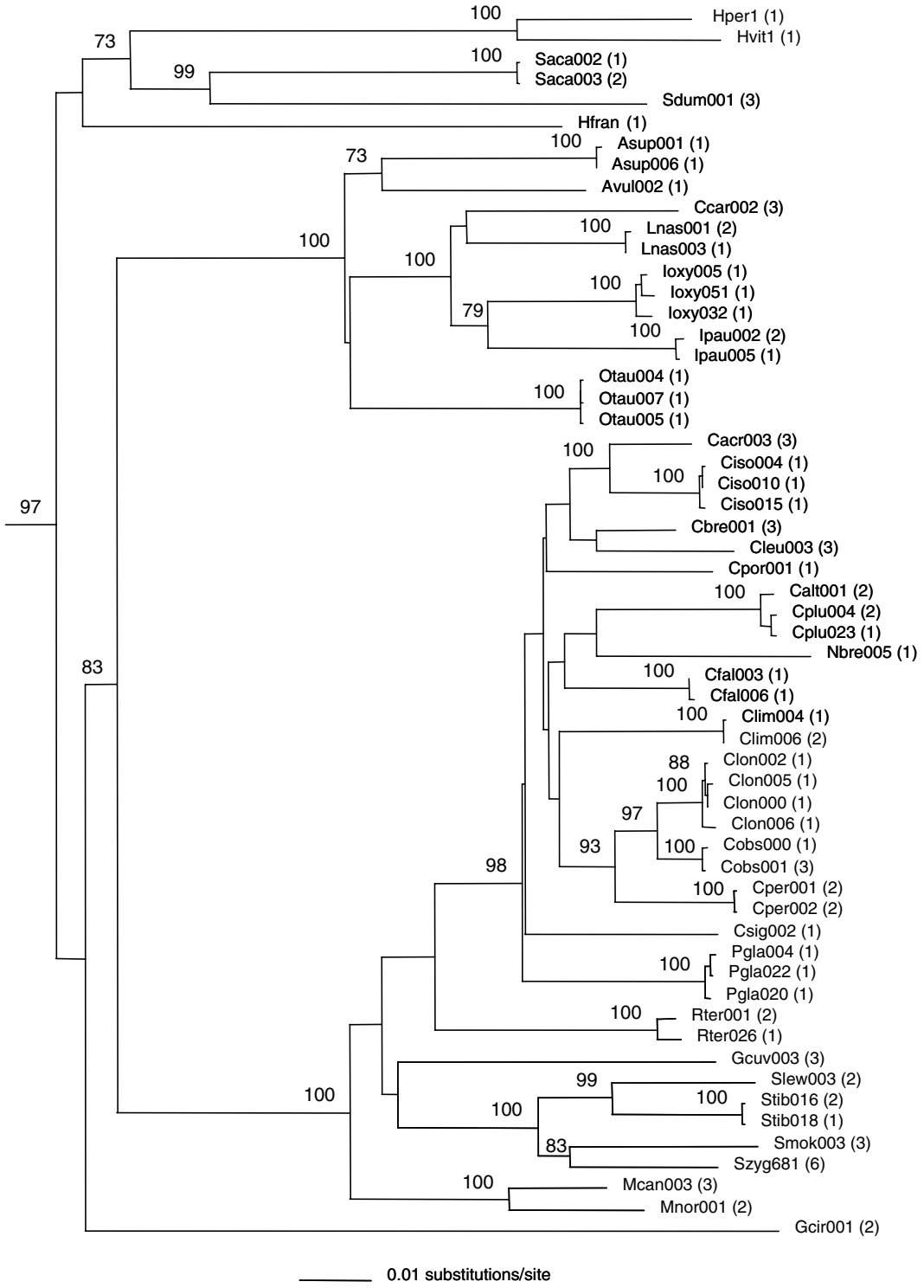

Figure 1

Neighbor-joining tree showing relationship of observed 12s–16s haplotypes among 36 species of shark. Codes are defined in Table 1 and numbers in parentheses indicate the number of individuals found with the indicated haplotype. Bootstrap support is indicated as numbers immediately above the relevant node (only values greater than 70% are shown). The phylogeny was rooted with several outgroup taxa (*Heterodontus francisci* (NC003137), *Raja radiata* (AF106038), *Chimaera monstrosa* (AJ310140), and *Rhinobatis lentiginosus* (AY830717)).

including a more comprehensive geographical survey of these four species to confirm that the genetic differences observed are diagnostic. However, it is clear that DNA sequence-based approaches appear more powerful in discriminating closely related species pairs and less likely to produce false positives than other DNA-based assays.

Although it was not our intent to conduct an exhaustive analysis of higher-order relationships among western North Atlantic shark species, some interesting results nonetheless deserve mention. First, the orders Carcharhiniformes and Lamniformes were strongly supported as monophyletic, as were the families Sphyrnidae, Triakidae, and Lamnidae that were included in the study. The order Hexanchiformes was likewise monophyletic, but bootstrap support for this grouping was low. The family Carcharhinidae was poorly supported as monophyletic, which is consistent with previous studies (Nalyor, 1992; Nelson, 1994; Musick et al., 2004). Interestingly, our phylogenetic hypotheses place the family Triakidae basal to all other families within the Carcharhiniformes, following Compagno (1988), but this position was not strongly supported and is predicated on limited sampling of Carcharhiniform familes (only four of eight were included in our analysis). Clearly this gene region contains some phylogenetically useful information regarding shark relationships, confined principally to higher-level groupings.

We are careful in judging the utility of a locus for species identification on the basis of phylogenetic signal alone. Clearly, rapidly evolving molecular markers are valuable tools for species identification but might not be appropriate for reconstructing phylogenetic relationships at certain scales. Conversely, those regions containing sufficient signal to generate reasonable phylogenetic reconstructions (i.e., general concordance with accepted phylogenetic relationships based on other independent characters) must be useful (and appropriate) markers for species identification. Further, these regions are amenable to the addition of uncharacterized species and the inclusion of intraspecific diversity (e.g., diverged mtDNA lineages within species). Importantly, however, DNA sequence-based approaches offer the potential to assign at least some level of taxonomic characterization to unknown or unrepresented samples. Although the use of DNA sequencing has historically been viewed as cost prohibitive, the genomic revolution over recent years has spawned cost-effective sequencing services, making routine sequencing of samples for species identification not only practical but optimal.

The size of the amplification product in the present study might place limitations on the application of this method to the poor-quality tissue and DNA often encountered in forensic studies. It has been our experience that the primers used in our study consistently have generated strong amplification products with DNA isolated from a variety of tissue types, including dried tissue and fins; however, we have yet to explore the range of amplifications possible using tissues more commonly encountered in forensic cases. To circumvent potential problems with large amplifications on degraded DNA samples, we have constructed a preliminary, searchable DNA-sequence database using the FASTA program (Univ. Virginia, Charlottesville, VA; Pearson, 1999) and the 12s–16s sequences presented in the present study. Our preliminary analyses indicate that all species examined in the study can be uniquely identified from approximately 400 bp of sequence generated by the 12SA-5′ primer. We are examining the limitations of sequence length in combination with the search accuracy of this informative fragment.

We are mindful of the restriction placed on these analyses due to limited within-taxon sampling (particularly within-family) and of the incomplete representation (notably the Pristophoriformes) of all orders of sharks and are aware that the phylogenetic affinities presented in this study could change with the addition of characters and taxa. These caveats notwithstanding, we believe that a taxonomically restricted DNA sequence database offers certain advantages over perhaps more rapid RFLP or multiplex PCR assays. DNA databases 1) can be "curated" (additions and access to the database can be selective) and distributed as an alignment suitable for further subsequent statistical or phylogenetic manipulation; 2) can be easily amended to include additional taxa, genetic variation within species, and additional gene loci more appropriate at various taxonomic scales; 3) allow for unequivocal assignment (subject to limits of discrimination of those loci included) of species identification while making available the raw data necessary for the development of more rapid assays (RFLP/Multiplex PCR) for select taxa (note that the opposite is not necessarily true); and, 4) facilitate the identification of those taxa not currently represented in the database through phylogenetic analysis.

In summary, we have found that the sequence of the 12S–16S region of the mtDNA that we examined contains ample information for discriminating between the shark species studied and shows promise for the placement of species not yet examined within the correct phylogenetic group (family). We are continuing to examine geographic variation within and among species and to assay genetic variability at nuclear loci in an effort to resolve potential introgression and (or) hybridization events. As information is added to our database, either in the form of additional species or loci, our species identification method will become more robust.

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